

**METHODS TO BREAK SEED DORMANCY OF *Rhynchosia capitata*, A SUMMER ANNUAL WEED**
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Dormancy of weed seeds is a significant feature contributing to their survival rate since it helps the weeds to avoid herbicides and other weeding practices along with unfavorable environmental conditions. We investigated the effects of different dormancy breaking treatments on the germination of *Rhynchosia capitata*, a common summer annual weed, which is emerging as a weed threat in Pakistan. Seeds were soaked in thiourea, KNO<sub>3</sub>, HCl, HNO<sub>3</sub>, and H<sub>2</sub>SO<sub>4</sub>, and they were also mechanically scarified (sandpaper). Results indicated that *R. capitata* seeds show signs of physical dormancy that is mainly due to the impermeability of their coat. Mechanical scarification and acid scarification (soaking of seeds in H<sub>2</sub>SO<sub>4</sub> for 60 and 80 min and in HCl for 12 and 15 h) were very efficient in breaking dormancy and promoting germination. Seed soaking in HNO<sub>3</sub> for 1 to 5 d showed little effect whereas various concentrations of thiourea and KNO<sub>3</sub> were ineffective in breaking *R. capitata* seed dormancy.

**Key words:** HCl, HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, dormancy, germination.

The genus *Rhynchosia* is widely distributed among several species occurring in the mountainous regions of the tropics. Basic similarities of various species of *Rhynchosia* are pinnate leaves, compressed pods, and non-accescent calyx (Adinarayana *et al.*, 1985). *Rhynchosia capitata*, a member of the *Fabaceae* family, is indigenous to Pakistan (Jahan *et al.*, 1994), India (Dogra *et al.*, 2009), and Sri Lanka (ILDIS, 2010). It has invaded the cultivated areas of Southern Punjab in Pakistan and is making things difficult for farmers.

In the field, the plant appears through the seed just after irrigation; it is an annual twinning prostrate plant with many branches spreading all around the root stock and rooting at every node. An approximately 1-mo-old plant starts flowering with oval-shaped pods with two seeds in each pod. Seeds are spherical and usually brown in color. Seeds mature within 3 mo when the plants also starts to dry (Sharma *et al.*, 1978). The growing season is from May to October with minimum and maximum average temperatures of 29/21 ± 3 °C and 39/29 ± 3 °C, respectively, and average rainfall of 650 mm.

Seed dormancy is an innate seed property that defines the environmental conditions in which the seed is able to germinate (Finch-Savage and Leubner-Metzger, 2006). An understanding of dormancy mechanisms is of ecological

and economic importance. Weed seed dormancy and germination are regulated by a complex interaction of environmental, edaphic, physiological, and genetic factors (Radosevich *et al.*, 1996). The relationship between seed dormancy and the success of a plant as an agricultural weed is significant. Weed seeds vary extensively with respect to degree, duration, and dormancy source. The existence of large weed seed populations with varying degrees and states of dormancy is the basis for the annual weed problem.

Dormancy is a common attribute of many weed seed populations and this usually hampers the task of predicting the timing and extent of weed emergence (Roberto *et al.*, 2000). Brasil (2009) examined that among 260 leguminoseae seeds, about 85% had tegument impermeable to water. Tegument impermeability is associated with the presence of impermeable layers of palisade cells (Baskin and Baskin, 1998). This kind of dormancy could be overcome by weakening the tegument by allowing water to pass and initiate germination (Cavalheiro *et al.*, 2007). This process can also occur through the action of acids during seed digestion by dispersing animals (Goddard *et al.*, 2009).

Many species of the *Fabaceae* family such as *Lupinus* spp. seeds exhibit dormancy that is primarily due to water impermeability of the seed's coat. Scarification of Texas bluebonnet (*Lupinus texensis* Hook) seeds with sulfuric acid for 30 to 60 min improved seedling emergence (Davis *et al.*, 1991). Acid scarification of big bend bluebonnet (*Lupinus havardii* S. Wats) seeds for 120 min and perennial lupine (*Lupinus perennis* Wats.)

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seeds for 45 min resulted in 100% germination (Mackay *et al.*, 1996). The concentrated sulfuric acid treatment has been widely used to improve seed germination of several hard seed coat species (Tigabu and Oden, 2001).

Weed control is an integral part of efficient crop production that is assisted by new methods of dormancy release (Gu *et al.*, 2004). Dormancy levels differ between individual seeds within a population. Changing weed seed dormancy levels is probably the most important process that determines weed emergence patterns under field situations. Predicting weed seed dormancy and consequently, timing and extent of weed emergence, is important in order to improve weed control strategies (Radosevich *et al.*, 1996).

Dormancy can distribute seed germination in the soil over the years, so weeds continue to emerge in the field even after years of thorough weed management practices. Weeds are difficult to eliminate in a field because seed banks provide an enormous seed collection with different dormancy levels. Thus, seed dormancy is a major cause of continuing weed problems, but no information is available about breaking seed dormancy of *R. capitata* worldwide. The present study was therefore conducted with the hypothesis of whether *R. capitata* seeds could be released from dormancy with different seed treatment methods. The objective of this research was to determine the effect of different methods and identify the best method to break seed dormancy and promote germination of *R. capitata* seeds.

## MATERIALS AND METHODS

### Seed collection

Mature *R. capitata* pods were collected from the *Vigna radiata* (L.) R. Wilczek field in October 2009. Immediately after collection, seeds were isolated from the pods, separated from the undesired materials and unripe seeds, and stored in sealed paper bags after drying for 1 wk in the shade under normal room temperature (25 to 30 °C); only mature and uniformly-sized seeds were used in various seed germination experiments.

### Experiment 1: Effect of seed treatment with KNO<sub>3</sub> and thiourea on dormancy release and germination of *R. capitata*

Seeds were soaked in different concentrations of potassium nitrate (KNO<sub>3</sub>) (0, 10 000, 20 000, 30 000, 40 000, 50 000, and 60 000 mg L<sup>-1</sup> and thiourea [(NH<sub>2</sub>)<sub>2</sub>C S] (0, 2 500, 5 000, 7 500, and 10 000 mg L<sup>-1</sup>) for 24 h at 30 °C. The concentration ranges were selected after preliminary trials in which seeds were immersed in KNO<sub>3</sub> at concentrations of 0, 500, 1 000, 2 500, 5 000, and 7 500 mg L<sup>-1</sup>, and thiourea at concentrations of 100, 200, 300, 400, 500, 600, and 1000 mg L<sup>-1</sup>, which had no effect on dormancy.

### Experiment 2: Effect of seed scarification with HCl and sandpaper on dormancy release and germination of *R. capitata*

Seeds were immersed separately in HCl (36%) for 3, 6, 9, 12, 15, and 18 h at 30 °C in addition to being rubbed against the rough surface of the sandpaper (the seed coat was sanded with a # 80 wood sandpaper at an area opposite from the embryo until the cotyledon was exposed). Immediately after the prescribed soaking period, seeds were removed and rinsed several times in clean distilled water. Untreated seeds were used as a control.

### Experiment 3: Effect of seed scarification with HNO<sub>3</sub> on dormancy release and germination of *R. capitata*

Seeds were treated separately with HNO<sub>3</sub> (65%) for 1, 2, 3, 4, and 5 d at 30 °C. Seeds were then rinsed several times in clean distilled water after the treatment with acids. Untreated seeds were used as a control.

### Experiment 4: Effect of seed scarification with H<sub>2</sub>SO<sub>4</sub> on dormancy release and germination of *R. capitata*

In this experiment, seeds were soaked in sulfuric acid (98%) for 20, 40, 60, 80, 100, and 120 min at 30 °C. These treatments were selected after conducting preliminary trials of soaking seeds in sulfuric acid for 2, 4, 6, 8, and 10 min, which had no effect on breaking dormancy. Thereafter, scarified seeds were rinsed several times in clean distilled water. Untreated seeds were used as a control.

### Germination test

After rinsing, seeds were allowed to dry on blotter paper at the laboratory temperature (30 °C) before being placed in Petri dishes in the abovementioned experiments. Seeds were surface sterilized by soaking in a 5% sodium hypochlorite (NaOCl) solution for 5 min and subsequently rinsed five times with sterilized water. Seeds were placed on double-layered Whatman N° 10 filter paper moistened with 10 mL of distilled water in sterilized 15 cm diameter Petri dishes. All the dishes were sealed with a strip of parafilm to reduce water loss and placed at 30/18 °C in a germinator.

Each experiment had a randomized complete block design with four replicates and with 25 seeds per Petri dish. Germination counts were made every day for 3 wk. A seed was considered germinated when the tip of the radicle (2 mm) had grown free of the seed. Each experiment was carried out twice and statistical analysis was performed on the mean of the two replicates. The germination index (GI) was calculated as described by the Association of Official Seed Analysts (1990) by the following formula:

$$GI = \frac{\text{N}^\circ \text{ of germinated seeds}}{\text{Days of first count}} + \dots + \frac{\text{N}^\circ \text{ of germinated seeds}}{\text{Days of final count}}$$

Time needed for 50% germination of seedlings ( $T_{50}$ ) was calculated according to the following formulae from Coolbear *et al.* (1984):

$$T_{50} = t_i + \frac{(N/2 - n_i)(t_j - t_i)}{n_j - n_i}$$

where  $N$  is the final number of germinated seeds,  $n_i$  and  $n_j$  are the cumulative number of seeds germinated by adjacent counts at times  $t_i$  and  $t_j$ , respectively, when  $n_i < N/2 < n_j$ .

Mean germination time (MGT) was calculated according to the Ellis and Roberts (1981) equation:

$$MGT = \frac{\sum (D_n)}{\sum n}$$

where  $n$  is the number of germinated seeds or emerged seedlings on day  $D$  and  $D$  is the total number of days counted from the beginning of germination.

Data were recorded for up to 14 d and then analyzed statistically by Fisher's ANOVA function of the MSTAT statistical computer package, and LSD at 5% probability was used to compare the treatment means (Steel *et al.*, 1997).

## RESULTS AND DISCUSSION

### Seed treatment with thiourea and $KNO_3$

Seeds of *R. capitata* showed no response to various concentrations of thiourea and  $KNO_3$  since thiourea and  $KNO_3$  failed to crack the seed coat and its imbibition. Seeds after the prescribed soaking treatments were still hard and viable, and they successfully germinated when scarified with sandpaper.

Since seeds did not germinate at various treatments with thiourea and  $KNO_3$ , data regarding these treatments were not included in the calculations.

### Scarification with HCl and sandpaper

Treatment with sandpaper was very effective in breaking seed dormancy (Table 1). Results indicated that the germination of seeds that were mechanically scratched with sandpaper significantly increased to 100% as compared to HCl treatments. In addition, seeds mechanically scarified with sandpaper had the minimum response time (0.66 d) and mean germination time (2.16 d) when compared to all other treatments. When seeds were scarified with HCl (36%) for 3, 6, 9, 12, 15, and 18 h, seed germination significantly ( $p < 0.05$ ) increased over the control (Table 1). Seeds treated with HCl for 12, 15, and 18 h had the minimum response time with 50% of the seeds germinating in all the replicates within 1.75, 1.13, and 1.20 d, respectively. Minimum MGT (2.94 and 2.95 d) was detected in seeds treated with HCl for 18 and 15 h, respectively. Both were statistically similar. Seeds treated with HCl for 3, 6, and 9 h had a significantly higher mean germination time than other treatments, but remained equal to each another. Maximum GI (7.75) was observed in sandpaper scarification and after 6 d when

**Table 1. Effect of seed scarification with HCl on breaking dormancy and germination of *Rhynchosia capitata*.**

| Treatments         | Germination | $T_{50}$ | MGT    | GI     |
|--------------------|-------------|----------|--------|--------|
|                    | %           |          |        |        |
| Control            | 0.00f       | 0.00g    | 0.00e  | 0.00g  |
| HCl (3 h)          | 17.50e      | 3.62a    | 4.08ab | 0.47f  |
| HCl (6 h)          | 25.00e      | 3.25b    | 4.37a  | 0.69f  |
| HCl (9 h)          | 35.00d      | 2.25c    | 4.15ab | 1.35e  |
| HCl (12 h)         | 65.00c      | 1.75d    | 3.88b  | 3.11c  |
| HCl (15 h)         | 90.00b      | 1.13e    | 2.95c  | 6.00b  |
| HCl (18 h)         | 35.00d      | 1.20e    | 2.94c  | 2.19d  |
| Sand paper         | 100.0a      | 0.66f    | 2.16d  | 7.75a  |
| LSD ( $P < 0.05$ ) | 8.8119      | 0.3669   | 0.4497 | 0.4668 |

Means followed by the same letter in a column did not differ significantly according to LSD test ( $P < 0.05$ ).

$T_{50}$ : Time needed for 50% germination; MGT: Mean germination time; GI: Germination index; LSD: Least significance difference.

seeds were treated with HCl for 15 h. However, there was no germination in the control treatment.

### Scarification with $HNO_3$

Soaking *R. capitata* seeds in  $HNO_3$  for 1 to 5 d had little effect on seed germination as compared to other treatments (Table 2). Total germination was not more than 18%, slow, and irregular. Percentages of seed germination in all  $HNO_3$  treatments were statistically equal to each other. Minimum ( $T_{50}$ ) germination and MGT were recorded in seeds treated with  $HNO_3$  for 3, 4, and 5 d, respectively. The control treatment (untreated) had no effect on germination. The remaining seeds were still hard and viable, but successfully germinated when scarified with sandpaper.

**Table 2. Effect of seed scarification with  $HNO_3$  on breaking dormancy and germination of *Rhynchosia capitata*.**

| Treatments         | Germination | $T_{50}$ | MGT    | GI     |
|--------------------|-------------|----------|--------|--------|
|                    | %           |          |        |        |
| Control            | 0.00b       | 0.00d    | 4.41a  | 0.28b  |
| $HNO_3$ (1 d)      | 12.50a      | 4.31a    | 3.91ab | 0.47a  |
| $HNO_3$ (2 d)      | 17.50a      | 3.25b    | 3.58ab | 0.59a  |
| $HNO_3$ (3 d)      | 17.50a      | 2.50c    | 3.33b  | 0.43ab |
| $HNO_3$ (4 d)      | 15.00a      | 2.50c    | 3.33b  | 0.59a  |
| $HNO_3$ (5 d)      | 17.50a      | 2.50c    | 3.33b  | 0.59a  |
| LSD ( $P < 0.05$ ) | 7.0031      | 0.7363   | 1.0325 | 0.1698 |

Means followed by the same letter in a column did not differ significantly according to LSD test ( $P < 0.05$ ).

$T_{50}$ : Time needed for 50% germination; MGT: Mean germination time; GI: Germination index; LSD: Least significance difference.

### Scarification with $H_2SO_4$

Scarification of *R. capitata* seed with  $H_2SO_4$  induced seed germination in all treatments (Figure 1). The seed germination percentage increased with increasing soaking time (up to 80 min) and began to decrease with the further increase in soaking time. Seeds soaked in  $H_2SO_4$  for 60 and 80 min had the most rapid response time with 50% of the seeds germinating in all replicates within 0.66 and 0.80 d, respectively (Figure 2). Minimum MGT (2.09 and 2.15 d) was detected with 60 and 80 min of soaking in  $H_2SO_4$ . Maximum GI was recorded when seeds were soaked in  $H_2SO_4$  for 60 min. There was no germination in the control treatment.

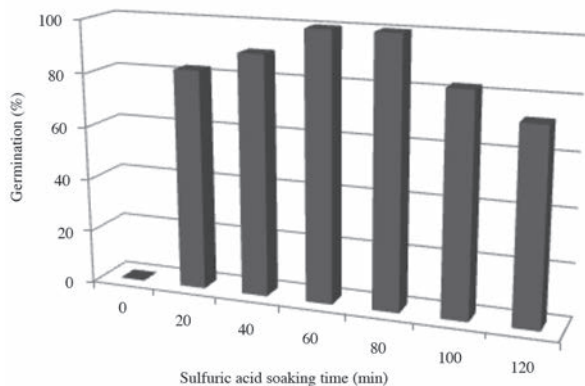
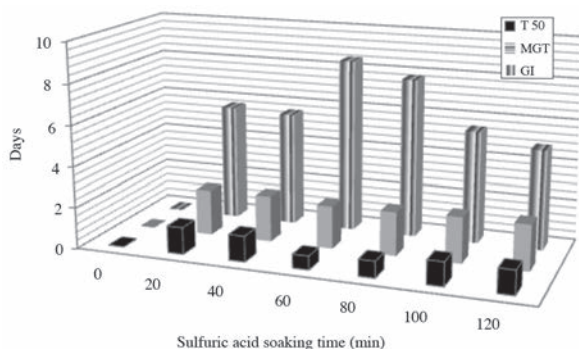


Figure 1. Effect of seed scarification with H<sub>2</sub>SO<sub>4</sub> on *Rhynchosia capitata* germination (LSD (P < 0.05) = 4.8134).



T<sub>50</sub>: Time needed for 50% germination; MGT: Mean germination time; GI: Germination index; LSD: Least significance difference.

Figure 2. Effect of seed scarification with H<sub>2</sub>SO<sub>4</sub> on T<sub>50</sub>, MGT, and GI of *Rhynchosia capitata* (LSD P < 0.05 for T<sub>50</sub> = 0.4397, MGT = 0.0576, and GI = 0.4386).

Mechanical constraint, including prevention of water and oxygen uptake, and retention or production of chemical inhibitors are some of the possible mechanisms that cause the strong inhibitory effect of the seed coat on seed germination (Taiz and Zeiger, 2002). The results of various treatments in our study confirm that *R. capitata* seeds exhibit dormancy due to their hard seed coat. Breaking down impermeability of the seed coat by scarification methods resulted in a considerable increase in the germination percentage (12 to 100%). The different chemicals (thiourea and KNO<sub>3</sub>) and acids (HCl, HNO<sub>3</sub>, and H<sub>2</sub>SO<sub>4</sub>) have been widely used for breaking dormancy of many hard seed coat species, such as European milkvetch (*Astragalus hamosus* L.), blackdisk medick (*Medicago orbicularis* [L.] Barta). (Patane and Gresta, 2006), and *Albizia* spp. (Tigabu and Oden, 2001). In the present study, the best treatment to remove hard seed dormancy causing the highest germination percentage was seed scarification with H<sub>2</sub>SO<sub>4</sub> and sandpaper. Similar results were obtained in experiments with African locust bean (*Parkia biglobosa*) seeds (Aliero, 2004), European milkvetch

and blackdisk medick seeds, (Patane and Gresta, 2006), and *Enterolobium contortisiliquum* (Vell.) Morong seed (Malavasi and Malavasi, 2004) in which seed dormancy was broken by soaking seeds in H<sub>2</sub>SO<sub>4</sub> and sandpaper scarification. Mechanical scarification provided rapid as well as the highest imbibitions and the germination percentage in the study as compared with untreated (control) seeds and completely overcame seed coat impermeability.

Hydrochloric acid was used to most closely imitate the stomach environment of animals. Total germination of the HCl-treated seeds increased when compared to the control over an extended period (up to 18 h) indicating a slow release from seed dormancy. These results are in line with Goddard *et al.* (2009) who found that Benghal dayflower seeds exposed to HCl soaking treatments successfully germinated with little loss of viability after each treatment. Seeds from the 18-h treatment were extremely soft and moldy at the end of the germination test, so lower germination was recorded in this treatment. The mechanism of possible seed germination influenced by H<sub>2</sub>SO<sub>4</sub> is due to its capability to break the seed coat that leads to water absorption and seed imbibition. A gradual increase in the germination percentage and GI and decrease in MGT and T<sub>50</sub> with an increase in the soaking time of seeds in HCl from 3 to 15 h and treatment with H<sub>2</sub>SO<sub>4</sub> for 20, 40, 60, and 80 min revealed that HCl and H<sub>2</sub>SO<sub>4</sub> were adequate to break the hard seed coat of *R. capitata* seeds in order to induce germination.

The decline in the germination rate at 100 and 120 min soaking in H<sub>2</sub>SO<sub>4</sub> and 18 h soaking in HCl was the result of the damaging effect to the seed embryo due to prolonged soaking time. A similar response was observed by Sadeghi *et al.* (2009) who found that the complete removal of the seed coat caused rapid imbibitions, which caused fracture and bursting of the endosperm. Similarly, Aliero (2004) reported that prolonged emersion of seeds in H<sub>2</sub>SO<sub>4</sub> injures the seeds since the acid can break vital parts of the embryo.

Thiourea has been known to stimulate germination by reducing the preventive effect of the seed coat in sweet cherry (*Prunus avium* L.) seeds (Çetinbaş and Koyuncu, 2006). Similarly, KNO<sub>3</sub> was very 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). Both these chemicals were unable to break dormancy in *R. capitata* seeds in the present study. This could be due to its excessively hard seed coat. These studies 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.

## CONCLUSIONS

The results of these experiments showed that *R. capitata* seeds were in a dormant state and softening the seed coat by soaking concentrated H<sub>2</sub>SO<sub>4</sub>, HCl, and HNO<sub>3</sub> significantly increased seed germination. Mechanical scarification has also been shown as the best method to overcome this coat-imposed dormancy. Seed coat was the major barrier to *Rhynchosia capitata* seed germination since the treatments that induce germination were those that can break the seed coat.

**Métodos para romper la dormancia de *Rhynchosia capitata*, una maleza anual de verano.** La dormancia seminal de las malezas es un rasgo significativo contribuyente a su tasa de supervivencia, puesto que ayuda a las malezas a evitar herbicidas y otras prácticas de desmalezado junto con condiciones ambientales desfavorables. Investigamos los efectos de diferentes tratamientos para romper dormancia sobre la germinación de *Rhynchosia capitata*, una maleza anual estival común en Paquistán. Las semillas se sumergieron en tiourea, KNO<sub>3</sub>, HCl, HNO<sub>3</sub> y H<sub>2</sub>SO<sub>4</sub> y además fueron escarificadas mecánicamente (papel lija). Los resultados indicaron que las semillas de *R. capitata* muestran signos de dormancia física principalmente debido a impermeabilidad de su cubierta. Escarificación mecánica y ácida (inmersión de semillas en H<sub>2</sub>SO<sub>4</sub> por 60 y 80 min y en HCl por 12 y 15 h) fueron muy eficientes para romper dormancia y promover germinación. Las semillas sumergidas en HNO<sub>3</sub> por 1 a 5 días mostraron poco efecto, mientras diversas concentraciones de tiourea y KNO<sub>3</sub> fueron inefectivas para romper dormancia seminal en *R. capitata*.

**Palabras clave:** HCl, HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, dormancia, germinación.

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