



SEED DORMANCY BREAKING AND GERMINATION OF POKEWEED AND LAMB'S EAR: TWO INVASIVE PLANTS

REZVANI M.^{1,*}, AMINI BENGAR J.¹, NIKKHAH KOUCHAKSARAEI H.¹

¹ Department of Agronomy and Plant Breeding, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran

*Corresponding author; telephone: +989367568324; e-mail: m_rezvani52@yahoo.com

(RECEIVED 20 MARCH 2020; RECEIVED IN REVISED FORM 9 AUGUST 2020; ACCEPTED 14 AUGUST 2020)

ABSTRACT - We investigated seed dormancy breaking techniques and some environmental factors affecting on seed germination of Pokeweed (*Phytolacca americana* L.) and Lamb's Ear (*Stachys byzantina* K. Koch) in a series of laboratory and green house experiments. The seed dormancy breaking treatments were consisted Gibberellic acid (GA₃), mechanical scarification and leaching duration. Environmental factors were included salt and drought stresses, pH and planting depth. The greater stimulatory effect on germination was observed at low concentrations of GA₃, while seed germination of both Pokeweed and Lamb's Ear was reduced with high concentrations of GA₃. With decreasing of water potential germination significantly reduced. Seed germination was inversely related to salt concentration. But, Lamb's Ear germinated in solutions with relatively high salinity. The Pokeweed seeds germination was greatly reduced at pH lower and higher than 7. Lamb's Ear seed germination was completely inhibited at pH lesser than 7. The maximum germination percentage of Pokeweed (62%) occurred at planting depth 5 cm after 30 days. Germination percentage of Lamb's Ear increased in comparison to the control as leaching duration enhanced. But it was declined as leached for 96 hours. The seedling emergence decreased with increasing of planting depth in both plants.

KEYWORDS: GERMINATION; INVASIVE PLANT; pH; OSMOTIC POTENTIAL; SALT STRESS; SEED DORMANCY.

INTRODUCTION

Pokeweed or Pokeberry (*Phytolacca americana* L.) belongs to the Phytolaccaceae family and considered as a common perennial herb (Ravikiran et al., 2011; Ding et al., 2012) that is abundant in open, disturbed habitats, as well as in forest edges and light gaps (Armesto et al., 1983). This plant grows as an invasive species at the edge of agroecosystems and natural ecosystem in Mazandaran Province, Iran.

The genus *Stachys* is from the Lamiaceae family that grows in south west of Asia such as Turkey and Iran. This genus consists of 250 species widespread throughout the world (Salimi et al., 2011). Lamb's Ear (*Stachys byzantina* K. Koch) is annual or short-lived perennial plants that invade to railroads, disturbed areas, and farmlands such as citrus orchards in Mazandaran Province, Iran.

As ecological perspective, seed dormancy is a mechanism that prevents seed germination during unsuitable environmental conditions. The dormancy mechanism allows a species to

synchronize its germination with favorable environmental conditions, which increases its probability of survival and establishment (Guleryuz et al., 2011). There are different type of seed dormancy including primary dormancy and secondary dormancy. Primary dormancy is established during seed maturation on the mother plant. While, secondary dormancy occurred as seed exposed to the specifying prolonged unfavorable situations after maturity (Buijs, 2020). Environmental signals that confer information about the seasonal changes can induce secondary dormancy (Footitt et al., 2019). There are techniques that help us to overcoming seed dormancy. Treating of seeds by Gibberellic acid (GA₃), potassium nitrate (KNO₃), cold stratification, physical and chemical scarification and leaching could be effective techniques to releasing seed dormancy and inducing germination (Rezvani and Fani Yazdi, 2013; Rezvani et al., 2014; Rezvani & Zaefarian, 2016).

The seed coat of pokeweed is not permeable to water and germination will not occur until the hard seed coat has been opened or softened to permit the entrance of moisture (Krochmal, 1970). Krochmal (1970) reported that the best germination can be achieved by storing seeds over winter in dry conditions at room temperature and then nicking or scarifying them with a needle, followed by germination treatment at about 24 °C. Hot burns facilitated germination of *Vitis* spp., *Rhus glabra*, and Pokeweed species in disturbed habitats (Glasgow & Matlack, 2007). Seed germination and seedling emergence are important processes for plant establishment and persistence in both natural and agricultural ecosystems (Henedina et al., 2014). Various environmental factors such as temperature, light, soil salinity, pH and moisture are the most important situations that effects on life cycle of plants (Rezvani & Zaefarian, 2016). Rezvani et al. (2017) and Rezvani et al. (2018) also indicated negative effect of drought and salt stresses on seed germination goat grass (*Aegilops cylindrica* Host.), rye grass (*Lolium perenne* L.) and greater bur-parsley (*Turgenia latifolia* Hoffm.).

In Iran, both Pokeweed and Lamb's Ear are invasive plants that damages to natural habitat, agroecosystems and roads. It is important to understand the ecological requirements for seed germination invasive plants. This information provides us valuable knowledge about some environmental factors effective in seed germination and also methods that help us to break seed dormancy both Pokeweed and Lamb's Ear. There is no enough information about seed germination requirements and seed dormancy breaking techniques. The objectives of this study were to investigate 1) effects of some seed breaking dormancy techniques including GA₃ and scarification leaching duration on overcoming seed dormancy 2) impact of some environmental factors affecting on seed germination such as pH, salt and drought stresses, and 3) effect of planting depth on seedling emergence of Pokeweed and Lamb's Ear.

MATERIALS AND METHODS

Seed collection and germination test

Seeds of Pokeweed and Lamb's Ear were harvested from natural matured plants from edge of crop fields and orchards at Qaemshahr, Mazandaran Province of Iran. Seeds were cleaned and stored in paper bags under ambient conditions (20±5 °C) in the laboratory until start the experiments. Morphological characteristics of seeds including length, width and thousand seed weight were determined in the laboratory. At the beginning of each trial, seeds were tested for viability using Tetrazolium Chloride solution (Peters, 2000). Tetrazolium test result showed that 98% of seeds of both species were alive.

In each experiment before germination test, seeds were sterilized with 1% sodium hypochlorite solution (NaOCl) for one minute and then rinsed with sterile deionized water 5-6 times. Thirty seeds were placed on two layers of filter paper in a petri dish with 9 cm diameter. The filter papers were moistened with 6 ml of deionized water or treatment solutions (in GA₃, pH salt and osmotic stress experiments) as described later. Petri dishes were sealed with two layers of parafilm in order to inhibit moisture losing and were placed in an incubator at temperature of 25/15 °C (day/night). The photoperiod was set at 12/12 hours (day/night). Fluorescent lamps were used to supply a light intensity of 300 μmol m⁻² s⁻¹. The number of germinated seeds was recorded 21 days after starting germination test. Seeds were determined as germinated when they produced visible radicle higher than 1 mm length (ISTA, 1985).

Dormancy breaking methods

Gibberellic acid and light regime

Effect of different concentration of GA₃ on seed dormancy breaking were evaluated in both 12/12-h day/night photoperiod and complete darkness regime. The GA₃ concentrations for treating of Pokeweed seed were included 0, 50, 100, 150, 200, 250, 500 and 1000 ppm. Lamb's Ear seed were treated with GA₃ concentrations consist of 0, 50, 100, 150, 200, 250 and 500 ppm. For darkness complete regime petri dishes were wrapped by two layers of aluminum foil to prevent light penetration. Seed germination tested in condition as mentioned in germination test section.

Mechanical scarification and stratification

The effect of mechanical scarification on stratified and non-stratified seeds of Pokeweed were studied. For stratification, Pokeweed seed was conserved at 5 °C for 5 months in fridge. Non-stratified seeds were stored in ambient conditions (20±5 °C) in the laboratory. Both non-stratified and stratified seeds were scarified by rubbing the seeds between two sheets of sandpaper for 1, 2 and 4 minutes. Seeds were place on filter paper and moistened with distilled water. Petri dishes were sealed with parafilm and were placed in incubator at alternating temperature 25/15 °C (day/night) and 12/12 hours (day/night) photoperiod.

Leaching duration

Pokeweed and Lamb's Ear seeds were placed in polyethylene bags and imposed to the leaching duration for 12, 24, 48 and 96 hours with tap water. After each leaching duration,

bags were exhumed and seeds incubated at germinator at 25/15 °C (light/dark) temperature with a period of 12/12 hours (light/dark).

Environmental factors treatments

Salt and drought stress

The effect of salt stress on seed germination of both plants was studied by adding of saline solutions in different concentration of 0, 25, 50, 100, 200 and 400 mM NaCl to petri dishes (Merck, Germany). Seed were placed on two layers of filter paper and treated with solutions. Sealed petri dishes with Parafilm tape were incubated at a temperature of 25/15 °C (day/night) with a photoperiod 12/12 hours (day/night). Germination evaluated as described in germination test section.

The effects of drought stress on seed germination of pokeweed and Lamb's Ear plants were evaluated in different osmotic potentials of 0, -0.1, -0.2, -0.4, -0.6, -0.8 and -1 MPa. The solutions were prepared by dissolving amount of 0, 4.34, 7.73, 10.16, 12.81, 15.06 and 17.04 g of polyethylene glycol (PEG 6000) in 100 ml distilled water, respectively. Petri dishes were moistened with polyethylene glycol solution and incubated at 25/15 °C (day/night) temperature with a photoperiod of 12/12 hours (day/night). Germination percentage evaluated as described in germination test section.

pH

The effects of different pH solutions including 3, 5, 7, 9 and 11 (Chachalis & Reddy, 2000) were evaluated on seed germination of Pokeweed and Lamb's Ear. Distilled water (pH 7.2) was used as control treatment. The seeds were incubated at 25/15 °C (light/dark) temperature with a photoperiod of 12/12 hours (light/dark). Seed germination determine with the method described in germination test section.

Planting depth

Thirty seeds of each plant were planted in plastic pots (20 cm in diameter) at depths of 0, 4, 8 and 12 cm. Control pots were considered to indicate that there was no background seed bank of both plants in the soil.

A silty soil (26.30% clay, 55.70% silt, 18.00% sand), with 7.6 pH and 0.63% organic carbon was used. The pots were irrigated as needed to maintain soil moisture in field capacity condition. Greenhouse temperature was set at 25/15 °C (light/dark) with a natural photoperiod. Emerging of two cotyledons was considered as seedling emergence. Seedling emergence was recorded up to 45 days after sowing. No seedling emergence was observed in the control pots that show there is not seed back ground of Pokeweed and Lamb's Ear in the soil.

Statistical analysis

All experiments were set as a completely randomized design with 5 replicates. Each experiment was conducted twice and the data of both experiments were used to analysis. Data were subjected to arcsine transformation to improve for homogeneity. Transformed data were used for statistical analysis.

Analysis of variance was conducted on the data GA_3 , scarification duration, leaching duration, pH, and planting depth experiments by SAS software. Means were compared using LSD test at $P=0.05$ and standard error bars. Regression analysis was used to determine the effect of salt and drought stresses on seed germination.

RESULTS AND DISCUSSION

General traits of seeds

Pokeweed seed thousand weight, length and width were 5.88 g, 3.59 mm and 3.23 mm, respectively. Initial seed germination percentage of pokeweed at light/dark and complete darkness was 43.05% and 12.29%, respectively. Seed thousand weight, length and width of Lamb's Ear were 1.65 g, 2.01 mm and 1.8 mm, respectively. Lamb's Ear germination at light/dark regime and complete darkness conditions were 50.83% and 54.09%, respectively. Armesto et al. (1983) reported that range of seed germination of 13 populations of pokeweed was varied from 24.7 to 98% as tested in 12/12 h light/dark regime, and 30/20 °C fluctuating temperature. But, Farmer and Hall (1970) showed germination of un-stratified seeds of pokeweed was lower than stratified. Guleryuz et al. (2011) observed that seeds of *Stachys germanica* L. subsp. *bithynica* (Boiss.) Bhattacharjee failed to germinate in the control treatment. Seeds also failed to germinate in moist chilling treatments with distilled water under both, continuous darkness and photoperiodic conditions. Dunn (1997) reported that Lamb's Ear seeds did not germinate immediately on shedding under natural conditions, and germination occurs almost exclusively under the parent plant and extends over several months and seeds may remain dormant for many years.

Dormancy breaking treatments

Impact of GA_3 concentrations and light regimes

Gibberellic acid concentrations at light/dark regime were significantly effective on seed germination of Pokeweed. A non-significant effect of GA_3 was observed on Pokeweed seed germination in complete darkness regime and Lamb's Ear in light/dark regime and complete darkness conditions (Table 1).

Table 1. ANOVA for the effect of treatments on seed germination of Pokeweed and Lamb's Ear.

Plant species	Pokeweed	Lamb's Ear
	Mean square	
Gibberellic acid concentrations at light/dark regime	*	ns
Gibberellic acid concentrations at complete darkness regime	ns	ns
Treatments		
Mechanical scarification on stratified	ns	-
Mechanical scarification on non-stratified	*	-
Leaching duration	ns	*
pH	*	*
Planting depth	*	*

ns: non-significant; *: significant at $p = 0.05$.

In the light/dark regime, a non-significant enhancement was observed as GA₃ concentration increased to 100 ppm. Concentration of GA₃ higher than 100 ppm reduced seed germination in comparison with control (Figure 1a). In the complete darkness regime, increasing GA₃ concentration from 50 ppm to 500 ppm significantly reduced pokeweed seed germination compared to control treatment. A non-significant difference was observed between the control and treatment of 1000 ppm. Without considering GA₃ concentration, seed germination of pokeweed at light/dark condition was greater than seed germination in complete darkness (Figure 1a).

According to Figure 1b, germination of Lamb's Ear seed was 51.3% at 500 ppm concentration of GA₃, which was slightly increased (0.9%) compared to control. The minimum germination percentage (43.12%) observed at 100 ppm concentration GA₃ under light/dark condition. In complete darkness regime, the maximum germination percentage (60.57%) was observed at 50 ppm concentration of GA₃ that shows about 11.98% enhancement compared to control. The minimum germination (53.19%) of Lamb's Ear under darkness condition was occurred in 100 ppm concentration of GA₃. Also, germination in darkness condition was more than the light/dark condition at all GA₃ concentrations (Figure 1b).

Seed germination of Pokeweed and Lamb's Ear was not markedly stimulated by GA₃ concentrations in both of light/dark and complete darkness conditions. Guleryuz et al. (2011) showed that treated seed of *S. germanica* with GA₃ or a combination of GA₃ and Kinetin (KIN) successfully

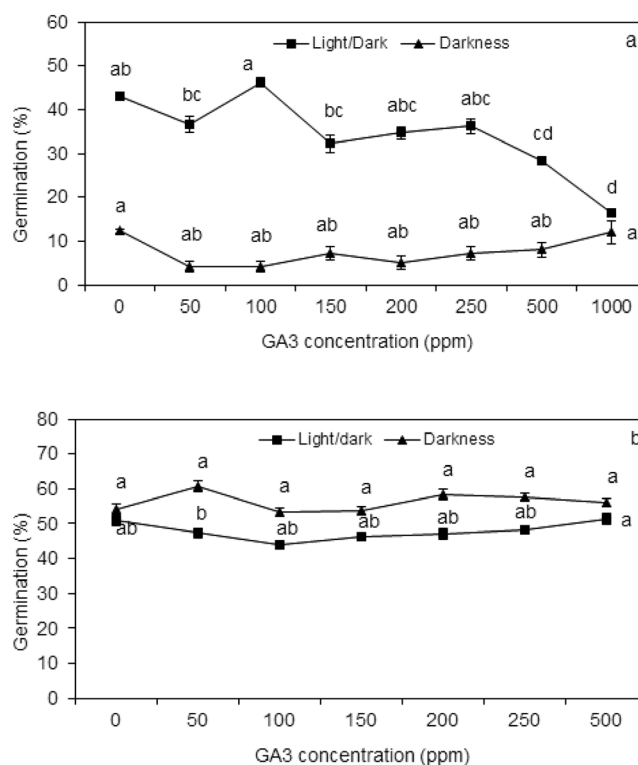


Figure 1. Impact of GA₃ concentrations on Pokeweed (a) and Lamb's Ear (b) seeds germination after 3 weeks of incubation at 25/15 °C light/dark temperatures and 12/12 hours light/dark photoperiod and darkness conditions. In each light regime, GA₃ concentrations with the same letter are not significantly different. Vertical bars represent standard errors of the means.

germinated, and maximum seed germination (37%) was observed. Nadjafi et al. (2006) reported that the maximum germination rate and percentage of *Teucrium polium* seeds were obtained at a range of concentration from 500 up to 2500 ppm concentration of GA₃.

Impact of scarification duration

Scarification significantly affected on pokeweed germination of stratified seeds (Table 1). In non-stratified seeds, scarification for 1 and 2 minutes declined germination comparing to control treatments, while germination of scarified seeds treated for 4 minutes was slightly higher than control (Figure 2). Stratified seed germination was lower than non-stratified, without considering scarification duration. Scarification did not significantly enhance seeds germination of both stratified and non-stratified seeds compared to control treatment (Figure 2). Scarification did not enhance seeds germination of both stratified and non-stratified seeds compared with control significantly. Cold stratification of seeds could be an effective treatment to seed dormancy releasing in many species.

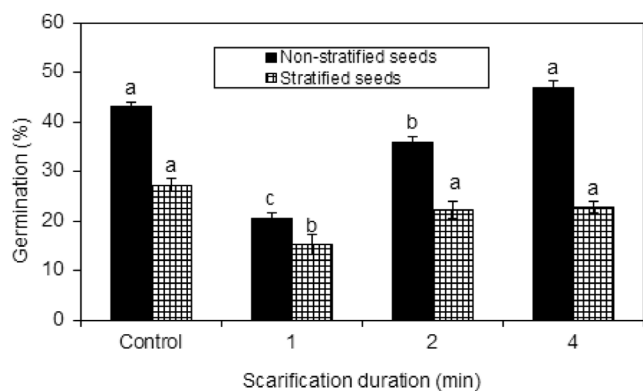


Figure 2. Impact of scarification duration on Pokeweed non-stratified and stratified seed germination after 3 weeks of incubation at 25/15 °C light/dark temperatures and 12 hours photoperiod. In each group, (i.e. non-stratified seeds or stratified seed) columns with the same letter are not significantly different. Vertical bars represent standard errors of the means.

But, naturally stratified seeds of *S. germanica* collected in spring, did not germinate in control treatment under darkness and photoperiod (Guleryuz et al., 2011). These findings suggest that naturally stratified seeds could have developed secondary dormancy in their habitat. Scarified seeds of pokeweed in 24 °C and 16 °C have shown 78.8% and 32% germination, respectively that indicated 97.46% and 93.75% enhancement comparing to control (Krochmal, 1970). Mechanical scarification and freezing were the most effective treatments in overcoming dormancy of *Medicago marina* L. seeds (Scippa et al., 2011).

Complete and rapid germination of Milkvetch (*Astragalus hamosus* L.) and button medic (*Medicago orbicularis* (L.) Bartal.) scarified seed by sandpaper showed that dormancy imposed exclusively by seed coat (Patané & Gresta, 2006). *Stachys alpina* L. seeds after ripening up to 20 weeks under dry storage did not germinate. No germination occurred with chilling duration less than 4 weeks. Chilling of seed for 12-20 weeks increased germination (20-30%) in *S. alpina* (Pinfield et al., 1972). Guleryuz et al. (2011) showed that *S. germanica* seeds that were imposed on natural chilling for 10 months, did not germinate.

Impact of leaching duration on germination

Effect of leaching duration was not significant on germination of Pokeweed (Table 1). Leaching of pokeweed seed for 12 hours to 96 hours reduced germination compared to control. At leaching duration for 96 hours, seed germination increased in comparison to 12 hours to 48 hours leaching duration (Figure 3a). Impact of leaching duration on Lamb's Ear seeds germination was significant (Table 1). With increasing

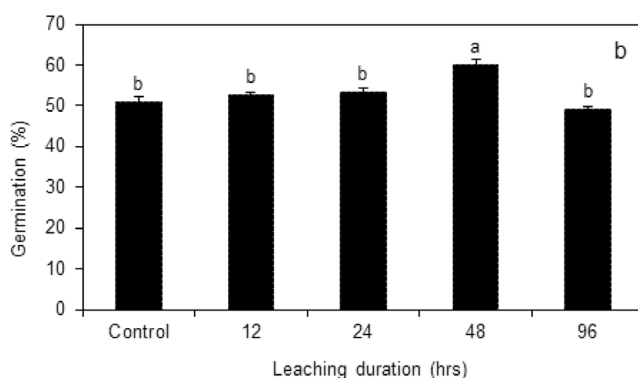
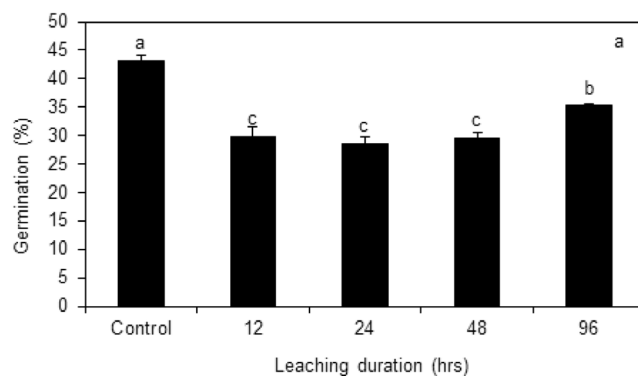


Figure 3. Impact of leaching duration on Pokeweed (a) and Lamb's Ear (b) seed germination after 15 days of incubation at 25/15 °C light/dark temperature and 12 hours photoperiod. Columns with the same letter are not significantly different. Vertical bars represent standard errors of the means.

of leaching duration, germination of seeds that leached for 48 h enhanced (15.12%) in comparison to control and reached to 60.03%. In contrast, enhancement of leaching duration up to 96 hours germination percentage decreased compared to control (Figure 3b). Nadjafi et al. (2006) reported that washing and chilling (5 °C) for a period of 14 days was effective on dormancy breaking in *Ferula gummosa* Biess.&Buhse. It seems that long term leaching treatments could be an effective method to extracting of germination inhibitors from seed and increasing seed germination. However, in this experiment, leaching reduced seed germination due to the inducing a secondary dormancy in Pokeweed.

Impact of environmental factors on seed germination

Impact of drought stress

The effect of osmotic potential on seed germination of Pokeweed was fitted by an exponential model (Figure 4a). Seed germination of pokeweed was reduced with decreasing osmotic potential. The maximum reduction in seed germination was observed as drought stress increased from 0

to -0.1 MPa (54.81% reduction). Osmotic potential -0.1 and -1 MPa showed the highest (19.45%) and lowest (4.20%) percentage of germination under drought stress, respectively. There was no significant difference in germination as drought stress increased from -0.4 to -0.8 MPa. Germination of 4.2% at -1 MPa shows that a low proportion of pokeweed seed can germinate under high water stress conditions (Figure 4a). Germination of Lamb's Ear seed was significantly reduced with increasing water stress. Seed germination reduced from 38.08% to 9.37% as osmotic potential declined in a range from -0.2 to -0.4 MPa, respectively. No seed germination was observed in osmotic potential -0.6 MPa. No seed germination of Lamb's Ear in osmotic potential -0.6 MPa shows the plant is sensitive to drought stress (Figure 4b). Decreasing of osmotic potential inversely impacted on germination of both Pokeweed and Lamb's Ear. Okcu et al. (2005) studied the effect of osmotic stress on Pea (*Pisum sativum* L.) germination and revealed that with decreasing of osmotic potential to -4 bar, germination percentage was declined to 94.7% and the lowest germination percentage (23.3%) was reported in -8 bar. Also, Kaya et al. (2006)

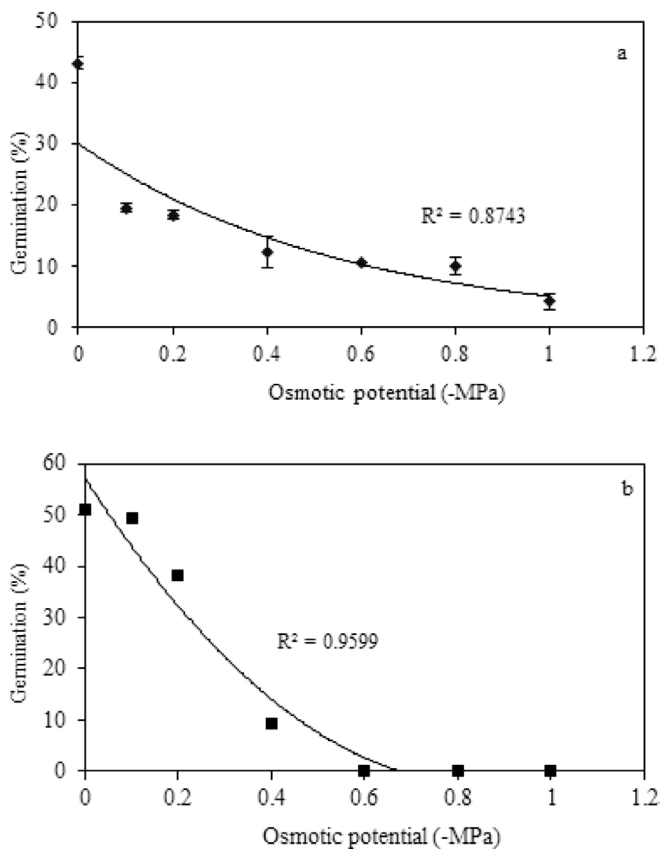


Figure 4. Impact of drought stress on Pokeweed (a) and Lamb's Ear (b) seed germination after 15 days of incubation at 25/15 °C light/dark temperatures and 12 hours photoperiod. Vertical bars represent standard errors of the means.

showed that seed germination of *Helianthus annuus* L. was observed at osmotic potential -1.2 MPa. No seed germination was observed in pepper (*Capsicum annum* L.) (Demir & Mavi, 2008) and *Tanacetum cinerariifolium* Sch.Bip. (Li et al., 2011) at osmotic potentials lower than -0.9 MPa.

Impact of salt stress

Seed germination of pokeweed significantly decreased as salt concentration increased. No seed germination was observed at 200 and 400 mM of NaCl concentration. Germination of seeds (17.27%) at 100 mM NaCl concentration indicated that pokeweed seed can germinate at moderate salinity conditions (Figure 5).

Saline stress negatively affected on pokeweed and Lamb's Ear germination. Germination of pokeweed seed occurred at moderate salinity conditions (Figure 5a). With increasing NaCl concentration, seed germination of Lamb's Ear reduced compared to control. Concentrations of 100 and 200 mM NaCl decreased the germination percentage about 13.1% and 79.16%,

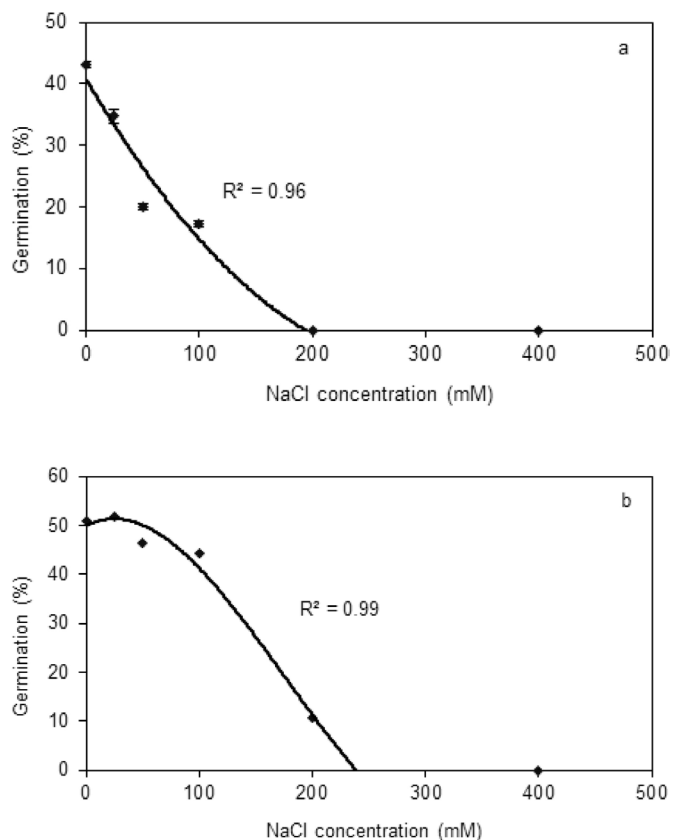


Figure 5. Impact of salt stress on Pokeweed (a) and Lamb's Ear (b) seeds germination after 15 days of incubation at 25/15 °C light/dark temperatures and 12 hours photoperiod. Vertical bars represent standard errors of the means.

respectively (Figure 5b). Germination did not occur in 300 and 400 mM NaCl (Figure 5b). Salinity stress were decreased germination percentage of *Parthenium argentatum* A. Gray (Sanchez et al., 2014), *Chloris virgata* Sw. and *Digitaria sanguinalis* (L.) Scop. (Zhang et al., 2012), *T. cinerariifolium* (Li et al., 2011), *Cucumis sativus* L. (Chang et al., 2010), *Salsola vermiculata* L. (Guma et al., 2010). No germination was observed in *Prosopis juliflora* (Sw.) D.C. seeds at 400 mM of NaCl (El-Keblawy & Al-Rawai, 2005). There is a variation between plants in salt tolerance for germination. Species with larger seed size are more successful than smaller ones for germination in high salinity levels (Easton & Kleindorfer, 2009).

Impact of pH

Effect of pH was significant on seed germination of Pokeweed and Lamb's Ear (Table 1). The maximum seed germination for Pokeweed (43.05%) was observed at pH 7 while reducing and/or increasing in pH from 7 decreased germination of pokeweed (Figure 6a). No seed germination

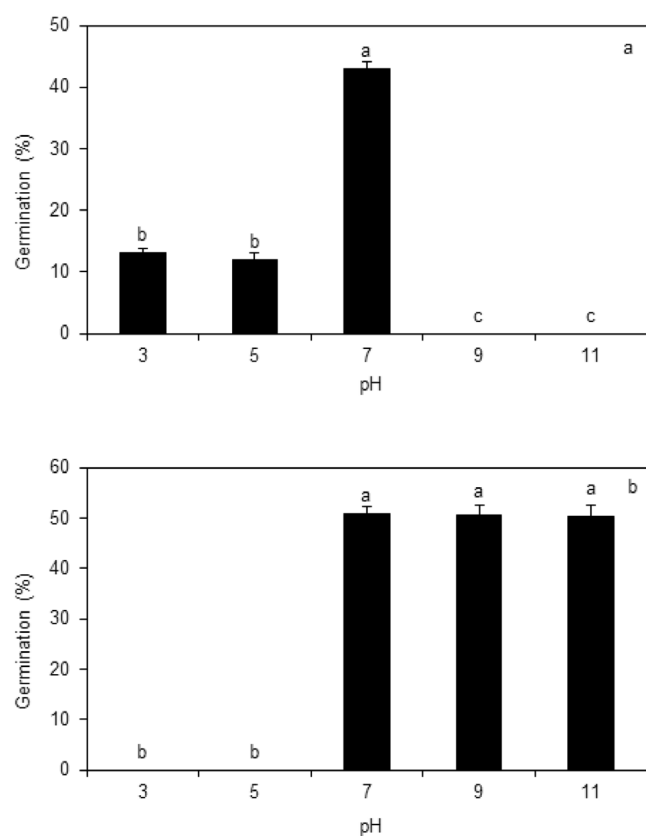


Figure 6. Impact of pH on Pokeweed (a) and Lamb's Ear (b) seed germination after 30 days of incubation at 25/15 °C light/dark temperatures and 12 hours photoperiod. Columns with the same letter are not significantly different. Vertical bars represent standard errors of the means.

was observed in pH 9 and 11. In Pokeweed, 13.18% and 12% of seeds germinated at pH 3 and 5, respectively (Figure 6a). The maximum Lamb's Ear seed germination was recorded at pH 7 to 11. No lamb's Ear seed germinated in pH 3 to 5 (Figure 6b). Our results showed that pH significant influenced on seed germination pokeweed and lamb's Ear. Our results suggest that no seed germination at pH lower and/or higher than 7 could be due to induction of secondary seed dormancy the plants. Soil pH plays an important role in seed dormancy breaking and germination (Pérez-Fernández et al., 2006). Goubitz et al. (2003) found that high pH negatively influenced on seed germination of *Pinus halepensis* Mill. In contrast, Killi (2004) proved germination enhancement of *Gossypium* spp. by pH enhancement.

Impact of planting depth on seedling establishment

Seedling emergence markedly impacted by planting depth (Table 1). With increasing of planting depth of both Pokeweed and Lamb's Ear, seedling establishment significantly decreased (Figure 7a and 7b). The maximum seedling emergence in Pokeweed and Lamb's Ear were 62.73% and 45.93% for seeds planted on the soil surface, respectively (Figure 7a, b). The minimum seedling emergence of pokeweed (12%) was observed at 12 cm sowing depth (Figure 7a).

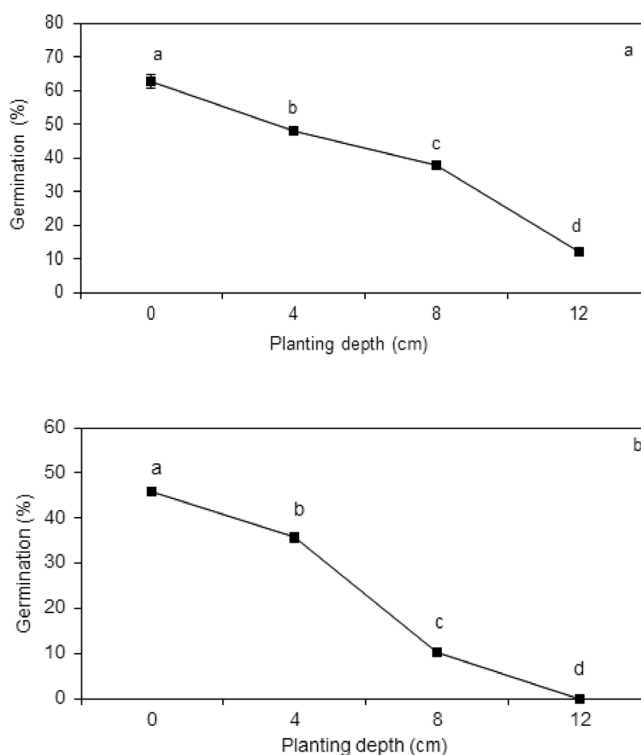


Figure 7. Effect of planting depth on Pokeweed (a) and Lamb's Ear (b) seedling emergence. Vertical bars represent standard errors of the means.

No seedling emergence of Lam's Ear was occurred at 12 cm planting depth (Figure 7b). The high percentage of seedlings emergence in soil surface shows that these seeds need to light for germination; therefore, these plants could be problematic in no-till and/or reduced tillage systems (Bolfrey-Arku et al., 2011). Cumulative germination percentages of *Ricinodendron heudelotii* (Baill.) Heckel seeds reduced with increasing in sowing depths (Anjah et al., 2013). *Rumex obtusifolius* L. seedlings did not emerge from seeds planted at depth of higher than 8 cm (Benvenuti et al., 2001). Seed of *Veronica hederifolia* L. retrieved from the soil surface germinated well initially, but germination decreased as depth of burial increased (Mennan & Zandstra, 2006). Using some agro-techniques including a shallow tillage and then herbicide for destroying seedlings and deep tillage after herbicide application in order to bury seeds can reduce presence of the weeds in the next crop (Bolfrey-Arku et al., 2011). Our result suggests that tillage deeper than 12 cm can be effective on reduction of seedling emergence of both weeds in arable field.

CONCLUSIONS

Gibberellic acid, scarification and leaching duration were not markedly effective in dormancy breaking of both Pokeweed and Lamb's Ear. The germination of pokeweed was tolerant to water deficit comparing to Lamb's Ear. Lamb's Ear germinated in soil with high salinity while Pokeweed seed germination reached to zero at moderately salt stress. According our results soil pH can be considered as a limiting factor for germination of Pokeweed and Lamb's Ear seeds. It seems adding lime to acidic soil could be an effective approach to reduction pokeweed growth. However, in areas with high pH of soil can be effective in the growth of Lamb's Ear by adding sulfur in order to reduce soil pH. Information obtained from the effect of salt and osmotic stress and pH could help us to predict the distribution of Pokeweed and Lamb's Ear in new regions. According to our results, Pokeweed seed is greater and heavier than Lamb's Ear seed. Therefore, Pokeweed seed might be buried deeper than Lamb's Ear. Also, seed germination of Pokeweed at a depth of 12 cm indicates the ability of the plant in order to escape from control with pre-emergence herbicides. The emergence of seedlings decreased with increasing planting depth in both plants which may be depending on the stored energy in seeds. The main reason of lack of seedling emergence in greater depth could be due to induction of the secondary dormancy. Also, mortality of seeds in high depth could be important in reduction of seedling emergence. Knowledge gained from this research will assist us to predict the distribution areas and development of effective strategies for management of Pokeweed and Lamb's Ear in the future.

REFERENCES

- Anjah G.M., Focho A.D., Dondjang J.P., 2013. The effects of sowing depth and light intensity on the germination and early growth of *Ricinodendron heudelotii*. *African Journal Agricultural Research* 8, 5854-5858.
- Armesto J.J., Cheplick G.P., McDonnell M.J., 1983. Observations on the reproductive biology of *Phytolacca americana* (*Phytolaccaceae*). *Bulletin of the Torrey Botany Club* 110, 380-383.
- Benvenuti S., Macchia M., Miele S., 2001. Light, temperature and burial depth effects on *Rumex obtusifolius* seed germination and emergence. *Weed Research* 41, 177-186.
- Bolfrey-Arku G.E.K., Chauhan B.S., Johnson D.E., 2011. Seed germination ecology of Itchgrass (*Rottboellia cochinchinensis*). *Weed Science* 59, 182-187.
- Buijs G.A. 2020. Perspective on secondary seed dormancy in *Arabidopsis thaliana*. *Plants* 9(6) 749.
- Chachalis D., Reddy K.N., 2000. Factors affecting *Campsis radicans* seed germination and seedling emergence. *Weed Science* 48, 212-216.
- Chang C., Wang B., Shi L., Li Y., Duo L., Zhang W., 2010. Alleviation of salt stress-induced inhibition of seed germination in Cucumber (*Cucumis sativus* L.) by ethylene and glutamate. *Journal of Plant Physiology* 167, 1152-1156.
- Demir I., Mavi K., 2008. Effect of salt and osmotic stresses on the germination of Pepper seeds of different maturation stages. *Brazilian Archives of Biology and Technology* 51, 897-902.
- Ding L.J., Ding W., Zhang Y.Q., Luo J.X., 2012. Bio-guided fractionation and isolation of esculentoside P from *Phytolacca americana* L. *Industrial Crops and Production* 44, 534-541.
- Dunn A.J., 1997. *Stachys germanica* L. *Journal of Ecology* 85, 531-539.
- Easton L.C., Kleindorfer S., 2009. Effects of salinity levels and seed mass on germination in Australian species of *Frankenia* L. (*Frankeniaceae*). *Environmental and Experimental Botany* 65, 345-352.
- El-Keblawy A., Al-Rawai A., 2005. Effects of salinity, temperature and light on germination of invasive *Prosopis juliflora* (Sw.) D.C. *Journal of Arid Environment* 61, 555-565.
- Farmer R.E., Hall G.C., 1970. Pokeweed seed germination: effects of environment, stratification and chemical growth regulators. *Ecology* 51, 894-898.

- Footitt S., Walley P.G., Lynn J.R., Hambidge A.J., Penfield S., Finch-Savage W.E., 2019. Trait analysis reveals DOG1 determines initial depth of seed dormancy, but not changes during dormancy cycling that result in seedling emergence timing. *New Phytologist* 225, 2035-2047.
- Glasgow L.S., Matlack G.R., 2007. Prescribed burning and understory composition in a temperate deciduous forest, Ohio, USA. *Forest Ecology and Management* 238, 54-64.
- Goubitz S., Werger M.J.A. Ne'eman G., 2003. Germination response to fire related factors of seeds from non-serotinous and serotinous cones. *Plant Ecology* 169, 195-204.
- Guleryuz G., Kirmizi S., Arslan H., Sakar F.S., 2011. Dormancy and germination in *Stachys germanica* L. subsp. *Bithynica* (Boiss.) Bhattacharjee seeds: effects of short-time moist chilling and plant growth regulators. *Flora* 206, 943-948.
- Guma I.R., Padron-Mederos M.A., Santos-Guerra A., Reyes-Betancort J.A., 2010. Effect of temperature and salinity on germination of *Salsola vermiculata* L. (*Chenopodiaceae*) from Canary Islands. *Journal of Arid Environment* 74, 708-711.
- Henedina A, Ramirez M., Jhala A.J., Singh M., 2014. Factors affecting germination of Citronmelon (*Citrullus lanatus* var. *citroides*). *Weed Science* 62, 45-50.
- ISTA., 1985. International rules for seed testing. *Seed Science Technology* 13, 307-513.
- Kaya M.D., Okcu G., Atak M., Cikili Y., Kolsarici O., 2006. Seed treatments to overcome salt and drought stress during germination in Sunflower (*Helianthus annuus* L.). *European Journal of Agronomy* 24, 291-295.
- Killi F., 2004. Effects of potassium humate solution and soaking periods on germination characteristics of undelinted Cotton seeds (*Gossypium hirsutum* L.). *Journal of Environmental Biology* 25, 395-398.
- Krochmal A., 1970. Germinating Pokeberry seed (*Phytolacca americana*). Forest Service, U.S. Dept. of Agriculture, Berea, Kentucky, USA.
- Li J., Yin L.Y., Jongsma M.A., Wang C.Y., 2011. Effects of light, hydropriming and abiotic stress on seed germination, and shoot and root growth of Pyrethrum (*Tanacetum cinerariifolium*). *Industrial Crops and Production* 34, 1543-1549.
- Mennan H., Zandstra B.H., 2006. The effects of depth and duration of seed burial on viability, dormancy, germination, and emergence of Ivy leaf speedwell (*Veronica hederifolia*). *Weed Technology* 20, 438-444.
- Nadjafi F., Bannayan M., Tabrizi L., Rastgoo M., 2006. Seed germination and dormancy breaking techniques for *Ferula gummosa* and *Teucrium polium*. *Journal of Arid Environment* 64, 542-547.
- Okcu G., Kaya M.D., Atak M., 2005. Effects of salt and drought stresses on germination and seedling growth of Pea (*Pisum sativum* L.). *Turkish Journal of Agriculture and Forestry* 29, 237-242.
- Patané C., Gresta F., 2006. Germination of *Astragalus hamosus* and *Medicago orbicularis* as affected by seed-coat dormancy breaking techniques. *Journal of Arid Environment* 67, 165-173.
- Pérez-Fernández M.A., Calvo-Magro E., Montanero-Fernández J., Oyola-Velasco J.A., 2006. Seed germination in response to chemicals: effect of nitrogen and pH in the media. *Journal of Environmental Biology* 27, 13-20.
- Peters J., 2000. Association of Official Seed Analysis Tetrazolium Testing Handbook, Contribution No. 29, 1st Revision, Association of Official Seed Analysis, Lincoln, NE, USA.
- Pinfield N.J., Martin M.H., Stobart A.K., 1972. The control of germination in *Stachys alpina* L.. *New Phytologist* 71, 99-104.
- Ravikiran G., Raju A.B., Venugopal Y., 2011. *Phytolacca americana*: a review. *International Journal of Research in Pharmaceutical and Biomedical Science* 2, 942-946.
- Rezvani M., Sadatian S.A., Nikkhahkouchaksaraei H., 2018. Factors affecting seed dormancy and germination of Greater bur-parsley (*Turgenia latifolia*). *Planta Daninha* 36:e018172841.
- Rezvani M., Taghinia N., Nikkhahkouchaksaraei H., 2017. Study of seed germination ecology of rye grass (*Lolium perenne* L.) and goat grass (*Aegilops cylindrica* Host.). *Thai Journal of Agricultural Science* 50, 146-154.
- Rezvani M., Zaefarian F., 2016. Hoary cress (*Cardaria draba* (L.) Desv.) seed germination ecology, longevity and seedling emergence. *Plant Species Biology* 31, 280-287.
- Rezvani M., Fani Yazdi S.A., 2013. Factors affecting seed germination of Black nightshade (*Solanum nigrum*). *Acta Botanica Hungarica* 55, 397-408.
- Rezvani M., Zaefarian F., Amini V., 2014. Effect of chemical treatments and environmental factors on seed dormancy and germination of Shepherd's purse (*Capsella bursapastoris* (L.) Medic.). *Acta Botanica Brasilica* 28, 495-501.

Salimi F., Shafaghat A., Sahebalzamani H., Habibzadeh H., 2011. Analysis and comparison of chemical composition of essential oils from *Stachy byzantina* C. Koch. wet and dried. *Archives of Applied Science Research* 3, 381-383.

Sanchez P.L., Chen M.K., Pessarakli M., Hill H.J., Gore M.A., Jenks M.A., 2014. Effects of temperature and salinity on germination of non-pelleted and pelleted guayule (*Parthenium argentatum* A. Gray) seeds. *Industrial Crops and Products* 55, 90-96.

Scippa G.S., Petrollini E., Trupiano, D., Rocco M., Falco G., Di Michele M., Chiatante D., 2011. Dormancy of *Medicago marina* (L.) seed. *Environmental and Experimental Botany* 72, 320-329.

Zhang H., Irving L.J., Tian Y., Zhou D., 2012. Influence of salinity and temperature on seed germination rate and the hydro-time model parameters for the halophyte, *Chloris virgata*, and the glycophyte, *Digitaria sanguinalis*. *South Africa Journal of Botany* 78, 203-210.