

SEED DORMANCY BREAKING TREATMENTS FOR AFRICAN PURSLANE (*Zaleya pentandra*)¹

Tratamentos de Quebra de Dormência de Sementes para Zaleya pentandra

MUNAWAR, S.², NAEEM, M.², ALI, H.H.⁴, JAMIL, M.², IQBAL, M.², NAZIR, M.Q.³, BALAL, R.M.⁴,
and SAFDAR, M.E.⁴

ABSTRACT - Understanding the mechanisms involved in releasing seed dormancy is crucial for effective plant management and renewal of species in the arid zone. *Zaleya pentandra* is an emerging invasive weed of the arid areas of Pakistan. We investigated the effects of different dormancy breaking treatments on the germination of *Z. pentandra* seeds. Seeds were treated with hot water (by placing them in boiling water for 5, 15, 30, 60, 90, 120, and 150 min), dry heat (by placing them in a preheated oven at 70 °C for 1, 2, and 4 hours; at 70 °C for 1, 2, 3, and 4 days, and at 200 °C for 5, 10, 15, 30, and 45 min) and stratification (by placing them at 2-5 °C in a refrigerator for 5, 10, 30, and 60 min; for 3, 6, and 12 hours, and for 1, 2, 4, 8, 15, and 30 days). Seeds also were soaked in thiourea ($[(\text{NH}_2)_2\text{CS}]$) (0, 2,500, 5,000, 7,500, and 10,000 mg L⁻¹ for 24 h at 30 °C) and in KNO₃ (0, 10,000, 20,000, 30,000, 40,000, 50,000, and 60,000 mg L⁻¹ for 24 h at 30 °C). Additionally, seeds were scarified with HCl (for 3, 6, 9, 12, 15, 18, and 21 h), HNO₃ (for 3, 6, 9, 12, 15, 18, and 21 h), and H₂SO₄ (for 20, 40, 60, 80, 100, and 120 min at 30 °C) and also mechanically scarified with sandpaper. *Zaleya pentandra* seeds showed typical signs of hard seed coat dormancy. Mechanical scarification and acid treatments promoted seed germination to a varying degree. Seed scarification with HNO₃ for 12 to 18 h as well as with HCl for 12 h and 15 h was efficient in breaking dormancy of *Z. pentandra* seeds, providing germination up to 92.5%. Seed scarification with H₂SO₄ from 20 to 120 min showed little effect, whereas hot water, dry heat, stratification and various concentrations of thiourea and KNO₃ were ineffective in breaking *Z. pentandra* seed dormancy.

Keywords: germination, scarification, stratification, dry heat, thiourea, arid zone.

RESUMO - Conhecer os mecanismos envolvidos na liberação da dormência de sementes é fundamental para o manejo da planta e recuperação de espécies na zona árida. A *Zaleya pentandra* é uma erva invasora emergente das áreas áridas do Paquistão. Neste estudo, os efeitos dos diferentes tratamentos da quebra de dormência foram investigados acerca da germinação de sementes de *Z. pentandra*. As sementes foram tratadas com água quente (colocadas em água fervendo por 5, 15, 30, 60, 90, 120 e 150 minutos), calor seco (colocadas em forno pré-aquecido a 70 °C por 1, 2, e 4 horas; a 70 °C por 1, 2, 3, e 4 dias e a 200 °C por 5, 10, 15, 30 e 45 min) e estratificação (colocadas num frigorífico de 2 a 5 °C por 5, 10, 30 e 60 min, por 3, 6 e 12 horas e por 1, 2, 4, 8, 15 e 30 dias). As sementes foram também inseridas em tioureia ($[(\text{NH}_2)_2\text{CS}]$) (0, 2.500, 5.000, 7.500, e 10.000 mg L⁻¹ por 24 ha 30 °C) e em KNO₃ (0, 10.000, 20.000, 30.000, 40.000, 50.000, and 60.000 mg L⁻¹ por 24 ha 30 °C). Além disso, as sementes foram escarificadas com HCl (por 3, 6, 9, 12, 15, 18, e 21 h), HNO₃ (por 3, 6, 9, 12, 15, 18, e 21 h), e H₂SO₄ (por 20, 40, 60, 80, 100 e 120 minutos a 30 °C) e também escarificada mecanicamente com lixa. As sementes de *Zaleya pentandra* demonstraram sinais típicos de dormência do revestimento duro da semente. Os tratamentos mecânicos com escarificação e ácido promoveram a germinação da semente, em grau variável. A escarificação das

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² University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan. ³ Department of Forestry, Range Management and Wildlife, University of Agriculture, Faisalabad, Pakistan; ⁴ University College of Agriculture, University of Sargodha, Sargodha, Pakistan, <haider3993@gmail.com>.



sementes em HNO_3 durante 12 a 18 h, bem como em HCl durante 12 h e 15 h, foi eficiente na quebra da dormência das sementes de *Z. pentandra*, proporcionando germinação de até 92,5%. A escarificação com H_2SO_4 de 20 a 120 min. surtiu pouco efeito, enquanto que a água quente, calor seco, a estratificação e várias concentrações de tiourea e KNO_3 foram ineficazes para quebrar a dormência da semente de *Z. pentandra*.

Palavras-chave: germinação, escarificação, estratificação, calor seco, tiourea, zona árida.

INTRODUCTION

Weeds are main constraints of crop production and one of the main reasons of yield losses in producing food and fiber crops (Malik et al., 2000; Mansoor et al., 2004). Weeds can grow in totally different settings (e.g. agricultural, urban, and natural) owing to certain characteristics, such as seed dormancy, which prevents germination when the probability of seedling survival is low, thus facilitating weed species adaptability (Roberto et al., 2000). Seed dormancy, a seemingly inactive or resting condition of the seed, slows down or stops seed germination of weeds over long time periods. Dormant weed seeds in the soil permit weeds to escape or stay away from exposure to control practices that target emerging and emerged weed seedlings (Ali et al., 2011). Radosevich et al. (1997) reported that one of the most important processes determining the emergence of a weed population under field conditions is most probably changes in seed dormancy levels.

Zaleya pentandra belongs to the family Aizoaceae, which consists of about 127 genera and 2,500 species (Schweingruber, 2011). The plant is believed to be native of Africa and from this continent it spread to Palestine, Arabian Peninsula, Iran, Pakistan, India (Akbar & Khatoon, 2012), and the Farasan islands, as well as Madagascar, Zambia, Zimbabwe, Malawi, Mozambique, and South Africa (Gonçalves, 1970). *Zaleya pentandra* is a prostrate annual herb, continuously invading cultivated areas of southern Punjab in Pakistan. It is a bit succulent and a multi-branched plant, with pubescent stem. Leaves differ in size and shape; usually they are elliptic to oblanceolate and opposite, generally 1-2 cm in length, with a slightly grey tinge on the surface along with dark green color, and more or less papilose (Alfarhan et al., 2005).

The period of flowering in Pakistan is from October to December (Akbar & Khatoon, 2012). The plant is found as a weed mainly in winter crops, along road sides, in overgrazed areas as well as in grasslands. Seed dormancy is a significant factor for the success of this species and it is considered the cause of its increasing infestations in arid agro-ecosystems of southern Punjab in Pakistan.

The relationship between seed dormancy and the success of a plant as a weed is considerable (Ali et al., 2012). The weed seeds vary extensively with respect to degree, duration, and cause of dormancy. Survival of large number of weed seeds with varying degrees of dormancy is the main cause for many weed problems every year (Ali et al., 2011). Seed dormancy owing to the presence of impervious layers of the palisade cells could be released by weakening the tegument, therefore allowing water to pass through the layers and enable germination (Cavalheiro et al., 2007). This succession can also occur when seeds pass through an animal digestive system by the action of the gastric acids (Goddard et al., 2009). Under natural conditions, release of dormancy imposed by the seed coat requires the interaction of a number of biological and physiological dormancy-breaking procedures (Kelly et al., 1992).

Weed control is a crucial part of an efficient crop production system, which can be facilitated by new methods to break seed dormancy (Gu et al., 2004). It is important to predict the weed seed dormancy in terms of timing and degree of weed emergence under field conditions to develop appropriate weed management approaches. Changes in the intensities of weed seed dormancy may delay seed germination in the soil profile over many cropping seasons (Ali et al., 2012). Therefore, weed seedlings continue to emerge from the

soil under field conditions even after comprehensive weed management practices are implemented.

No information is available in the international literature about the seed dormancy characteristics of *Z. pentandra*. Thus, this present study was conducted to assess characteristics of dormancy of *Z. pentandra* seeds. In particular, the purpose of this study was to determine the effects of hot water, dry heat, stratification, and scarification on the release of dormancy in *Z. pentandra* seeds.

MATERIALS AND METHODS

Collection of seeds for the experiments

Mature plants of *Z. pentandra* were collected from Islamia University of Bahawalpur, southern Punjab, Pakistan in November 2013. After collection of plants, seeds were separated and the undesired material and unripe/damaged seeds were removed. Healthy seeds [weighed in lots of 100 seeds (mean \pm standard error, $0.115 \text{ g} \pm 0.0025$)] were kept under room temperature ($28 \text{ }^\circ\text{C} \pm 2$) for a period of 7 days. Viability of the seeds was confirmed through the tetrazolium salt test. Only mature and uniform in size seeds were used for the germination experiments.

Experiment 1 (dry heat treatment)

Seeds of *Z. pentandra* were placed in shallow containers in a preheated oven according to given temperature and duration, i.e. $70 \text{ }^\circ\text{C}$ for 1, 2, and 4 hours; $70 \text{ }^\circ\text{C}$ for 1, 2, 3, and 4 days and $200 \text{ }^\circ\text{C}$ for 5, 10, 15, 30, and 45 min. After each treatment, the seeds were immediately cooled and placed for germination.

Experiment 2 (hot water treatment)

Seeds of *Z. pentandra* were placed in boiling water for 5, 15, 30, 60, 90, 120, and 150 min. After the prescribed period, seeds were removed immediately from the boiling water and kept at room temperature (at about $30 \text{ }^\circ\text{C}$) to cool down before placing them for germination.

Experiment 3 (cold treatment)

Seeds of *Z. pentandra* were placed in a tightly closed glass jar and stored in a refrigerator at a temperature of $2\text{-}5 \text{ }^\circ\text{C}$ for 5, 10, 30, and 60 min; 3, 6, and 12 hours, and 1, 2, 4, 8, 15, and 30 days.

Experiment 4 (chemical treatment)

Seeds of *Z. pentandra* were soaked in different concentrations of potassium nitrate (KNO_3) (0, 10,000, 20,000, 30,000, 40,000, 50,000, and $60,000 \text{ mg L}^{-1}$) and thiourea [$(\text{NH}_2)_2\text{CS}$] (0, 2,500, 5,000, 7,500, and $1,000 \text{ mg L}^{-1}$) for 24 h at $30 \text{ }^\circ\text{C}$.

Experiment 5 (acid treatment)

Seeds of *Z. pentandra* were soaked separately in HNO_3 (65%) for 3, 6, 9, 12, 15, 18, and 21 h; in HCL (36%) for 3, 6, 9, 12, 15, 18, and 21 h as well as in sulphuric acid (98%) for 20, 40, 60, 80, 100, and 120 min at $30 \text{ }^\circ\text{C}$ in addition to being rubbed against the coarse surface of a sandpaper. Immediately after the prescribed soaking period in the different acids, seeds were removed and rinsed several times with distilled water. Untreated seeds were used as control.

Germination test

After rinsing, seeds were dried on blotter paper at the laboratory at a temperature of $30 \text{ }^\circ\text{C}$ prior to being placed in the Petri dishes in the above mentioned experiments. The seeds were surface sterilized by soaking in a 5% sodium hypochlorite (NaOCl) solution for 5 min and then rinsed five times with sterilized water. After sterilization, the seeds were placed on single-layered Whatman No 10 filter paper moistened with 10 mL of distilled water in sterilized 15 cm-diameter Petri dishes. Afterwards, all the dishes were sealed with a strip of parafilm to decrease water loss and placed at $30/18 \text{ }^\circ\text{C}$ in a germinator. Every experiment was arranged in a completely randomized design with four replicates (Petri dishes) and 25 seeds per Petri dish. Germination counts were made every day for 3 wk. A seed was considered germinated when the tip of the radical (2 mm)



had grown free of the seed. Every experiment was carried out twice and statistical analysis was performed over the two runs. Data regarding Germination index (GI), Germination percentage (GP), (Association of Official Seed Analysis, 1990), Time to 50% germination (T_{50}) (Coolbear et al., 1984), and Mean germination time (MGT) (Ellis & Roberts, 1981) were determined and then were analyzed statistically using one-way analysis of variance (ANOVA) of the MSTAT statistical computer package. The least significant difference (LSD) at 5% probability was used to compare the treatment means (Steel et al., 1997).

RESULTS AND DISCUSSION

Dry heat, hot water, and stratification seed treatment

Seed treatments with dry heat, hot water, and stratification showed no effect on breaking dormancy; thus, data for these treatments are not presented. The non-germinated seeds with these treatments were hard and viable, except from the dry heat treatments in the oven at 200 °C. All the non-germinated seeds with these treatments germinated successfully only when scarified with sandpaper. Rigorous heat applied to seeds may rupture their hard coats or may soften their waxy coverings (Tarrega et al., 1992). However, in our study a range of dry heat treatments had no effect on breaking dormancy of *Z. pentandra* seeds. Seed treatments with hot water were found to improve germination of hard seed coat species by uplifting water and O₂ permeability of the testa (Teketay, 1998; Aydýn & Uzun, 2001). However, in our study, various hot water seed treatments failed to promote *Z. pentandra* seed germination. The response of *Z. pentandra* seeds to a range of stratification treatments was similar to that reported by Susko et al. (2001) in kudzu [*Pueraria lobata* (Willd.) Ohwi]. They reported that keeping kudzu seeds at 5 °C for 0-6 weeks did not affect seed germination.

Seed treatment with thiourea and KNO₃

Seeds of *Z. pentandra* showed no response to various concentrations of thiourea and

KNO₃, probably because thiourea and KNO₃ failed to crack the seed coat and thus to facilitate imbibition. Seeds after the prescribed soaking treatments were hard and viable, and they successfully germinated only when scarified with sandpaper.

Scarification with HNO₃ and sandpaper

The treatment with sandpaper was very effective in breaking seed dormancy (Table 1). The germination of seeds that were mechanically scratched with sandpaper significantly increased to 100% when compared to HNO₃ treatments. In addition, seeds that were mechanically scarified with sandpaper showed the minimum time to 50% germination (0.9 d) and MGT (2.1 d) when compared to all other treatments. When seeds were treated with HNO₃ (36%) for 3, 6, 9, 12, 15, 18, and 21 h, seed germination significantly increased ($P < 0.05$) over control (Table 1). Seeds treated with HNO₃ for 12, 15, and 18 h showed the minimum response time, with 50% of the seeds germinating in all the replicates within 2.0, 2.0, and 2.1 d, respectively. Minimum MGT (2.6 to 3.0 d) was detected in seeds treated with HNO₃ for a period of 12 to 18 h. Seeds treated with HNO₃ for 6 or 9 h had a significantly higher MGT than the other treatments. Sandpaper scarification had maximum GI value (7.7). No germination was found in the control treatment.

Table 1 - Effect of seed scarification with HNO₃ and sandpaper on germination parameters of *Zaleya pentandra*

Treatment	Germination (%)	T ₅₀ (d)	MGT (d)	GI
Control	0.0f	0.0e	0.0 e	0.0 f
HCL (3 h)	37.5 e	1.6 bc	2.5 c	1.6 e
HCL (6 h)	50.0 d	2.2 a	3.3 a	1.9 de
HCL (9 h)	60.0 cd	2.1 a	3.3 a	2.6 d
HCL (12 h)	75.0 b	2.0 ab	3.0 b	3.7 c
HCL (15 h)	92.5 a	2.0 ab	2.6 c	5.3 b
HCL (18 h)	70.0 bc	2.1 a	2.9 b	4.8 b
HCL (21 h)	35.0 e	1.1 cd	1.9 d	2.5 d
Sandpaper	100.0 a	0.9 d	2.1 d	7.7 a
LSD _{0.05}	10.9	0.4	0.3	0.7

Means followed by the same letter in a column do not differ significantly according to LSD test ($P < 0.05$). T₅₀: Time needed for 50% germination; MGT: Mean germination time; GI: Germination index; LSD: Least significance difference.

Scarification with H₂SO₄

Soaking *Z. pentandra* seeds in H₂SO₄ from 20 to 120 min had little effect on seed germination when compared to other acid treatments (Table 2). Germination was slow and irregular, reaching a total percentage of no more than 20%. Percentages of seed germination in all H₂SO₄ treatments were better than control, but were very low (< 20%) without striking differences. Minimum time to 50% germination and MGT were recorded in seeds treated with H₂SO₄ for 120 min. The remaining seeds were hard and viable, and successfully germinated only when scarified with sandpaper.

Table 2 - Effect of seed scarification with H₂SO₄ on germination parameters of *Zaleya pentandra*

Treatment	Germination (%)	T ₅₀ (d)	MGT (d)	GI
Control	0.0c	0.0c	0.0d	0.0 c
H ₂ SO ₄ (20 min)	12.5 ab	0.6 ab	4.4 a	0.2 b
H ₂ SO ₄ (40 min)	17.5 a	0.8 a	3.9 ab	0.4 ab
H ₂ SO ₄ (60 min)	17.5 a	0.8 a	3.3 b	0.5 a
H ₂ SO ₄ (80 min)	15.0 a	0.7 a	3.5 ab	0.4 ab
H ₂ SO ₄ (100 min)	17.5 a	0.8 a	3.3 b	0.5 a
H ₂ SO ₄ (120 min)	7.5 b	0.3 b	2.0 c	0.3 b
LSD _{0.05}	6.9	0.3	0.9	0.2

Means followed by the same letter in a column do not differ significantly according to LSD test ($P < 0.05$). T₅₀: Time needed for 50% germination; MGT: Mean germination time; GI: Germination index; LSD: Least significance difference.

Scarification with HCL

Soaking *Z. pentandra* seeds in HCL for 3 to 21 h had pronounced effect on seed germination (Table 3). Minimum time to 50% germination and MGT were recorded in seeds treated with HCL for 3 h. The remaining seeds were hard and viable, and successfully germinated only when scarified with sandpaper.

Overall, the results of our study showed that *Z. pentandra* seeds exhibited dormancy due to their hard seed coat. Scarification is an important factor influencing seed germination of different species in different habitats. Mechanical constriction, comprising prevention of water and oxygen uptake by the

Table 3 - Effect of seed scarification with HCL on germination parameters of *Zaleya pentandra*

Treatment	Germination (%)	T ₅₀ (d)	MGT (d)	GI
Control	0.0 e	0.0 d	0.0 c	0.0g
HCL (3 h)	17.5 d	0.8 c	4.0 a	0.4 fg
HCL (6 h)	25.0 d	1.5 b	4.3 a	0.6 ef
HCL (9 h)	35.0 c	2.7 a	4.1 a	1.5 d
HCL (12 h)	65.0 b	2.2 a	3.8 a	3.6 b
HCL (15 h)	92.5 a	1.3 bc	2.9 b	5.9 a
HCL (18 h)	35.0 c	1.5 b	2.9 b	2.1 c
HCL (21 h)	22.5 d	1.2 bc	2.8 b	1.1 de
LSD _{0.05}	8.5	0.5	0.6	0.6

Means followed by the same letter in a column do not differ significantly according to LSD test ($P < 0.05$). T₅₀: Time needed for 50% germination; MGT: Mean germination time; GI: Germination index; LSD: Least significance difference.

seed, and retention or even production of chemical inhibitors are possible mechanisms causing strong inhibitory effect of the seed coat on germination (Taiz & Zeiger, 2002). Seed dormancy is one of the most important mechanisms of viability in plants. Generally, seed dormancy is seen little in plants that are domesticated from ancient times compared to wild and native species. Water absorption, enzymatic activity, embryo growth, seed coat rupture and plant growth are important steps of germination (Schmidt, 2002). Each desert plant species has its own set of mechanisms that allow it to start under a broad variety of conditions. Acid scarification proved to be highly effective in improving germination of species with hard seed coats (Shaltout et al., 1989). In this present study, breaking the impermeability of the seed coat by scarification methods resulted in a substantial increase in the germination percentage (from 10 to 100%) of *Z. pentandra* seeds. The different chemicals (thiourea and KNO₃) and acids (HCL, HNO₃, and H₂SO₄) have been widely used for breaking dormancy of many hard seed coat species, such as European milkvetch (*Astragalus hamosus*), black-disk medick (*Medicago orbicularis*) (Patane & Gresta, 2006), and *Albizia* spp. (Tigabu & Oden, 2001). In the current study, the superlative treatments to release hard seed coat dormancy that caused the maximum germination percentages were seed scarification with sandpaper, HNO₃ and HCL. Mechanical scarification



(sandpaper) provided quick germination when compared to the untreated (control) seeds and absolutely overcame seed coat impermeability. Scarification with sandpaper also provided 100% germination, with the minimum time to 50% germination (0.9 d) and MGT (2.1 d) when compared to all other treatments. Germination of seeds treated with HCl increased when compared to control, but only over an extended period of treatment (i.e., up to 15 h), demonstrating a slow release of seed dormancy. These results are in line with those of Goddard et al. (2009) who found that benghal dayflower (*Commelina benghalensis*) seeds that were subjected to HCl soaking treatments effectively germinated with little loss of viability after each treatment. Seeds from the 21 h treatment were highly soft and moldy at the end of the germination test; so, very low germination was recorded in this treatment. Similarly, total germination of the HNO₃ treated seeds (up to 15 h) increased, while the minimum germination was recorded after treatment with HNO₃ for 21 h. This was the minimum germination when compared to other acid treatments by H₂SO₄, with maximum germination percentages from 10 to 20%. A gradual increase in the germination percentage and GI values, but decrease in MGT and time to 50% germination with an increase in the soaking time of seeds in HCl from 3 to 15 h and with an increase in the soaking time of seeds in HNO₃ for 3 to 18 h revealed that sandpaper, HCl and HNO₃ were sufficient to break the hard seed coat of *Z. pentandra* seeds and induce germination. The decline in the germination rate at 21 h soaking in HNO₃ and 18 to 21 h soaking in HCl was the result of the damaging effect to the seed embryo due to the prolonged soaking time. Thiourea is known to stimulate germination by dropping the preventive effect of the seed coat in sweet cherry (*Prunus avium*) seeds (Çetinbas & Koyuncu, 2006). Likewise, KNO₃ was found effective in breaking dormancy of many species (Previero et al., 1996), and it has been stated as being a growth-regulating substance in *Salvia* species (Yücel, 2000). However, both these chemicals were unable to break dormancy in *Z. pentandra* seeds in this present study. This could be due to the excessively hard seed coat of this species (*Z. pentandra*).

Our experiments indicated that the success of this species is largely attributed to the occurrence of seed dormancy, which allows the seed to persist for long periods in the soil and thus escape the effects of post-germination weed control measures.

In general, the results of these experiments showed that *Z. pentandra* seeds exhibited hard seed coat dormancy. Softening the seed coat by soaking the seeds in acids (HNO₃, HCl, and H₂SO₄) significantly increased seed germination. Mechanical scarification (sandpaper) was found to be the best non-chemical treatment to overcome this coat-imposed dormancy in the seeds of this species. Given that treatments to induce *Z. pentandra* seed germination in this study were ones that could effectively break the seed coat, it can be concluded that the seed coat was the major barrier to *Z. pentandra* seed germination.

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