JJBS

Volume 9, Number 2, June .2016
ISSN 1995-6673
Pages 131 - 138

Jordan Journal of Biological Sciences

Germination and Emergence Characteristics of Annual Ground Cherry (*Physalis divaricata*)

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Received: January 20,2016 Revised: May 19, 2016 Accepted: May 26, 2016

Abstract

Recently, annual ground cherry *Physalis divaricata* (*P. divaricata*) has become a very serious damaging weed in a wide range of summer annual crops and some minor crops in western Iran. However, data regarding the seed germination and seedling emergence behavior of this weed are limited. Therefore, laboratory experiments were conducted in Razi University, Kermanshah, Iran during 2013 to evaluate the effect of temperature, KNO_3, GA_3, light, burial depth, pH, osmotic and salt stresses on seed germination and emergence of *P. divaricata* collected from Kermanshah, Islamabade-Gharb, Sarpole-Zahab. Results indicated that the seeds of *P. divaricata* germinated over a relatively wide range of temperatures. Though, the greatest germination occurred under the alternating temperature regime of (20/35°C 16 h low and 8 h high). Generally, light had a stimulatory effect on seed germination of *P. divaricata*. Emergence of *P. divaricata* decreased with the increase in burial depth. The highest emergence percentage was recorded for seeds buried at soil depth of 0 and 1 cm (75 and 55, respectively). The seeds failed to emerge when they were buried deeper than 4 cm. The salt concentration 46.09 mM NaCl resulted in 50% inhibition of seed germination. An osmotic potential of 0.43 MPa led to the inhibition of seed germination by 50%. Germination positively responded to increasing pH level. The maximum percentage of seed germination was shown at pH 10. Overall, germination increased when GA_3 and KNO_3 were added to the germination media. The results revealed that *P. divaricata* has the potential to become a noxious weed in further areas in Iran. Using cultivation, especially conducted during night, to deprive photoblastic seeds of *P. divaricata* from light would be beneficial in reducing its seed germination and removing newly established seedlings.

Keywords: burial depth, light, osmotic stress, salt stress, temperature.

1. Introduction

Annual ground cherry (*Physalis divaricata* L.) belongs to the family of solanaceae. It is an invasive erect weed with a length of 15-60 cm. Flowers are solitary with yellow color and cup-shaped, which appear from May to July. *P. divaricata* produces 4 to 70 seeds per berry depending on environmental conditions. The number of seeds produced can range from 126 to 16,300 per plant. This weed prefers nutrient-rich soils. According to the observations and preliminary tests of the present stufy (data not published), the freshly harvested seeds showed high levels of dormancy. Recently, *P. divaricata* has become a very serious damaging weed in a wide range of summer annual crops, such as sugar beet (*Beta vulgaris* L.), tomato (*Solanum lycopersicum* L.), potato (*Solanum tuberosum* L.), maize (*Zea mays* L.), and some minor crops in western Iran. In addition to the substantial reduction in crop yield, it causes harvest problems and reduces crop quality due to sticky materials released from its berries. Seed germination is one of the most important stages in the life cycle of *P. divaricata*. Like other nightshade family weeds, *P. divaricata* seeds apparently have no special tools to disperse for short and long distances. Therefore, its survival highly depends on seed dormancy (Defelice, 2003; Zhou *et al.*, 2005; Taab & Andersson, 2009; Stanton *et al.*, 2012). Factors, such as seed burial depth (Penny & Neal, 2003; Wilson *et al.*, 2006), temperature (Fandrich & Mallory-Smith, 2005; Foley, 2008), light (Malik *et al.*, 2010; Huebner, 2011), osmotic potential (Boyd & Van Acker, 2004) and their interactions with internal conditions of seeds can highly affect seed germination and seedling emergence. Because *P. divaricata* is a very close relative to other Solanaceae crops, its chemical control is difficult, especially in the fields of these crops (Gorski & Wertz, 1987). A better understanding of the factors affecting seed germination and seedling emergence of *P. divaricata* could help to develop effective control measures, predict its invasion potential and make

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critical weed management decisions (Ghersa & Holt, 1995).

In spite of the fact that *P. divaricata* is a problematic and invasive weed for farming systems in western Iran, data describing the response of seed germination and seedling emergence of this weed to environmental factors is limited (Alam *et al.,* 2011; 2013). Hence, the aim of the present study is to determine the effect of some major environmental factors, including temperature, KNO3, GA3, light, burial depth, pH, osmotic and salt stresses on seed germination and emergence of *P. divaricata.*

2. Materials and Methods

2.1. Plant Material

The seeds used in the present study were collected from an infested maize field of Research Farm of Paradise of Agricultural and Natural Resources, Razi University, Kermanshah (34°18′N47°03′E; elevation 1519 m), Iran, during early September 2013. Seeds were also collected from an infested potato field located in Islamabade-gharb city (34°03′N46°40′E; elevation 1335 m), Iran, during late September 2013. In addition, seeds were collected from a tomato field belonged to Sarpole-Zahab city (34°35′N45°50′E; elevation 536 m), Iran, in July 2013. Meteorological conditions of these regions are given in Fig. 1. These different sites, which are heavily infested by P. divaricata, were chosen to test the relationship between seed germination of *P. divaricata* and environmental conditions. Seeds were stored at room temperature for10 days.

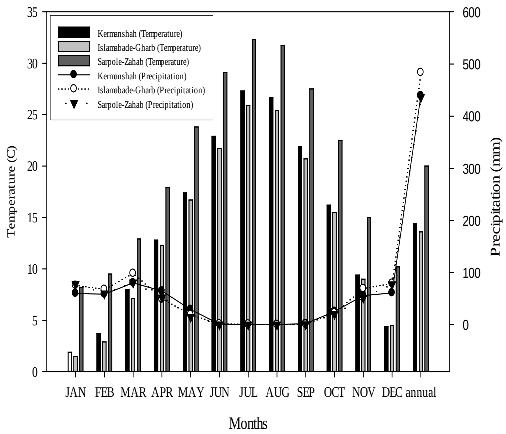


Figure 1. Long-term precipitation and temperature for Kermanshah, Sarpole-Zahab and Islamabad-Gharb during (1955-2014)

2.2. Germination Test

Four replications of 25 seeds of *P. divaricata* were placed in 9-cm Petri dishes lined with two layers of Whatman No. 1 filter paper, which were moistened with either 5 ml deionised water or other solutions when required. The Petri dishes were sealed with parafilm to prevent evaporation and then placed in a germination chamber in the physiology laboratory of the campus of Agricultural and Neutral Resources, Razi University, Kermanshah, Iran. Germination tests were conducted for 14 days at a light/dark temperature range of 20/35°C with the high temperature during the light cycle. Seeds were considered to have germinated when the radicle emerged. The number of germinated seeds was counted daily until day 14 of germination. The temperature and light conditions used here are according to the preliminary tests (data not shown).

2.3. Effect of Light and Temperature on Germination

Incubation temperatures consisted of constant (10, 20, 30 and 40°C) and fluctuating (5/10, 10/20, 15/25 and 20/35°C) (8 h low/16 h high) temperatures under light/dark cycle and continuous dark. For light treatments, dishes were sealed in transparent polyethylene bags while dark controls were wrapped in aluminum foil. All treatments were chosen according to the preliminary tests (data not shown).

Preliminary experiments showed no differences in germination among ecotypes collected from Islamabade-gharb, Sarpole-Zahab and Kermanshah in response to salt stress, pH, osmotic stress and burial depth treatments. Therefore, all experiments were carried out with seeds collected from Kermanshah.

2.4. Effect of Salt Stress on Seed Germination

Seeds were exposed to ten levels of salinity using solutions containing 0, 2, 4, 8, 16, 32, 64, 128, 256 and 512 mM NaCl. The experiment was carried out under the conditions described in the general procedure for germination test above.

2.5. Effect of pH on Seed Germination

Evaluated pH values were 4, 5, 6, 7, 8, 9 and 10. Buffered solution of different pH values were prepared based on Chen et al. (2009). The pH of 2 mM potassium hydrogen phthalate buffer was adjusted to 4 with 1 N HCl. The pH of 2 mM MES [2-(N-morpholino) ethanesulfonic acid was adjusted to 5 and 6 with 1 N NaOH. The pH of 2 mM solution HEPES [N -(2-hydroxymethyl) piperazine- N-(2-ethanesulfonic acid)] was adjusted to 7 and 8 with 1 N NaOH. Buffer solutions of pH 9 and 10 were prepared with 2 mM Mtricine [N -Tris (hydroxymethyl) methylglycine]. Petri dishes were incubated under light and temperature regimes as described for the germination test above.

2.6. Effect of Osmotic Stress on Seed Germination

The effect of osmotic stress on germination was assessed by incubating the seeds in polyethylene glycol solutions (PEG-6000) with the osmotic potentials of 0, -0.1, -0.2, -0.4, -0.6, - 0.8 and - 1.0 MPa. The solution was prepared based on the method described by Boyd and Hughes (2011). The seeds were incubated in the light and temperature regimes as mentioned for the germination test above.

2.7. Effect of Burial Depth on Seedling Emergence

The burial depths consisted of 0, 1, 2, 3, 4, 6, 8 and 10 cm. Thirty *P. divaricata* seeds were placed at the specified depths in a cup. Cups were watered daily and kept at room temperature (~25 °C). Emergence was counted weekly for 28 d or until emergence no longer occurred. Then, soil was sieved to find germinated seeds which could not reach the soil surface (suicidal germination). Seedlings were considered emerged when the two cotyledons could be seen at the soil surface.

2.8. Effect of GA3 and KNO3 on Seed Germination

Seeds were placed on filter paper moistened with 7 ml of distilled water containing GA3 (5 and 10 mM) and KNO3 (25 and 50 mM). In the control Petri dishes, only the distilled water was used. Petri dishes were then incubated according to the germination test above.

2.9. Statistical Analysis

All experiments were conducted as a completely randomized design with four replications and repeated twice. Unless otherwise noted, data were pooled across runs because of the lack of the significant run by-treatment interactions. Data were analyzed using PROC GLM in SAS and means were compared using LSD at the 0.05 level of probability. The following model was fitted to the data obtained from the salt and osmotic stress experiments using Sigma Plot software (version 12.0, SyStat Software, Inc., Point Richmond, CA, USA):

G (%) = Gmax/[1+(X/X50) Grate] (1)

where, G: Total germination (%) at NaCl concentration or osmotic potential x, Gmax: maximum germination (%), X50: the NaCl concentration or osmotic potential for the 50% reduction in the maximum germination (%) and Grate: germination rate or the slope of the curve (Chauhan *et al.*, 2006b)

The seedling emergence data resulted from the burial depth experiment were fitted to the following sigmoidal model (Norsworthy & Oliveira, 2006):

E(%) = Emax/(exp(-(X-X50/Erate)) (2)

where, E: seedling emergence percentage at burial depth x, Emax: maximum seedling emergence, X50: the soil depth at which emergence is reduced by 50% and Erate: emergence rate or the slope of the curve.

3. Results

Seed Germination under Different Conditions

Light and Temperature

Preliminary experiments (data not shown) demonstrated that the freshly harvested mature seeds of *P. divaricata* exhibited dormancy. Germination of seeds from three ecotypes of *P. divaricata* (Islamabade-Gharb, Sarpole-Zahab and Kermanshah) was studied over a series of constant and alternating temperatures under dark and light/ dark regimes. Seeds collected from Sarpole-Zahab with mean germination percentage of 15.77 showed the highest percentage of seed germination (Fig. 2).

In general, for all temperature regimes light had stimulatory effect on seed germination of *P. divaricata* and its effect was more obvious at the lower temperatures (Fig. 2).

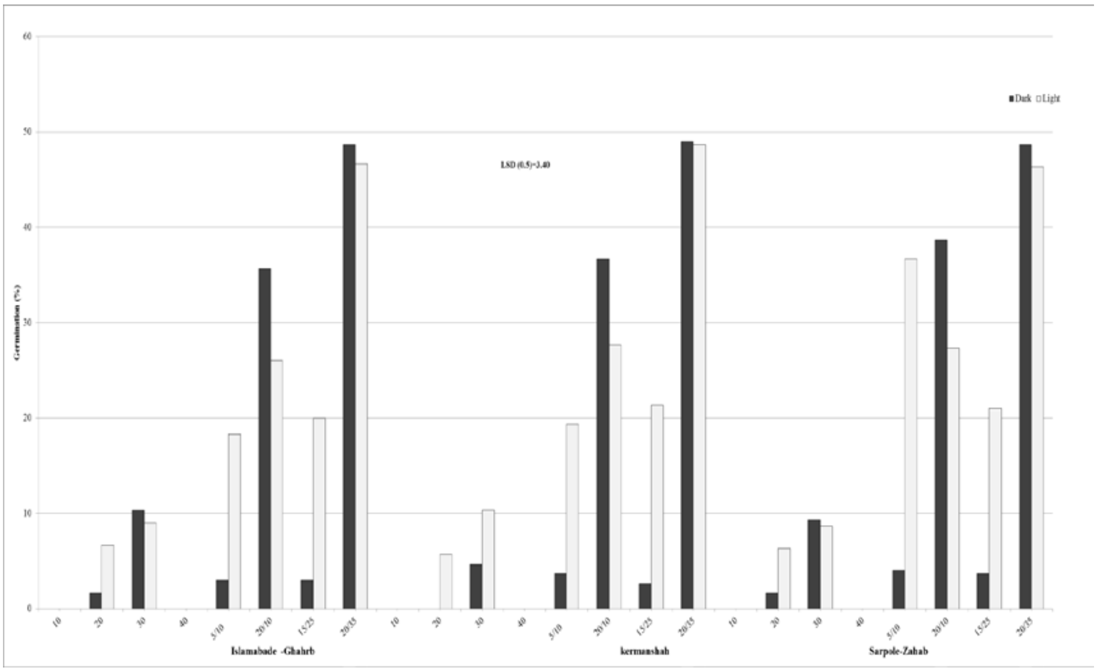


Figure 2 Seed germination of *P. divaricata* collected from different locations as influenced by different light and temperature regimes

Moreover, seeds exposure to a cycle of 8 h dark/ 16 h light enhanced germination percentage by 4.15 compared with the continuous darkness (Fig. 2). Different ecotypes responded to light regime identically so that under light/dark cycle germination percentage of all ecotypes was higher than that under complete darkness (3.04, 4.38 and 5.04%, respectively) (Fig. 2).

Burial Depth

Emergence of *P. divaricata* decreased with an increase in burial depth. At Day 14 After Burying (DAB), the higher emergence percentages (75 and 55) were recorded for the burial depth of 0 and 1 cm, respectively (Fig. 3).

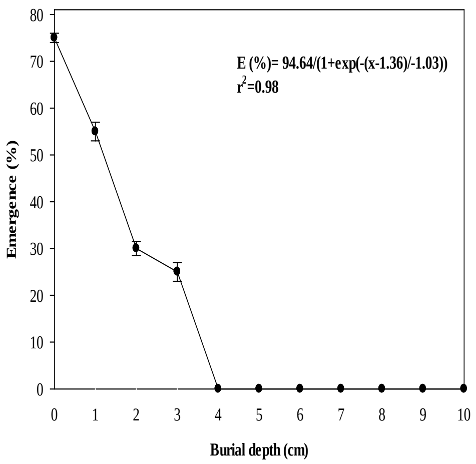


Figure 3 .Effect of different seed burial depths on seedling emergence of *P. divaricata*.

Salinity Stress

A three-parameter logistic model described well the relationship between germination of *P. divaricata* and salt concentration (Fig. 4). The germination occurred over a wide range of the salt concentration (from 0 to near 180 mM NaCl). A significant decrease in seed germination was shown with the increase in salt concentration (seed germination of 76% at the 0 mM to 0% at the 180 mM) (Fig. 4).

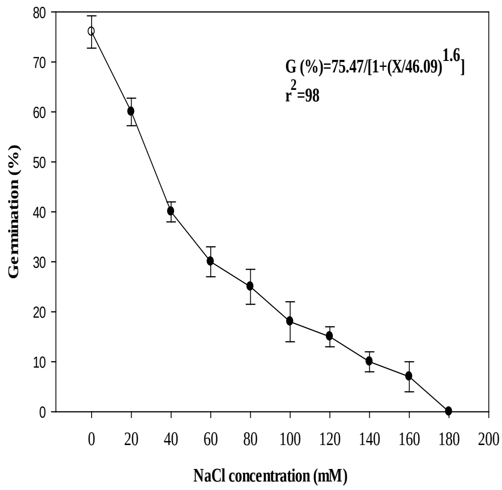


Figure 4. Seed germination of *P. divaricata* as influenced by different NaCl concentrations.Vertical bars represent standard error of the mean.

At the lowest concentration of NaCl (10 mM), seed germination of *P. divaricata* was reduced by 8.1% compared with the control (the 0% of salt concentration). However, *P. divaricata* seed germination was more than 40% at NaCl concentrations up to 40 mM. Even at 100 mM NaCl, seed germination was 18% and it reached 10% at140 mM (Fig. 4).

Osmotic Stress

A functional three-parameter logistic model was fitted to the germination data (%) obtained under different osmotic levels (Fig. 5). Germination decreased as osmotic stress level was increased (Fig. 5). Significantly, the greater number of seeds (80%) germinated in the control treatment (distilled water) when compared with other osmotic treatments (Fig. 5). Germination rapidly decreased when osmotic potential reached -0.5 MPa. Very limited number of seeds could germinate at osmotic potentials greater than -0.8 MPa and above -1.0 MPa, no germination occurred.

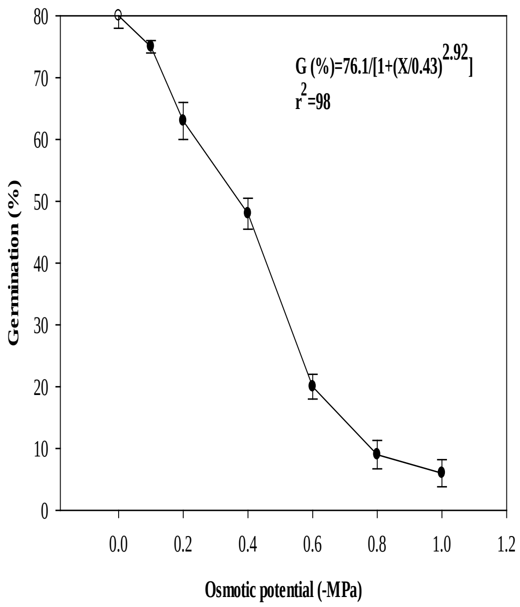


Figure 5 Seed germination of *P. divaricata* as influenced by different osmotic stress levels

pH

P. divaricata seeds showed a germination value more than 44% over a pH range of 4 to 10 (Fig. 5). Maximum and minimum germination percentages were recorded at pH values of 4 and 10 (44.7 and 86 %), respectively. Seed germination increased linearly in response to increasing pH (y=5.86X+25.29, R2=95). In general, *P. divaricata* seeds notably performed better in alkaline pHs, as the higher germination percentage were recorded at the pH levels above 7 with a maximum value at pH 10 (Fig. 6). Acidic condition (pH values lower than 7) significantly decreased *P. divaricata* germination.

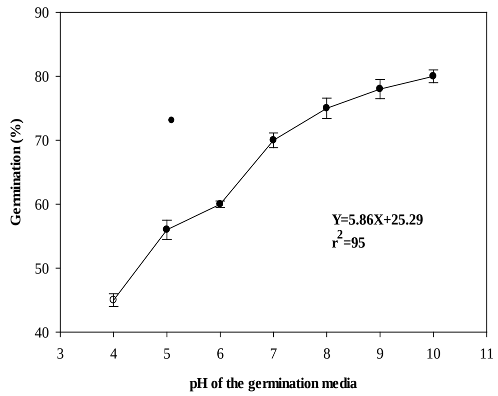
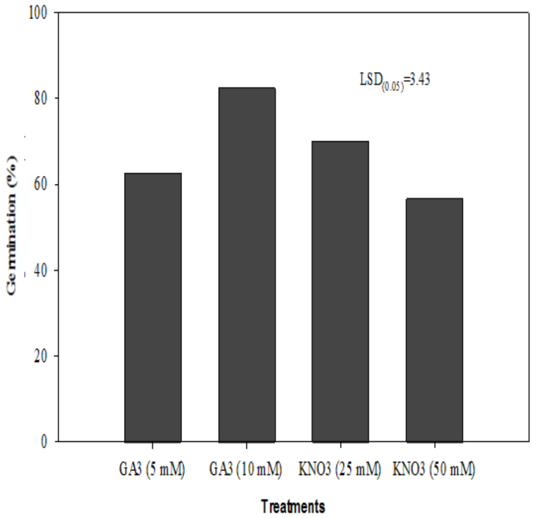


Figure 6 . Seed germination of *P. divaricata* as influenced by different pH levels.Vertical bars represent standard error of the mean

KNO3 and GA3

KNO3 at concentration of 25 and 50 mM stimulated germination to a maximum level of 70.22 and 56.70 %, respectively (Fig. 7). In general, germination declined with concentrations of KNO3 greater than 50 mM (data not shown). Overall, germination increased when GA3was added to the germination solution (Fig. 7). Addition of 10 mM of GA3 increased seed germination by 82.43% relative to the control.



Figuer 7. Effect of GA_3 and KNO_3 incubation on Germination percentage of *P. divaricata.*

4. Discussion

There was statistically substantial difference between seed germination (%) of ecotypes collected from Kermanshah and Islamabade-Gharb ecotypes with that of Sarpole-Zahab. In opposition to our results, an earlier work conducted on seed dormancy and germination of 44 ecotypes of Johnson grass [*Sorghum halepense* (L.) Pers.] by Taylorson and McWhorter (1969) indicated a negligible difference among the different ecotypes. Similar to our results, several works have demonstrated that seed germination varies greatly among ecotypes of different species (Meyer & Monsen, 1991; Galloway, 2002; Pita Villamil *et al.*, 2002). As a general rule, the conditions under which seeds mature on the mother plant can determine the level of seed dormancy (Meyer & Allen, 1999; Baskin & Baskin, 2014). The most important factor in this regard is temperature (Sharif-Zadeh & Murdoch, 2000). Consistent with our results, seeds produced by plants grown at higher temperatures have a lower dormancy level than those produced at lower temperatures. It is probably due to the fact that the environmental conditions can affect seed germination by affecting their chemical composition and seed provisioning (Galloway, 2002). Sarpole-Zahab is located in a warmer climatic condition relative to Kermanshah and Islamabad and *P. divaricata* seeds collected from this region had lower dormancy level which could be attributed to its warmer weather conditions.

Generally, light had a stimulatory effect on the seed germination of *P. divaricata*. Similar results obtained by De Cauwer et al. (2014) who demonstrated that Galinsoga spp. seeds required light for germination as well as light dependency varied among populations. Likewise, Malik et al. (2010) reported that wild radish (*Raphanus raphanistrum* L.) requires light for optimum seed germination. However, the study conducted by Del Monte and Dorado (2011) revealed the negative effect of light on seed germination in great brome (Bromus diandrus Roth.). Stimulatory and inhibitory effects of light on seed germination have also been reported for licorice weed (Scopa riadulcis L.) (Jain & Singh, 1989) and wild oat (Avena fatua L.), respectively (Sharma & Born, 1978). Previous studies demonstrated that seed germination of nightshade species, which have close botanical relationships with *P. divaricata*, was not sensitive to light (Taab & Andersson, 2009; Stanton *et al.*, 2012). This is in contrast to seed germination of P. divaricata, which was significantly enhanced by light. The light, either by the phytochrome system or by altering the balance of germination promoters and inhibitors in the embryo, may affect germination of P. divaricata. Both mechanisms could guarantee that germination occurs far away from other plants and close to the soil surface (Schutte *et al.*, 2014). The seeds of *P. divaricata* germinated over a relatively wide range of temperatures. The greatest germination occurred under the alternating temperature of 20/35°C, irrespective of lightness regime. Among the constant temperature treatment, the seeds of *P. divaricata* failed to germinate at the 10 and 40°C. It is well documented in several very old and recent studies that fluctuating temperatures greatly improve seed germination of different weed species (Morinaga, 1926; Ghersa *et al.*, 1992; Lee *et al.*, 2011; Wu *et al.*, 2015). The higher seed germination of *P. divaricata* at temperature regime of 20/35°C can be a major component of the mechanism that enables its seeds to detect soil depth. Usually, in a soil profile the most temperature fluctuation occurs in the upper layers of soil and it diminishes in response to increasing soil depth. Therefore, the *P.*

divaricata seeds located in the upper layers have more chance for germination and seedling formation. This can be explained by the relatively small size of *P. divaricata* seeds. Commander et al. (2008) also reported that alternating temperatures improved the germination of a range of Solanum species which their seed size is almost similar to that of P. divaricata.

The emergence of *P. divaricata* decreased sharply when seeds were buried deeper than 3-cm. This might be due to the lack of the seed germination stimulants such as light and fluctuating temperatures at the deeper layers of a soil profile. Diminishing seedling emergence with increasing burial depth has also been reported in other weed species (Prostko *et al.,* 1998, Sabila *et al.,* 2012; Schutte *et al.,* 2014). It can be concluded that *P. divaricata* germinates predominately near the soil surface, with a few seedlings emerging from the seeds buried beyond 3 cm. This confirms that *P. divaricata* seeds can germinate better at the presence of light and fluctuating temperatures as shown in the present study. These conditions usually exist at the surface layers of soil. *P. divaricata* emergence drastically reduced at the soil depth more than 3 cm and reached zero at the 4 cm. A practical finding is that burying seeds through tillage may effectively reduce the *P. divaricata* infestation in crop fields. This can explain the reason why this weed species is more dominant in less disturbed habitats such as gardens, roadside and edge of tilled fields in western Iran (personal observation).

The salt concentration required to inhibit the germination of *P. divaricata* was 250 mM. This suggests that *P. divaricata* can tolerate some levels of salt stress and even at the salinity levels up to 180 mM a portion of its seeds may still germinate. It is concluded that this weed species can pose a serious invasion threat for habitats of salty soil. This conclusion is strongly supported by the fact that the farms, located in the western Iran having a more or less saline soil, are heavily infested by *P. divaricata*. Further studies are needed to dissect the mechanisms involved in salt tolerance of P. divaricata. The seeds of other weed species, such as rigid rye grass (Lolium rigidum Gaudin) and common reed (Phragmites australis (Cav.) Trin ex Steudel), have shown some level of tolerance to salinity as they germinated by 50% at the 40 mM of NaCl (Chauhan *et al.,* 2006c). Chauhan et al. (2006a) also reported a germination percentage of 7% for the annual sow thistle (Sonchus oleraceus L.) (a common weed species in western Iran) at salt concentration of 160 mM.

Very limited number of seeds could germinate at osmotic potentials more than -0.8 MPa. This shows that *P. divaricata* can tolerate low water potentials and germinate under very dry and intense water stress conditions. Other weed species have shown varying sensitivity to water stress. Reddy and Singh (1992) reported that the germination of hairy beggar ticks (*Bidens pilosa* L.) decreased linearly with increasing osmotic stress. In another study, hemp dogbane (*Apocynum cannabinum* L.) germination rapidly decreased at osmotic potentials below -0.25 MPa (Webster & Cardina, 1999). However, tolerance to severe water stress conditions has been reported in some weed species, such as turnip weed (*Rapistrum rugosum* (L.) All.) and members of the Brassicaceae family (Ray *et al.,* 2005). It is worth noting that turnip weed and Brassicaceae family are dominant weed species in western parts of Iran, especially in Kermanshah province, which is also the habitat for P. divaricata. Therefore, under low soil water potentials and not dryland farming, *P. divaricata* is expected to grow well and compete vigorously with the irrigated summer crops.

Acidic conditions (pH values lower than 7) significantly decreased seed germination of P. divaricata. Oliveira and Norsworthy (2006) reported that optimal germination of other weedy members of solanaceae family was at pH 6 to 8. Our findings are compatible with those obtained by Ahmadi (1999), based on the fact that most of the soils infested by *P. divaricata* are alkaline. This compatibility can intensify the invasion potential of *P. divaricata* in western regions of Iran, the regions with soil pH higher than 7. However, the results of the present study cannot be fully conclusive and further research needs to be conducted to assess the impact of pH on seed germination of this weed under soil conditions.

Generally, germination increased when GA3 and KNO3 were added to the germination media. GA3 is known to reduce the adverse effects of germination inhibitors (Zhou *et al.,* 2005). The increase of seed germination by GA3 has been demonstrated in other weed species. This is because GA has an important role in the mobilization of food reserves in the seeds to nourish the growing embryo (Toyomasu, 1993). Nevertheless, results revealed that light was a stimulatory factor of seed germination of *P. divaricata*. Therefore, this response is likely to be due to the breakage of physiological dormancy in the seeds of this species. It is well documented that KNO3 and GA3 can overcome seed dormancy (Fawcett & Slife, 1978; Foley & Chao, 2008). From the results of this experiment, it could be argued that the application of N-containing fertilizers may stimulate the germination of *P. divaricata* seeds under field conditions. This agronomic operation is common across infested areas with P. divaricata. Bouwmeester and Karssen (1993) also reported stimulation in seed germination of hedge mustard (*Sisymbrium officinale* L.) by the addition of nitrate.

5. Conclusion

The simple and most important result of the present study is that seeds of *P. divaricata* have innate dormancy and this dormancy could be alleviated by some seed dormancy breaking factors. This is contrary to the results reported by Mousavi and Ahmadi (2008) who suggested that the reason of seed dormancy of *P. divaricata* was the mucilage content of the berries, which inhibits seed germination. Therefore, washing out mucilage by normal irrigation and by rain will result in a high germination percentage. In addition, the berries are decomposed during autumn and winter seasons, which eventually lead to a dormancy breakage and a seed germination in the following season.

As it is well demonstrated, the survival of weed seeds in the soil is important, since potential weed problems exist as long as weed seeds remain alive in the soil (Schutte *et al.*, 2014). The survival of weed seeds during log time surely could not depend only on mucilage content of its fruits, because they cannot be eradicated from the field easily by irrigation or by destroying the emerging seedlings. Seed dormancy ensures that the seeds do not germinate under closed canopies and in very deep depths of soil profile by detecting light quality and temperature fluctuations (Ghersa *et al.*, 1992).

In conclusion, *P. divaricata* is an emerging problem in summer crops in the western parts of Iran and this problem is exacerbated by seed dormancy and the absence of an efficient herbicide-based management system. Further research is required to elucidate the details of *P. divaricata* seed germination, especially those related to the effect of real soil conditions. However, the present study provides preliminary information on the effect of some factors on seed dormancy germination. Long-term studies are needed to determine the impact of management and climatic factors on the persistence of *P. divaricata* seed banks. This important information is required for the development of management strategies for this weed species.

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