

Research Article

Dodartia orientalis reproduction under temperature, salinity, and burial constraints

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Abstract: Dodartia *Dodartia orientalis* L. (Scrophulariaceae) is an invasive perennial weed that has been investigated as a medicinal plant species. There are no comprehensive studies on the biology of this weed. Seven experiments on seeds and rhizomes were conducted in the laboratory and greenhouse at the Weed Research Department of the Iranian Research Institute of Plant Protection in 2018 and 2019. The results showed that dodartia seed viability was 62.5%. The optimum constant and alternating temperatures for seed germination were 20 °C and 15/25 °C, respectively. Alternating temperature was more appropriate than constant temperature for dodartia seed germination. The use of concentrated sulfuric acid (98%) for 2 minutes was the best stimulus for Dodartia seed germination. Conversely, gibberellin did not stimulate seed germination. The seeds germinated at NaCl concentrations < 300 mM. The study of dodartia vegetative reproduction under the effect of various environmental factors showed that the optimal planting depths and lengths for rhizome emergence were 2.5 cm and 15 cm, respectively. Rhizomes did not emerge in response to salt stress (for all studied NaCl concentrations).

Additionally, the planting depth and smaller rhizome size lead to a longer time to shoot emergence. The study of cold stress effects revealed that Dodartia rhizome emerged after 24 and 48 hours of incubation at 0 °C and -5 °C. Dodartia rhizomes emerged after 24, 48, and 72 hours at 25 °C, and after 48 hours at 0 °C and -5 °C. However, keeping rhizomes at -10 °C resulted in their loss of viability. Our findings indicate that breaking rhizomes into small pieces and deep burial are crucial for effectively controlling Dodartia growth.

Keywords: Invasive weed, Seed germination, Vegetative reproduction, Weed biology

Introduction

Weed biology provides the foundation for

successful weed management systems. It is essential to understand the biology of weeds and develop effective management strategies based on current and emerging knowledge (Bhowmik,

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1997). Research in weed biology continues to explore seed dormancy and germination, weed seed bank dynamics, root reserves, tuber dormancy, the evolution of weeds, modeling of weed seedling emergence, morphology and physiology of growth, competitive ability, and reproductive biology (Norris, 1997). The biological traits, ecological processes, and management of the weed are discussed in relation to its distribution, biology, and ecology. Both seed germination (SG) and dormancy play significant roles in regulating weed seed banks (Bajwa et al., 2019). Traditionally, effective weed management has depended on farmers gaining an understanding of the characteristics of the weeds they manage. For effective weed management, further research into weed biology is essential (Van Acker, 2009).

Vegetative reproduction is a typical trait of perennial weeds. The importance of perennial weeds stems from their ability to reproduce asexually (Soteres and Murray, Vegetative propagation can be accomplished by means of stolons, rhizomes, tubers, bulbs, corms, roots, and stems (Bhowmik, 1997). One of the major barriers to perennial weed control is the re-emergence of reproductive parts. The majority of the world's 18 most dangerous weeds (61%), such as field bindweed Convolvulus arvensis L., purple nutgrass Cyperus rotundus L., Bermuda grass Cynodon dactylon L. Pers, and Johnson grass Sorghum halepense, reproduce vegetatively (Ghersa and Martinez-Ghersa, 1991). There are several research studies on sexual and asexual reproduction in perennial weeds (Meighani et al., 2021). Wilcut et al. (1988) reported high levels of rhizome emergence under drought conditions in Bermuda grass C. dactylon, cogon grass Imperata cylindrica, torpedograss Panicum repens, and Johnson grass S. halepense. Dexter (1937) studied the effects of freezing on the underground organs of some perennial weeds. He observed that although the underground organs of Bermuda grass C. dactylon, field bindweed C. arvensis, and cheatgrass Bromus tectorum didn't regenerate when the temperature decreased to -8 °C, 30% of their roots survived

at -6 °C. Dunham *et al.* (1956) reported that quackgrass *Agropyron repense* rhizomes were destroyed at -7 °C. McWhorter (1972) found that Johnson grass tolerated soil and growth chamber temperatures of up to -3 °C for 24 hours, but not below -3 °C.

Dodartia *Dodartia orientalis* L. is the sole species of dodartia belonging to the Phrymaceae family. Dodartia is a perennial, xerophytic, herbaceous weed with 25-40 cm height, bare, and sometimes finely and shortly pubescent in the lower part.

Dodartia rhizomes are thick and give rise to offspring. The stems` are numerous, straight, and branched from the base. The leaves of dodartia are flat, sessile, spaced, serrated, and quickly fall off. Lower leaves are opposite, ovate or oblong, and widened at the base; the rest are alternate, linear-lanceolate, or lanceolate. The leaf plate is 1.5-2.5 cm long and 0.2-0.5 cm wide (Sharipova 2020). This species was first named in 1634 by a French physician and botanist (Denis Dodart). Dodartia seeds are tiny, oval, and light to dark brown. Seed germination is epigeal (Li *et al.*, 2001).

Dodartia has been present in Iran for several years and has been found in the provinces of Guilan (Talesh), North Khorasan (Bojnourd), West Azerbaijan, Sistan and Baluchestan (Iranshahr), and Tehran (Varamin Shahriyar). This weed has been introduced into cotton and alfalfa fields, as well as roads and irrigation canals (Sohrabi et al., 2014). It is also used for livestock feeding (Chen et al., 2018). In addition to Iran, this species has been reported in Turkmenistan, Afghanistan, the Caucasus, Central Asia, Mongolia (Mozaffarian, 1996), Uzbekistan (Sharipova, 2020), Western Siberia (Sokolov et al., 2020), and Poland (Wrzesien et al., 2016).

Although Dodartia maintains its survival through both vegetative and reproductive propagation, seeds are the primary means of its spread. There are reports on the botanical properties (Amiri and Jabarzadeh, 2010; Wang et al., 2017), phylogeny (Liu et al., 2014), medicinal properties (Li et al., 2001), and molecular biology of dodartia (Schaferhoff et

al., 2010). Up to now, too limited research studies have been carried out, and little information exists about the environmental effects on sexual and asexual reproduction of dodartia. Therefore, we investigated the impact of certain environmental factors on the SG and vegetative reproduction of Dodartia. This knowledge may help complete the basic information for weed management. The findings of this study, if effectively linked to practical weed management, can help predict how this response weed evolves in to varied environmental conditions.

Materials and Methods

Seed collection

Dodartia ripe fruits were collected from parent plants (at least 30 adult plants were randomly selected) in cotton fields of Varamin (Tehran Province) (latitude of 35°21′N, longitude of 51°37′ E with an altitude of 927 m above the sea level) during the 2018 and 2019 growing seasons.

To ensure uniform seed drying, fruits were stored at room temperature for four weeks after harvest. Then, the seeds were separated from the fruits, cleaned by hand, and stored in paper bags at room temperature for two months.

To investigate Dodartia's sexual and asexual reproduction in response various environmental factors, seven experiments were conducted on two separate organs (seed and rhizome) in the laboratory and greenhouse at the Weed Research Department, Iranian Research Institute of Plant Protection, in 2018 and 2019. All experiments were conducted over a period of two years. To break the hard coat, Dodartia seeds were soaked in concentrated sulfuric acid (98%) for 2, 5, or 10 minutes, then rinsed with distilled water for 5 minutes to remove the sulfuric acid before commencing the other trials.

Seed germination (SG) Effect of gibberellin on SG

Seeds were treated with concentrated sulphuric acid (98%) (based on the results of the scarification experiment), then placed in 9-cm

diameter Petri dishes containing Whatman paper (No: 42) moisturized with 8 ml distilled water (control), or gibberellin (0, 10, 20, 50, and 100 ppm) and incubated in a germinator with a temperature (T) range of 25/15 °C and photoperiod of 16 h light/8 h dark (Demir and Mavi, 2008). The experiment was conducted as a completely randomized design (CRD) with four replications.

Effect of constant and fluctuating temperatures on SG

Seeds, treated with concentrated sulphuric acid (98%) (based on the results of the scarification experiment), were placed in 9-cm diameter Petri dishes containing Whatman paper (No: 42) moisturized with 8 ml distilled water and incubated in a germinator at different constant temperatures (5, 10, 15, 20, 25, 30, and 35 °C) and at fluctuating temperature (T) (20/10, 25/15, and 30/20 °C and a photoperiod of 16/8 h) (Demir and Mavi, 2008). The experiment was conducted using a CRD factorial design with four replications.

Effect of NaCl on SG

To determine the behaviour of SG to salt stress, 2-min-sulphuric acid (98%) treated seeds were placed in 9-cm petri dishes containing Whatman paper (No: 42) moistened with 8 ml distilled water (control), or NaCl solutions (0, 100, 200, 300, 400, 500, 600 and 700 mM) and incubated in a germinator at 25/15 °C and a photoperiod of 16/8 h. At the end of the experiment, germination sensitivity was calculated as the ratio of the germination percentage of treated seeds (under salt stress) to that of non-treated seeds (control) (Demir and Mavi, 2008). The experiment was conducted using a CRD factorial design with four replications.

Shoot emergence (Rhizome collection)

Rhizomes of dodartia were collected from 40 to 50 parent plants from a natural population in a cotton field in Varamin (Tehran Province) (latitude of 35°21′N, longitude of 51°37′ E with an altitude of 927 m above the sea level) in June 2018 and 2019 by the process of chopping down

part of the stand, and digging up from 30 cm depth the rhizomes. The rhizomes were placed into paper bags and stored at room temperature for two months. Before planting, the rhizomes were surface-sterilized in a 20% v/v sodium hypochlorite (NaOCl) solution for 8 minutes (Jose and Thomas, 2015).

Effect of rhizome length and planting depth on shoot emergence

We studied the effect of planting depth (2.5, 5, 10, 15, and 20 cm) and rhizome size (2.5, 5, 10, and 15 cm) on shoot emergence (SE) in plastic pots (30 cm diameter, 35 cm depth) filled to 5 cm with sterilized 1: 1: 2: 2 perlite, pneumatic sand, livestock manure, and farm soil, respectively, in a randomized complete block design with six replications/treatment. One piece of rhizome with at least one node was horizontally placed in each pot. A layer of soil (i.e., 2.5, 5, 10, 15, and 20 cm) was added to create a burying depth after the rhizomes were sown into the soil. Water was slowly and carefully supplied to the individual pots (Dalbato et al., 2014). In the greenhouse, the pots were arranged at random, and the temperature was maintained at 30 °C during the day and 25 °C at night (16/8 h). The pots were irrigated twice per week to provide adequate soil moisture to emerging plants (Besancon, 2019). After two months, if seedlings emerged, they were counted (Mangoale et al., 2020). Emergence was recorded for seedlings that reached 1 cm or more (Mangoale and Afolayan, 2020). The experiment was conducted using a CRD factorial design with four replications.

Salinity stress (NaCl) effect on shoot emergence

Based on the results of the previous experiment, 15 cm length rhizomes were sown at a depth of 2.5 cm for each of the NaCl concentrations studied (0, 100, 200, 300, 400, 500, 600, 700, and 800 mM), with six replicates. The pots were irrigated once with this solution. The control irrigation (0 mM NaCl) was with tap water.

Cold stress effect on shoot emergence

The rhizomes (15 cm length-rhizome) were incubated in the dark at 0, -5, and -10 $^{\circ}$ C in a

paper bag for 0, 24, 48, 72, 96, 120, 144, and 168 hours (Pumisutapon *et al.*, 2012). Then, they were planted in pots at a depth of 2.5 cm under the greenhouse conditions. The experiments were performed as a factorial experiment in a randomized complete block design with six replications (pots). Factor A was the cold stress at three levels (0, -5, and-10 °C), and factor B was the storage duration of rhizomes (0, 24, 48, 72, 96, 120, 144, and 168 hours).

Effect of drying and freezing on rhizome emergence of dodartia

Dodartia rhizomes were divided into 15 cm pieces and incubated for 24, 48, 72, 96, 120, 144, and 168 hours at 25 °C in the oven. Additionally, rhizomes were placed at temperatures of 0, -5, and -10 °C in a freezer. The rhizomes were then transferred to pots and kept in the greenhouse. For each treatment, four pots (4 repetitions) were considered. The pots were placed at 25-30 °C under a 16/8-h photoperiod. 4 weeks after planting, the number of emerged shoots per rhizome was recorded (Peters et al., 2000). The experiment was conducted as a factorial, randomised complete block design with four replications. Factor A was exposure temperature at four levels (25, 0, -5, and -10 °C), while factor B was the duration of rhizome storage at these temperatures with seven levels (55. 48, 72, 96, 120, 144, and 168 hours).

Statistical analysis

For data analysis, PROC ANOVA in SAS (Ver. 9.2) was used. The averages of two years were used for analysis. The mean comparisons were performed using the PROC MEANS procedure and the Duncan multiple-range test (DMRT) at the 0.05 significance level. The graphical representation and fitting of the three-parameter model were performed using SigmaPlot software (version 11).

Results

Seed germination behaviour Effect of scarification with sulphuric acid on seed germination

The results showed that sulphuric acid had a

significant effect on SG (%). However, non-scarified seeds did not germinate (Fig. 1). The 2-minute sulfuric acid treatment yielded the highest germination rate (more than 50%). Conversely, SG was 21.5% when the seeds were treated with sulphuric acid for 5 min. Increasing the treatment time from 5 to 10 min inhibited dodartia SG. Hence, the two treatments were selected for the subsequent experiments.

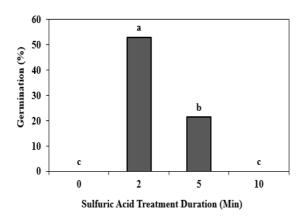


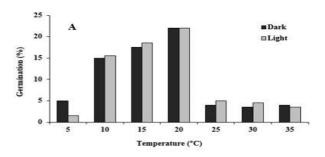
Figure 1 The effect of sulfuric acid on *Dodartia* orientalis seed germination.

Effect of gibberellin on seed germination

The results of the analysis of variance showed that there was no significant difference in gibberellin levels or in their effect on Dodartia SG (p > 0.05). Consequently, gibberellin had no stimulatory effect on dodartia SG.

Effect of constant and fluctuating temperatures on seed germination

Results showed that SG was lowest at 5 °C, reaching 18.5% and 17.5% in light and dark conditions, respectively, at 15 °C. At 20 °C, SG hit a peak of 22% and then decreased with increasing temperature. Actually, at $T \ge 25$ °C, SG decreased significantly, reaching 3.5% and 4% in the light and dark conditions, respectively. Consequently, the threshold temperature for dodartia SG is 20 °C (both in light and dark) (Fig. 2A).



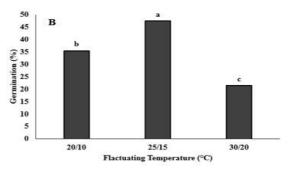


Figure 2 Effect of constant temperatures in light and dark (A) and fluctuating temperature (B) on *Dodartia orientalis* seed germination.

The effect of fluctuating temperature on dodartia seed germination is presented in Figure 2B. The maximum seed germination (47.5%) was recorded at alternating temperatures of 25/15 °C. Dodartia SG at alternating temperature was higher (about 2.5 times) than at a constant temperature (the maximum value was about 20% (Fig. 2). It seems that alternating temperatures can increase dodartia SG%.

Effect of salinity stress (NaCl) on seed germination

Salinity stress decreased significantly SG (Fig. 3). The highest germination (47%) was observed in the control treatment (0 mM NaCl). However, at 100 and 200 mM NaCl SG was 15.33 and 14.66%, respectively. Besides, at concentrations \geq 300 mM NaCl, SG was inhibited. Therefore, Dodartia seeds tolerate low salinity.

Effect of rhizome length and planting depth on shoot emergence

The time to the first emergence ranged from

5 to 25 days, depending on burial depth and rhizome size. Deeper sowing and smaller rhizome size lead to a longer time to shoot emergence (SE). In fact, for the 12.5 cm burial depth treatment, the first emergence shoot occurred after 25 days. The shortest time to the SE was 5 days, when the rhizome was buried to a depth of only 2.5 cm, regardless of the rhizome length. Thus, the SE and the onset of growth required this time period (data not shown). At the end of the experiment (after 60 days), emergence had occurred in 80% of pots. From the initially buried fragments, 15% had decayed at the end of the experiment.

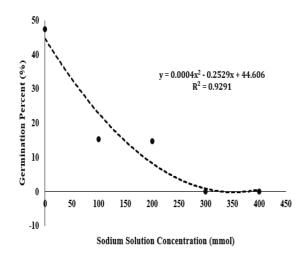


Figure 3 *Dodartia orientalis* seed germination in response to different NaCl concentrations.

Shoot emergence

The effect of burial depth and rhizome length on SE% is shown in Table 1. The SE% decreased significantly (p < 0.05) with increasing burial depth for all studied rhizome lengths. In fact, for a rhizome length of 2.5 cm, SE decreased from 71.3 to 34.5% at a depth of 2.5 and 12.5 cm, respectively. Conversely, SE increased significantly with increasing rhizome length (i.e., 71.3% vs. 93.4% for rhizome lengths of 2.5 and 15 cm, respectively).

Table 2 Effect of cold stress by storing rhizomes at subzero temperature on shoot emergence of *Dodartia orientalis*.

	0 (°C)	-5 (°C)	-10 (°C)
0	93.4aA	93.4aA	93.4aA
24	68.0 bA	55.0 bB	0 bC
48	50.0 cA	42.0 aA	0 bA
72	0 dA	0 aA	0 aA
96	0 dA	0 aA	0 aA
120	0 dA	0 aA	0 aA
144	0 dA	0 aA	0 aA
168	0 dA	0 aA	0 aA

Means with uppercase superscripts (A > B > C > D) in the same rows are significantly different (p < 0.05) while means with lowercase superscripts (a > b > c > d) within a column are significantly different (p < 0.05) Values are means of n = 6.

There was no significant difference in rhizome length between 5 and 10 cm. The 15 cm rhizome length produced the highest shoot emergence (93%). A significant difference in shoot emergence was observed among different lengths at a 5 cm planting depth (from 60.5 to 80.1%). There was no significant difference in rhizome length between 5 and 15 cm. A significant difference in shoot emergence was observed at 7.5 and 10 cm planting depths. At 7.5 cm planting depth, there was no significant difference between the rhizome lengths 2.5, 5, and 10 cm. At a planting depth of 10 cm, no significant difference was observed between rhizome lengths of 5 and 10 cm. Similarly, there was no significant difference in shoot emergence among different rhizome lengths at the deepest planting depth (12.5 cm). The shoot emergence (two-year mean) decreased with increasing planting depth.

Effect of salinity stress (NaCl) on shoot emergence

Dodartia rhizomes were not capable of producing new shoots when treated with NaCl concentrations (100, 200, 300, 400, 500, 600, 700, and 800 mM). However, 94% of the rhizomes emerged in the control treatment (Table 1). This feature indicates that dodartia rhizomes are sensitive to salinity stress, and that the activity of buds on the rhizomes is strongly affected by it.

Effect of cold stress on shoot emergence

The results showed that as freezing storage time increased, SE decreased significantly at the same freezing temperature (Table 2). At 0 °C, the SE declined and reached 93.4%, 68%, and 50% for incubation periods of 0, 24, and 48 hours, respectively. With storage times exceeding 72 h, no SE occurred. Similarly, SE decreased significantly at -5 °C with increasing incubation time, with no SE observed after 72 h. However, no SE registered for rhizomes kept at -10 °C even after 24 h (Table 2).

Table 1 Effect of rhizome size and rhizome planting depth in the soil on shoot emergence of *Dodartia orientalis*.

Rhizome length (cm)	Shoot emergence (%)					
	2.5 (cm)	5 (cm)	7.5 (cm)	10 (cm)	12.5 (cm)	
2.5	71.3 cA	60.5 cA	50.5 bB	40.5 cC	35.4 aC	
5	84.1 bA	73.4 aB	70.6 aB	55.3 bC	39.5 aD	
10	80.5 bA	68.3 bB	65.5 aB	63.2 bB	41.2 aC	
15	93.4aA	80.1 aB	68.2 aC	77.3 aB	42.6 aD	

Means with uppercase superscripts (A > B > C > D) in the same rows are significantly different (p < 0.05) while means with lowercase superscripts (a > b > c > d) within a column are significantly different (p < 0.05) Values are means of n = 6.

Effect of drying and freezing on the emergence of the rhizome dodartia

By increasing the storage time of dodartia rhizomes at 0 °C and -5 °C, their emergence decreased, and this decrease was significant 2 days after storage. Twenty-four and 48 hours after keeping the rhizomes at 0 °C, their percentage emergence was Additionally, 72 and 96 hours after storing the rhizomes at 0 °C, their germination rates were 4% and 0%, respectively (Fig. 4). Creating droughts of varying duration and temperature is considered an approach for managing dodartia and perhaps other perennial weeds. Freezing prevents rhizome growth and effectively kills them. Therefore, the use of winter ice water is considered an approach for the integrated management of dodartia.

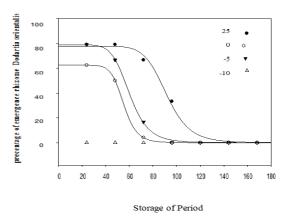


Figure 4 Effect of drying and freezing on the rhizome emergence percentage of the *Dodartia orientalis*.

Discussion

The 1000-seed weight (unpublished data) showed that dodartia has very light seeds, indicating that this species is propagated through rhizomes. Consequently, Dodartia will regain its vegetative reproductive ability within a few weeks, and the underground organs will be responsible for establishing the new plant (Khalilova and Eshchanov, 2020). Since this plant is perennial and propagated by rhizomes, it spreads by a few seeds and establishes itself in a new environment. Our results also showed that Dodartia SG is enhanced through scarification by concentrated sulphuric acid for a 2-minute incubation. However, increasing this duration will lead to SG inhibition. This could be due to damage to the embryo caused by this treatment (Bradbeer, 1988).

Some chemicals appear to affect the embryo indirectly by altering the seed coat, such as scarification with sulphuric acid. This study showed that increasing the duration of sulphuric acid seed treatment can prevent germination by damaging the seed embryo.

Plant growth substances were considered prime suspects as dormancy regulators, including gibberellins, which are known to break dormancy (Bradbeer, 1988). Nevertheless, our results showed no effect of gibberellins on SG.

Generally, understanding the optimal germination temperature of weed seeds can be

beneficial for weed management, especially in determining the appropriate timing for preemergent herbicide applications (Zimdahl, 2007). This study showed that the threshold temperature for dodartia SG is 20 °C (both in light and dark).

Although no significant difference was observed between light and dark effects on SG at 15 and 20 °C, the effect of light and dark on SG was significantly different at 5 °C, which could be due to the phytochrome balance in seeds. The effect of light at a T < to the optimum one can be due to the interaction of the phytochrome Pfr and low T on the cell membrane. Moreover, SG decreases in light at high T is probably due to the destructive effect of phytochrome Pfr at high T (Baird and Dickens, 1991).

Our results showed that dodartia seeds can germinate in both light and dark, which is considered an advantage for this weed. In other words, dodartia seeds are not photoblastic. Similarly, the same result has also been reported for Creeping Thistle Cirsium arvense and Black-jack Bidens pilosa (Singh and Achhireddy, 1984). For species with this feature, the high density of the canopy of other plants cannot prevent these seeds from germination (Ghersa and Martinez-Ghersa, 1991).

Alternating temperatures can increase seed germination in some weeds and break dormancy in others (Zimdahl, 2007). For instance, changing the constant temperature to an alternating one increased the germination rate of water hemp Amaranthus tuberculatus from 30 to 90% (Leon and Knapp, 2004). A similar result has been reported for Chinese millet Setaria faberi seeds (Leon and Knapp, 2004), corroborating our findings. Seeds in the soil are subject to alternating temperatures under natural conditions. In fact, Thompson and Grime (1983) investigated the effect of alternating temperatures on seed germination in species of herbaceous plants and 112 demonstrated a positive effect. Additionally, Bradbeer (1988) showed that the SG of 46 species was stimulated by alternating light and temperature conditions.

Determining the SG behavior under saline stress conditions would help predict germination under salt stress (Demir and Mavi, 2008). According to our results, this weed's salt sensitivity prevents it from spreading in saline areas.

The first exposure of a plant to the salinity stress usually occurs at the germination stage (Passam and Kakouriotis, 1994). The salt damage is attributed to various factors, including reduced water availability, changes in the mobilization of stored reserves, and alterations in the structural organization of proteins (Machado Neto et al., 2004). Actually, under salt stress, seeds require greater water uptake during germination due to the accumulation of soluble solutes, which increases osmotic pressure. This phenomenon causes an excessive uptake of Na+ and Cl-, resulting in toxicity (Jones, 1986). However, Na+ can, to some extent, play an osmotic role, thereby maintaining a water potential gradient to ensure water uptake until the seed germinates. Although the inhibition of growth and yield by salinity has been reported in several species, its effect on germination, particularly in differentially matured pepper lots, is not well understood (Demir and Mavi, 2008).

Agreeing with our results, burial has also been shown to delay emergence in *Calystegia sepium* (Dalbato *et al.*, 2014). Bhowmik (1997) reported that the percentage of tubers emerging from the soil is higher, and that shoot emergence is faster from tubers growing nearest to the soil surface. Minshall (1977) found that common milkweed with smaller rhizome fragments took longer to emerge. The delay in weed emergence should allow for good crop establishment and a significant competitive effect against weeds (Bhowmik, 1997).

The current study demonstrated that longer rhizome fragments led to greater shoot emergence. Other authors (Klimes *et al.*, 1993) have suggested that longer rhizome fragments have more carbohydrate reserves. The plants that emerged from short fragments had less competitive vigor than those from long pieces. The rhizome length may be positively correlated with the amount of

stored carbohydrates, thereby facilitating the survival and growth of fragments. This suggests that carbohydrate reserves stored in the longer rhizomes can be remobilized and reused to increase plant growth (Wang et al., 2019). Weber (2011) also found that the length of rhizome fragments plays a crucial role in shoot formation. The shoot growth vigor of Tussilago farfara from a rhizome fragment is approximately related to the rhizome fragment length (Dalbato et al., 2014). In Sonchus arvensis, which has a shallower root system, the time to emergence and the time from plant development to seed set decreased with increasing root fragment length (Anbari et al., 2011).

Our work showed that SE was higher at 2.5 cm than at greater depths (> 2.5 cm), regardless of rhizome fragment length. Previous studies on Physalis viscosa have also reported that deeper burial depth decreased SE (Shen et al., 2005). In agreement, Soltani et al. (2006) showed that shoots had a higher percentage of emergence in shallow planting depths. Thus, it is clear that planting depth is a significant factor influencing SE. Planting depth may have been a major environmental stress on the SE, as it can significantly alter abiotic conditions, including temperature, photosynthetically active radiation. moisture, and soil organic matter (Mangoale and Afolayan, 2020). It has been reported that rhizomes buried deeper in the soil may deplete all stored carbohydrates before new shoots reach the soil surface, thereby increasing the risk of plant regeneration (Mangoale and Afolayan, 2020). If 1 cm fragments are buried at depths of 4–5 cm or more, the shoots will not reach the soil surface (Dalbato et al., 2014). It has been reported that purple nutsedge *C. rotundus* tubers can germinate from soil depths of 30-50 cm, thereby defeating the purpose of tillage in controlling weeds (Roozkhosh et al., 2017, 2023). Previous studies on Alternanthera philoxeroides (Mart.) Standl. and Physalis viscosa L. also reported that greater burial depth decreased the rate of shoot emergence (Shen et al., 2005). The survival and growth of rhizome fragments buried in deeper soils may primarily

rely on the utilisation of reserves stored in the rhizomes when carbohydrates cannot be supplied by photosynthesis (Klimes *et al.*, 1993).

Rhizome length and depth were found to influence the establishment of many weeds. The burial of the rhizome may significantly affect the survival and growth of clonal fragments by altering biotic and abiotic conditions. The survival and growth of rhizome fragments buried in deeper soils may largely depend on the utilization of reserves stored in the rhizomes when carbohydrates cannot be supplied by photosynthesis (Klimes *et al.*, 1993). Factors generally regarded as important in controlling perennial weeds are the extent of fragmentation and burial depth (Dalbato *et al.*, 2014).

There are some perennial weeds for which control involves cutting the rhizomes or roots and attempting to bury the fragments. As shown by Turner (1968), 54% of 2.5-cm-long rhizome pieces of Elytrigia repens died when buried to a depth of 10 cm, while 28% of 7.5-cm fragments died.

Osmotic and salinity stresses are the most common abiotic stresses that limit plant growth and productivity, especially in arid and semiarid regions of Iran. Osmotic and salinity stresses not only limited plant growth and seed yield, but also affected seed quality and vigor. It has been well documented that abiotic stresses, such as osmotic and salinity stress, can affect the biochemistry and physiology of growth, and that the plant's native habitat may also influence these effects (Hammami et al., 2020). Salt damage to plants is attributed to various factors, including reduced water availability, changes in the mobilization of stored reserves, and effects on the structural organization of proteins (Demir and Mavi, 2008).

To the best of our knowledge, no study has been done on the effect of salinity on dodartia vegetative reproduction. Based on the present results, this weed can be classified as a sensitive species to salt stress. Indeed, no SE was observed when rhizome was salt-treated. It was reported that among the vegetative organs, Leymus chinensis rhizome had the highest

accumulation of Na⁺ and Cl⁻ and the lowest levels of K⁺, followed by roots (Li *et al.*, 2014).

Our results showed that freezing inhibits rhizome vegetative propagation, especially at temperatures below -5 °C. Similarly, couch grass and Johnson grass rhizomes (Dunham et al., 1956) and yellow nut grass tubers (Stoller, 1973). While the physiological principles of cold tolerance in plants are not fully understood, it is hypothesized that fatty acid confer cold tolerance. saturation can Unsaturated fatty acids solidify at lower temperatures than saturated ones. Therefore, tissues that contain more unsaturated fatty acids have lower freezing points and, consequently, cold higher tolerance. Consequently, understanding the levels of saturated and fatty acids unsaturated in vegetative propagation organs can reveal their cold tolerance in different plants. For instance, the greater tolerance of couch grass rhizomes A. repens can be attributed to higher fat concentration and a higher proportion of unsaturated fatty acids. Stoller and Sweet (1987) studied the effect of chilling on yellow and purple nut grass tubers by placing them at 0 °C to -10 °C for 4, 8, 16, and 48 hours and observed that purple nut grass tubers disappeared in winter and did not germinate during the growing season.

There are also differences between laboratory and farm conditions. For instance, Johnson grass (Sorghum halepense) rhizomes are destroyed in the laboratory at -3 and -5 °C but survive in the field at -9 °C. The length, diameter, and thickness of the root shell, as well as storage time freezing temperature. indispensable roles in cold tolerance. Roots with thick skin freeze later and tolerate cold better. The climate and ecotypes of the region are also important, as plants in these regions are more tolerant due to physiological adaptations to cold temperatures. Accumulation of carbohydrates, water, lipids, and amino acids occurs at low temperatures to prevent the formation of ice in the cell and, consequently, cell damage (Schimming and Messersmith, 1988). Freezing will reduce soil viability at all

depths. In addition, cold duration plays a vital role in rhizome viability and growth (Stoller and Sweet, 1987). In general, drought with freezing can significantly damage the organs near the soil surface (Hakansson, 2003). In the present study, to achieve a definitive result, field testing is necessary to determine whether the temperatures that destroy Dodartia weed rhizomes in the laboratory have the same effect in the field.

Conclusion

The viability of Dodartia seeds is moderate (62.5%), and the seeds can be widely distributed due to their light weight. Dodartia seeds can germinate in both light and dark conditions. This feature is one of the most important factors that enable it to germinate under different environmental conditions. Scarification with sulphuric acid (98%) for two minutes is the most suitable stimulus to break the dormancy of dodartia seeds. The optimum constant and fluctuating temperatures for seed germination were 20 °C and 25/15 °C, respectively. Alternating temperature was more suitable for seed germination of Dodartia compared to constant temperature. The SG decreases at NaCl concentrations greater than 200 mM. The optimal planting depth and length for rhizome emergence were 2.5 cm and 15 cm, respectively. The SE from rhizomes submitted to NaCl was inhibited. Therefore, dodartia is relatively sensitive to salt stress. Dodartia rhizomes kept at 0 °C and -5 °C for either 24 or 48 h were able to regenerate. However, no SE was reported for rhizomes incubated at -10 °C. Therefore, dodartia is relatively sensitive to salt and cold stress. These results could be important in predicting dodartia distribution to new regions. The results of this study demonstrated that deeper burial of Dodartia by plowing could inhibit and potentially extirpate the weed. Our results showed that fragmentation into short rhizome pieces and deep burial are essential for controlling dodartia growth. We emphasize that we know too little about dodartia vegetative reproduction, and the present data is the first report about this weed's asexual reproduction.

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تولیدمثل گونه Dodartia orientalis در شرایط محدودکننده دما، شوری و دفنشدن

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چكىدە: بزچرە .Dodartia orientalis L يك علف هرز مهاجم چندساله از خانواده Scrophulariaceae است که در تمامی مطالعات انجام شده، این گیاه را به عنوان یک گیاه دارویی مورد بررسی قرار دادند. اما تاكنون مطالعه جامعي روى زيستشناسي اينگونه علف هرز صورت نگرفته است. در همینخصوص هفت آزمایش متفاوت روی بذر و ریزوم این گیاه در آزمایشگاه و گلخانه بخش تحقیقات علفهای هرزمؤسسه گیاهپزشکی ایران در سالهای ۱۳۹۷ و ۱۳۹۸ انجام شد. نتایج این بررسی نشان داد که بهترین دمای ثابت و متناوب برای جوانه زنی بذر بهترتیب ۲۰ و ۲۵/۱۵ درجه سلسیوس بود. دمای متناوب نسبت به دمای ثابت برای جوانهزنی بذر بزچره مناسبتر بود. استفاده از اسید سولفوریک غلیظ (۹۸ درصد) بهمدت ۲ دقیقه بهترین محرک برای جوانهزنی بذر بزچره بود و از طرف دیگر محرک جیبرلین هیچ تأَثیری بر جوانهٔزنی بذر اینگونه نداشت. بذرها در غلظت NaCl < 300 میلی مولار جوانه زدند. بررسی تکثیر رویشی بزچره تحت تأثیر برخی عوامل محیطی نشان داد که بهترین عمق و طول ریزوم برای سبز شدن ریزوم بهترتیب ۲/۵ و ۱۵ سانتیمتر بود. ریزوم ها در پاسخ به تنش شوری (در تمام غلظتهای NaCl مورد مطالعه) قادر به جوانهزنی نبودند. همچنین نتایج بررسی اثر تنش سرما نشان داد که ریزوم در بزچره پس از انکوباسیون ۲۴ و ۴۸ ساعته در دمای ۰ و ۵- درجه سلسیوس ظاهر شد. اما نتایج نشان داد زمانیکه ریزم در دمای -1درجه سلسیوس قرار گیرد قدرت جوانهٔزنی خودش را از دست می دهد. همچنین نتایج نشان داد که دو فاکتور افزایش عمق کاشت و اندازه کوچکتر ریزوم، منجر به طولانی تر شدن زمان سبز شدن اندام هوایی میشود.ریزومها در مدت زمان ۲۴، ۴۸ و ۷۲ ساعت و پس از نگهداری در دمای ۲۵ درجه سلسیوس و ۴۸ ساعت در دماهای ۰ و ۵- درجه سلسیوس قادر به رویش بودند، اما نگه داری ریزوم ها در تیمار با دمای ۱۰- درجه سلسیوس باعث از بین رفتن آنها شد.

واژگان کلیدی: علف هرز مهاجم، جوانهزنی بذر، تولیدمثل رویشی، زیستشناسی علفهای هرز