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SPECIAL TOPICS RESEARCH ARTICLE

Climate change, an emerging threat to weed management

The effect of environmental factors on seed germination and emergence of cutleaf geranium

Zahara Mahmoodi Atabaki[a], Javid Gherekhloo[a*], Farshid Ghaderi-Far[a], Omid Ansari[a], Saeid Hassanpour-bourkheili[a], Rafael De Prado[b]

[a] Department of Agronomy, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
[b] Department Agroforestry, Biochemistry and Molecular Biology, University of Cordoba, 14014 Cordoba, Spain.

Abstract: Background: Cutleaf geranium (*Geranium dissectum* L) is a weed found in cereals and grasslands. Knowledge about the germination response of weeds under various environmental conditions is critical for their effective management, whereas such study has not been carried out thoroughly on this species.

Objective: This study was carried out to investigate the effect of some environmental factors on seed germination and emergence of cutleaf geranium.

Methods: The seeds were subjected to various environmental factors including water potentials, salinity, pH, high temperatures, burial depth and flooding at the temperature resulting in the highest germination (10 °C). All experiments were carried out twice, which were done consecutively.

Results: Germination percentage was halved at a water potential of -0.6 MPa, and no germination was observed at -2 MPa. *Geranium dissectum* seeds had negligible germination at 200 mM NaCl concentration. Seeds germinated over a pH range of 5-8, with the highest germination at pH between 6 and 7. Germination of this species reached from 85.0% after treatment at 80 °C but no seeds germinated after exposure to 120 °C. Maximum emergence was estimated 88.8%, which was halved at 2.2 cm depth. No emergence was observed at 4 cm or greater depths. Germination was 88.4% to 0% 15 days after flooding.

Conclusions: This species may not usually be found in areas with poor environmental conditions, and a semi-deep tillage or inclusion of rice in crop rotation may be used to control this weed.

Keywords: Burial depth; Flooding; *Geranium dissectum*; PEG 6000; pH; Temperature

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* Corresponding author:
<gherekhloo@gau.ac.ir>

1. Introduction

Seed germination is a crucial stage in the life-cycle of plants, which determines the success of plant establishment and further growth. This process is defined as the events that start with the uptake of water by the seed and end with radicle protrusion (Talská et al., 2020). Germination of non-dormant seeds is influenced by various environmental factors, including temperature, water potentials, salinity, light and darkness, pH and burial depth (Hassanpour-bourkheili et al., 2021b).

Cutleaf geranium (*Geranium dissectum* L., Geraniaceae), a weed species native to Europe but also found in other continents, is an invasive weed in the Northern provinces of Iran (Sohrabi et al., 2020), and drastic yield losses have been reported in some crops due to its infestation. For instance, this weed resulted in approximately 50% reduction in biological yield and grain yield of canola in Mazandaran province. Furthermore, *G. dissectum* has been described as a "difficult to weed" species and can reduce the yield and growth of canola by competing for resources such as light, water and nitrogen (Nouralizadeh Otaghsara, 2015).

Germination of *G. dissectum* has been studied previously and Gama-Arachchige et al. (2012) reported that the physical dormancy of *G. dissectum* seed was eliminated at temperatures higher than 20 °C , although the seeds were unable to germinate under high temperatures. Guillemin et al. (2013) estimated that the base temperature and base water potential of *G. dissectum* were 0.6 °C and -3.31 MPa, respectively. Atabaki et al. (2021) estimated the cardinal temperatures of germination for *G. dissectum* and reported that segmented and dent models were the best models to determine the cardinal temperatures of this species. However, more information is required about the germination response of *G. dissectum* to environmental factors to devise more effective weed management programs. Thus, the present study was carried out to investigate the effect of some environmental factors on seed germination and emergence of non-dormant *G. dissectum*.

2. Material and Methods

2.1 Plant material and study area

The present study was conducted in Golestan Province, which is located in the North of Iran. The region is very diverse regarding environmental conditions such as rainfall and soil salinity. Soil pH ranges from neutral to alkaline (Khormali and Kehl, 2011). Canola is often sowed in this region in rotation with wheat, followed by rice cultivation in summer. Also, many farmers burn the residues for seedbed preparation (Kazemi et al., 2016; Hassanpour-bourkheili et al., 2021a).

Seeds were collected in May of 2021 from 50 plants of *G. dissectum* growing in the canola fields of Aliabad-e-Katul township, Golestan province, Iran. The seeds were pooled after sampling and were kept in a store at room temperature during the experiment period, which began immediately (Atabaki et al., 2021).

2.2 Seed pre-treatment

According to Atabaki et al. (2021), an after-ripening pre-treatment results in the highest germination percentage for *G. dissectum* seeds. For this purpose, the seeds of *G. dissectum* were kept under dry-storage conditions at room temperature for 140 days to obtain the maximum germination percentage. Then, the seeds were transferred to the lab for use in experiments.

2.3 General germination protocol

All experiments were performed based on a completely randomized design with four replications. Twenty-five pre-treated seeds disinfected with sodium hypochlorite were placed in 9 cm Petri dishes and covered with Whatman No. 1 paper, and each Petri dish was considered as one replicate. Five millilitres of distilled water or other solutions (see below) were added to each Petri dish. Then, the Petri dishes were placed in an incubator at the temperature that led to the highest germination (10 °C) (Atabaki et al., 2021). To prevent evaporation of the solutions, the Petri dishes were covered with plastic freezer bags and were inspected 2-6 times a day for 10 days. The seeds were considered as germinated when the radicle (1 mm length) emerged, and the germinated seeds were removed. All experiments were carried out twice, which were done consecutively in October and November of 2021.

2.4 Response of *Geranium dissectum* seeds to water potentials

Polyethylene glycol 6000 (PEG 6000) solutions were prepared according to Michel and Kaufmann (1973). Then, the solutions with a water potential of 0 (distilled water), -0.2, -0.4, -0.6, -0.8, -1.0, -1.5 and -2 MPa were added to the Petri dishes. Other conditions were the same as described in the "General germination protocol" section.

2.5 Response of *Geranium dissectum* seeds to salinity stress

Solutions with 0 (distilled water), 40, 80, 120, 160, 200 and 240 mM NaCl concentration were added to the Petri dishes. Other conditions were the same as described in the "General germination protocol" section.

2.6 Response of *Geranium dissectum* seeds to pH

The germination response of *G. dissectum* seeds to various pHs was investigated using the method described by Susko et al. (1999). The seeds were incubated in solutions with a pH of 4, 5, 6, 7, 8, and 9 values and in distilled water (pH = 6.2) (Chauhan, Johnson, 2009). Other conditions were the same as described in the "General germination protocol" section.

2.7 Response of *Geranium dissectum* seeds to high temperatures

This experiment was done to simulate the response of seeds to burning of the residues for seedbed preparation (Chauhan, Johnson, 2009). Twenty-five seeds were wrapped in aluminum foils and then exposed to 80, 90, 100, 110, 120, 150 and 200 °C in an oven for 5 minutes (Chauhan, Johnson, 2009). Other conditions were the same as described in the "General germination protocol" section. Tetrazolium (TZ) test was used to investigate the viability of the seeds after exposure to high temperatures. First, seeds were soaked for 18h in distilled water at 20 °C. Then, seeds were cut through the embryo axis using a razor. 1% 2,3,5-triphenyl-2H-tetrazolium chloride was applied on seeds for 18 hours at 30 °C. Seeds that were completely stained red were considered viable (Akbari-Gelvardi et al., 2021).

2.8 Response of *Geranium dissectum* seeds to burial depth

The experiment was performed based on a completely randomized design with three replications in a greenhouse. The greenhouse temperature was 22/16 °C (day/night) with a 12/12 h of light/darkness and relative humidity of approx. 60%. The pots were filled with a certain amount of silty-loam soil (with a Clay: Silt: Sand ratio of 24: 62: 14 percent). Then, twenty-five seeds were placed on the surface of the soil in plastic pots. The pots were then filled with the same type of soil to achieve the burial depths of 0, 1, 2, 4, 6, 8 and 10 cm. The pots were irrigated using a trigger sprayer. The seedlings were considered as emerged when the cotyledons became visible on the soil surface. The emergence was recorded every 24 h, and the experiment lasted for 21 days. The pots were checked upon the completion of the test to see whether the non-emerged seeds had not germinated or the epicotyl was not able to reach the soil surface. For this purpose, the soil in the pots was removed with care and non-germinated seeds were extracted. Seeds that did not germinate were given a TZ test to check their viability (similar to high temperature experiment).

2.9 Response of *Geranium dissectum* seeds to flooding

First, plastic pots (15 cm diameter and 20 cm depth) were filled with a certain amount of silty-loam soil. Then, twenty-five *G. dissectum* seeds were placed in fabric bags. The fabric bags were sewn and buried at 1 cm depth (depth which resulted in the highest emergence). There were three pots per treatment, and three bags were placed in each pot. The pots containing the seeds were placed inside larger plastic pots (20 cm diameter and 30 cm depth) to maintain 3 cm water above the soil (Siahmarguee et al., 2020). The bags were extracted from pots 0 (control), 2, 5, 10, 15, 20, and 30 days after burial. The experiment lasted for 30 days. The soil containing *G. dissectum* seeds was then washed through a sieve, and the seeds were subjected to a germination test as described in the "General germination protocol" section.

2.10 Statistical analysis

After testing for the homogeneity of variances using Bartlett's test, a combined analysis was done based on a completely randomized design. Water potential, salinity, pH and high temperature experiments were done with four replications, and burial depth and flooding experiments had three replications. In each experiment, treatments (e.g. 0-240 mM NaCl in salinity experiment) were considered as the factor levels, and experimental run had two levels (first and second run). In all experiments, no interaction was observed between the treatments and the experimental runs, so the data from the two experimental runs were pooled.

Statistical analysis for water potential, salinity, burial depth and flooding experiments was done using SigmaPlot (v. 13, Systat Software, San Jose, CA 95110) software. The three parameter sigmoidal function (Equation 1) was fitted to the data related to cumulative germination percentage over time:

$$G = Gmax / (1 + \exp(-(x - x_{50}) / b)) \qquad (1)$$

where G is the percent of seeds germinated at time x, $Gmax$ is maximum germination percentage, x_{50} is the time to 50% maximum seed germination, and b is the slope of the curve at x_{50}. Analysis of variance for pH and high temperature experiments was done with the use of SAS (v. 9.0; SAS Institute, Cary, NC, USA) software. Comparison of means for these two experiments was done using the LSD method at $p<0.05$. Finally, Microsoft Excel 2013 (Microsoft, Redmond, WA) was used for the preparation of figures for pH and high temperature experiments. It must be noted that dead seeds were removed from the experiment prior to statistical analysis for these two experiments.

3. Results and Discussion

3.1 Response of *Geranium dissectum* seeds to water potentials

The highest germination of *G. dissectum* seeds was estimated 92.0%. Germination percentage decreased with increasing water potentials and was halved at a water potential of -0.6 MPa. No germination was observed at -2 MPa water potential (Figure 1). According to the results, although *G. dissectum* can germinate in relatively dry environments, this species has limited germination in soils with very low water content. Therefore, *G. dissectum* can potentially emerge in moist and arid regions. In Golestan province, most winter crops are sown in October- November, which coincides with the start of rainfalls (Atabaki et al., 2021). The ability of this weed to germinate- although not very much- under low water potential conditions can result in earlier seed emergence and thus, establishment (Chauhan, 2016).

Water potential is the most important factor in water uptake and seed imbibition, and drought stress reduces water uptake (Toscano et al., 2017). According to Thompson et al. (2021), germination of rigid ryegrass (*Lolium rigidum* Gaud.) populations from Australia declined drastically with increasing water potentials induced by PEG 8000. They reported that the water potential required for 50 percent reduction in germination percentage in these three populations ranged from 0.39 to 0.46 MPa, and no germination was observed at 0.8 MPa or higher water potentials.

3.2 Response of *Geranium dissectum* seeds to salinity stress

The highest germination was 91.9%. Increasing NaCl concentrations resulted in a decline in the germination percentage of cutleaf geranium. A NaCl concentration of 116.8 mM led to a 50% reduction in maximum germination percentage. Germination percentage was negligible at 200 mM and reached zero at 240 mM NaCl (Figure 2). Soils with 40–100 mM NaCl are considered moderately saline (Chauhan, Johnson, 2009) and soils that contain more than 100 mM of NaCl are classified as saline (Chauhan, 2016). Therefore, although high soil salinity reduces *G.*

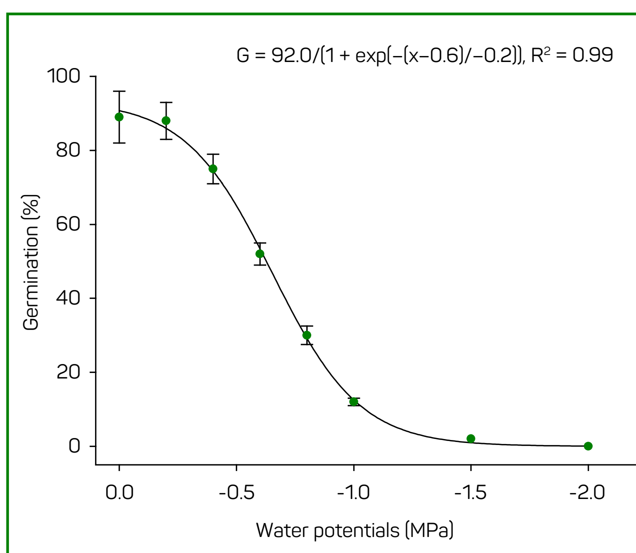

Figure 1 - Germination of *Geranium dissectum* as affected by different water potentials

dissectum seed germination, this weed may still be found in saline soils, which are common in some parts of the region as well as the world.

Salinity stress leads to inhibition of seed germination by reducing the availability of water. Also, salinity changes the mobilization of the reserves stored in the seed and alters protein structure (Ibrahim, 2016). Salinity stress also increases accumulation of Na^+ and Cl^- ions, which have toxic effects on germination (Murillo et al., 2002). These events ultimately culminate in poor plant establishment (Foti et al., 2019). Increasing levels of salinity stress led to a reduction in the germination of Carolina geranium (*Geranium carolinianum* L.) seeds, and germination percentage was almost halved at 80 mM NaCl and reached zero at 160 mM NaCl (Liu et al., 2018). The NaCl concentration required for 50% reduction in *L. rigidum* seed germination was 130mM and a NaCl concentration of 250 mM resulted in 0% germination (Thompson et al., 2021).

3.3 Response of *Geranium dissectum* seeds to pH

According to the results, the highest germination was obtained at pHs of 6 to 7 with 82-88%, and no significant difference were observed between these treatments. Germination declined drastically in higher and lower pHs, and was 38 and 35% in 5 and 8 pHs, respectively. Also, no germination was observed at pH 4 and 9 (Figure 3). Since this weed species is relatively sensitive to the pH of the medium, its presence in acidic and basic soils will be limited. Soil pH in most of the region ranges from 6 to 8 (Shahbazi, Besharati, 2013), and is therefore relatively suitable for the growth of *G. dissectum*. However, this weed can potentially be present in soils with lower pHs.

The pH range in which the seeds are able to germinate varies among plant species. The seeds of winter wild oat (*Avena sterilis* subsp. *ludoviciana* (Durieu) Gillet & Magne) were able to germinate over a wide range of pHs from 4 to 9 (Hassanpour-bourkheili et al., 2021b). The optimum pH for germination of little seed canary grass (*Phalaris minor* Retz.), shortspike canary grass (*Phalaris brachystachys* Link.) and hood canary grass (*Phalaris paradoxa* L.) seeds was 7.5. However, the seeds of these species were able to germinate over a pH range of 4 to 8. (Rezvani et al., 2021).

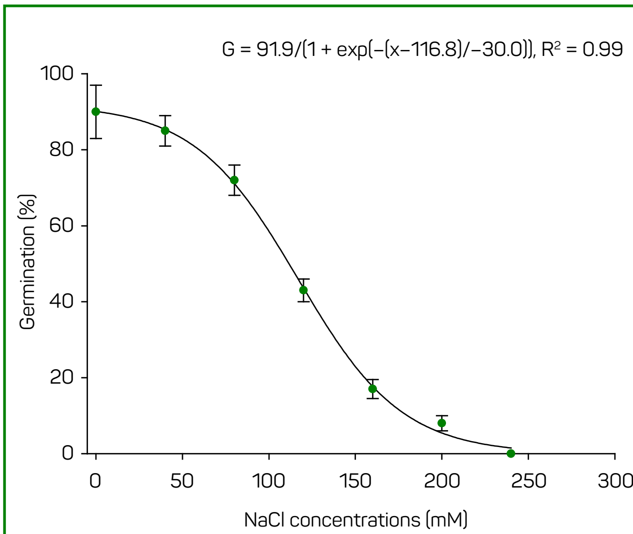

Figure 2 - Germination of *Geranium dissectum* as affected by different NaCl concentrations

3.4 Response of *Geranium dissectum* seeds to high temperatures

The highest germination for *G. dissectum* seeds was 85% and was recorded for seeds exposed to 80 °C. Thereafter, germination percentage declined after seeds were subjected to increasing high temperatures and reached zero at 120 °C temperature (Figure 4). Therefore, residue burning- which is common in some parts of the region, may be used to control *G. dissectum*.

Burning of the residues for preparation of seedbed is a common practice in some parts of the world (Patel et al., 2021), which may increase the temperature at the soil surface up to 200 °C. This temperature declines drastically as the soil depth increases, but it may still damage the seeds buried under shallow depths. However, seeds at great burial depths may still germinate if they reach the soil surface afterwards (Chauhan, Johnson, 2009). *Geranium dissectum* seeds were very sensitive to high temperatures, so weed management methods such as soil steaming, composting of the weed seeds and solarization may be suitable ways to deplete the soil seed bank of this weed. More information is needed on the effect of fire and high temperatures on the seed bank of *G. dissectum*, however.

Germination of *Ipomoea hederacea* Jacq. was severely reduced as a result of exposure to high temperatures. The maximum germination percentage of this species was halved at a temperature of 119 °C, and no germination was observed at temperatures higher than 130 °C (Siahmarguee et al., 2020). Sohrabi et al. (2016) reported that the germination of *Cucumis melo* L. is inhibited when the seeds are subjected to long periods of heat at 90 °C. However, exposure to 120 °C temperature for merely 5 minutes is sufficient to inhibit the germination of this species.

3.5 Response of *Geranium dissectum* seeds to increased burial depth

The maximum emergence of *G. dissectum* was 88.2% at 1 cm. However, no significant difference was observed between the maximum emergence percentage at 0 cm (soil surface) and 1 cm. This result could indicate that light did not affect germination and seedling emergence in this species. Seedling emergence declined drastically with increasing burial depth. It was reduced by 50% at 2.2 cm depth, was negligible at 4 cm and zero at greater

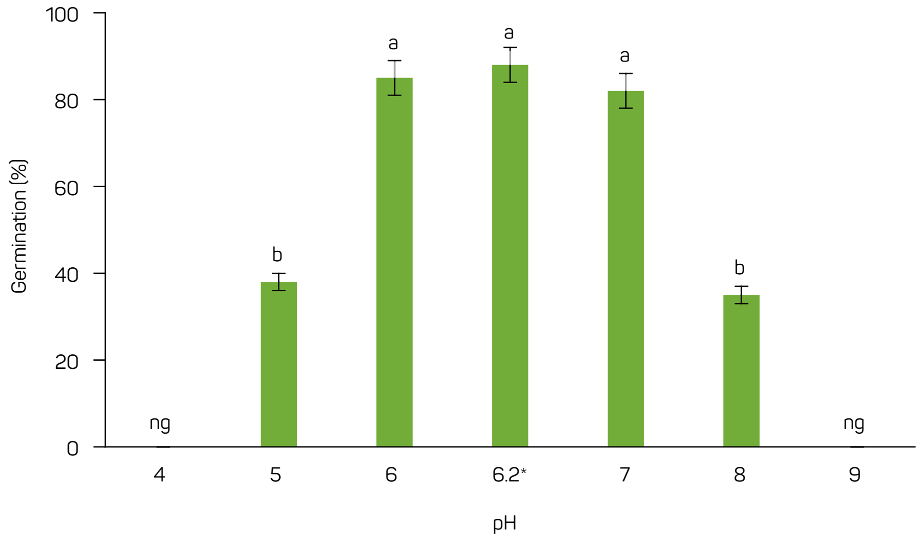

Figure 3 - Germination of *Geranium dissectum* as affected by different pHs. The asterisk (*) denotes the pH of distilled water. Similar letters denote non-significant difference at p<0.05. Treatments with the label "ng" had no germination and thus, were excluded from analysis

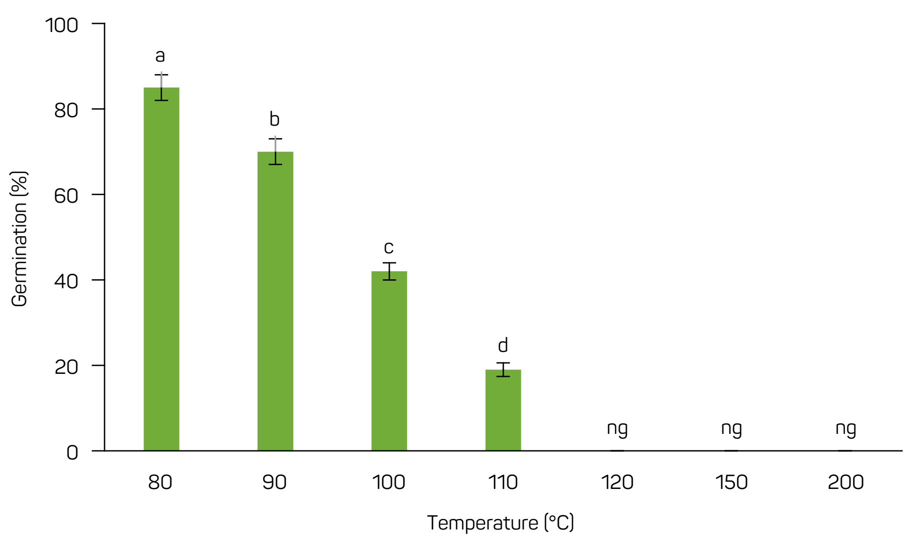

Figure 4 - Germination of *Geranium dissectum* as affected by high temperatures. Similar letters denote non-significant differences at p<0.05. Treatments with the label "ng" had no germination and thus, were excluded from analysis

depths (Figure 5), because seedlings were unable to reach the soil surface (Figure 6). However, the percentage of seeds which were unable to germinate was similar at all burial depths (Figure 6). At the end of the experiment, the non-germinated seeds were subjected to TZ test to investigate their viability. According to the TZ test, these seeds were dead.

The reduction in seedling emergence may be due to depletion of seed reserves (Mennan, Ngouajio, 2006). Also, oxygen deficiency and limitation of gas diffusion are among the factors that may reduce the emergence percentage and rate at greater depths (Wang et al., 2019).

According to the results obtained, it may be concluded that the emergence of *G. dissectum* seedlings may be higher in reduced tillage or no-tillage systems. Thus, the emergence of *G. dissectum* may be controlled by tillage operations to bury the seeds at soil depths of 4 cm or more. Emergence of *G. carolinianum* seedlings was higher than 80% at zero and 1 cm depths, whereas no emergence was observed when the seeds were buried under 7 cm of soil (Liu et al., 2018). Similarly, emergence of *A. sterilis* seeds was the highest when buried at 0 to 2 cm, but declined thereafter and was nil at 15 cm (Hassanpour-bourkheili et al., 2021b).

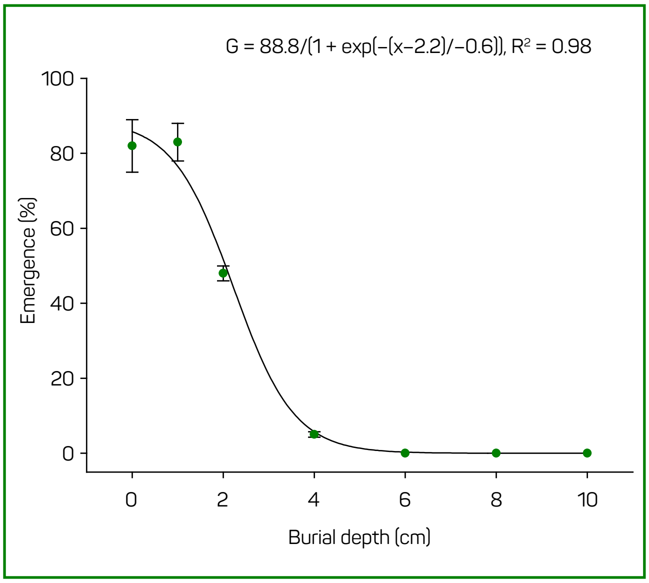

Figure 5 - Emergence of *Geranium dissectum* as affected by different burial depths

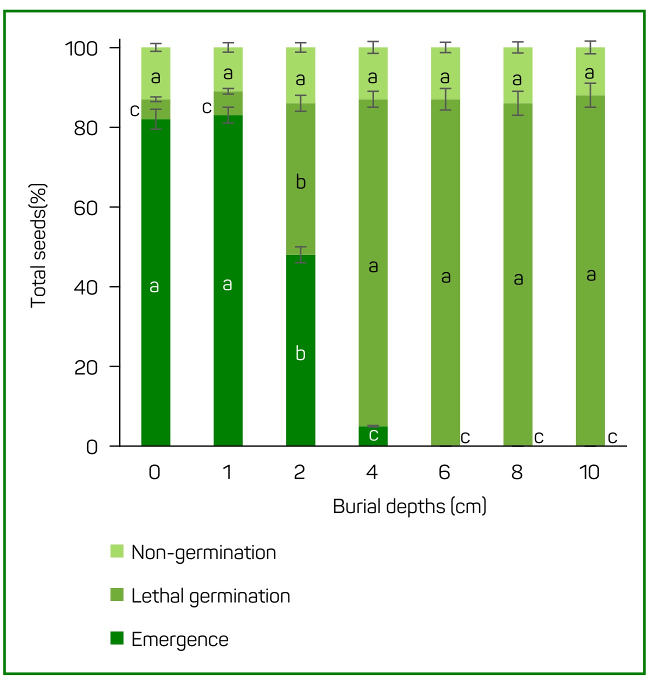

Figure 6 - Percentage of the emerged and non-germinated seeds as well as the germinated seeds that were not able to reach the soil surface in *Geranium dissectum* at different burial depths. Similar letters in each column section denote non-significant differences at p<0.05

3.6 Response of *Geranium dissectum* seeds to flooding

Since the highest emergence percentage was observed at 1 cm burial depth (Figures 5 and 6), the seeds were buried at this depth for the flooding experiment. According to the results, maximum germination was 88.4%, which was recorded at the start of the experiment (day zero).

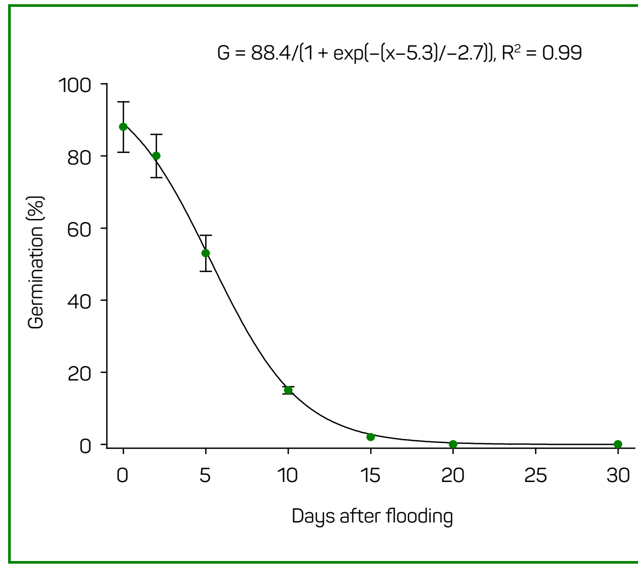

Figure 7 - Germination of *Geranium dissectum* as affected by flooding duration

No significant difference was observed between 0 and 2 days after treatment. However, germination percentage plummeted thereafter with increasing flooding duration, and, was halved when the seeds were flooded for 5.3 days. No germination was observed after 15, 20 and 30 days of flooding (Figure 7). Therefore, *G. dissectum* is highly sensitive to flooding conditions, and since a considerable area of Golestan province is suitable for lowland rice cultivation due to high rainfall and poor soil drainage (Akbari-Gelvardi et al., 2021), inclusion of rice in crop rotation may be an option to control this weed species.

Flooding stress may negatively affect the viability of seeds depending on the species and flooding duration (Kolb, Joly, 2010). Germination of *G. carolinianum* decreased drastically when the seeds were exposed to flooding stress (Liu et al. 2018). The seeds of *Caperonia palustris* (L.) A.St.-Hil were able to survive the flooding condition and showed a germination percentage of 25 percent (Koger et al., 2004). The seeds of *C. melo* were also able to retain their viability under flooding stress up to 90 days (Sohrabi et al., 2016).

4. Conclusions

Overall, germination of seeds of *G. dissectum* is negligible at -1.0 MPa water potential. A NaCl concentration of 116.8 mM led to a 50% reduction in the maximum germination. The seeds of this species only germinated over a pH range of 5-8. Germination percentage reached zero as a result of 5 minutes of exposure to 120 °C and higher temperatures. The seedlings emerged well from zero and 1 cm soil depths, but no emergence was observed when the seeds were buried at 4 cm or greater depths. Flooding severely reduced the germination percentage of *G. dissectum*, and no germination was observed in 15 days of flooding treatment.

This information may be used to devise weed management plans and predict the areas that are prone to presence of this species.

It must be noted that only one population was studied in this paper. Since different weed populations may exhibit different seed germination behavior, the results obtained in the present study are most relevant to *G. dissectum* population collected in Golestan province, Iran. Although the results may be applicable to regions with similar climatic conditions, further studies on different populations of *G. dissectum* from different environments is recommended to better understand the germination characteristics of this species.

Author's contributions

All authors read and agreed to the published version of the manuscript. JG and FG: conceptualization of the manuscript and development of the methodology. ZMA: data collection. OA and SH: data analysis. JG, FG, and SH: data interpretation. JG: supervision. SH: writing the original draft of the manuscript. JG, FG, SH, and RD: writing, review and editing.

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