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EVALUATION OF SOME SEED DORMANCY BREAKING METHODS ON GERMINATION OF *Rhynchosia capitata* (Roth DC)

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ABSTRACT

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 (Roth DC), a common summer annual weed, which is emerging as a weed threat in farming areas of Southern Punjab, Pakistan. Different dormancy breaking methods i.e. dry heat, hot water, stratification, soaking in HCl, HNO_3, and H_2SO_4 were evaluated in 2010 at the department of Agronomy, the University of Agriculture, Faisalabad, Pakistan. Results indicated that R. capitata seeds show signs of physical dormancy that is mainly due to the impermeability of their coat. Acid scarification treatments were very efficient in breaking dormancy and promote germination. Various seed treatments with dry heat, hot water and stratification were ineffective in breaking dormancy of R. capitata seeds.

Key words: Dormancy, germination, HCl, HNO_3, H_2SO_4, stratification.

INTRODUCTION

The genus *Rhynchosia* of *Fabaceae* family is widely distributed in most parts of the world. *Rhynchosia capitata,* an emerging annual summer season weed, 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 of Pakistan and is increasingly becoming a problematic weed in farming systems (Ali *et al*., 2011). Under field condition, this weed emerges through the seed just after irrigation. It is twinning prostrate plant with many branches spreading all around the rootstock. An approximately one month old plant starts flowering and the plant has oval-shaped pods with two seeds in each pod (Sharma *et al*., 1978). The growing season is from May to October with minimum and maximum average temperature of 29/21 ± 3 °C and 39/29 ± 3 °C, respectively, and average rainfall of 650 mm (Ali *et al*., 2011).

Weeds are the primary pests that cause yield losses in producing the nation's food and fiber crops (Malik *et al*., 2000; Mansoor *et al*., 2004). Weeds flourish in agricultural, urban, and natural settings because they have certain characteristics, such as

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seed dormancy, that provide for their persistence (Roberto *et al*., 2000). Seed dormancy, which denotes a relatively inactive or resting condition, slows down or stops weed seed germination. The dormant seeds in the soil allow weeds to escape or avoid exposure to control practices that target emerging and emerged weed seedlings. An understanding of weed seed dormancy mechanisms is of ecological and economic importance. Interactions of many environmental, edaphic, physiological and genetic factors regulate weed seed dormancy in an agricultural system (Radosevich *et al.*, 1996). The relationship between seed dormancy and success of a plant as a weed is significant. Weed seeds vary extensively with respect to degree, duration, and basis of dormancy. The existence of large population of weed seeds with varying degrees and states of dormancy is the main reason for the annual weed problem.

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 (Mackay *et al.,* 1996). 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.) 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). Khudahibergenov and Mikhahilova (1972) showed that untreated seeds of *Glycyrrhiza uralensis* L. have 11% germination in the laboratory and 9% in the field. Treatment with concentrated sulphuric acid increased germination from 60 to 94%. Seeds germinated most vigorously at 40 to 50°C.

Seed dormancy is major motive towards the success of this species, which permit the seeds of this species to persist for long periods in the soil and thus escape the effects of post-emergence weed control measures (Ali *et al*., 2011). The most successful weed management programs are developed on the foundations of adequate ecological understandings. Knowledge of seed dormancy of *R. capitata* would facilitate the development of an effective weed control program. Keeping in view the importance of weed seed dormancy and increasing problem of *R. capitata*, the present study was planned to determine the dormancy behavior of *R. capitata* seeds in order to evolve effective control strategy.

MATERIALS AND METHODS

Seed collection

Mature *R. capitata* pods were collected from the mungbean [*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 of *R. capitata* were used in various seed dormancy breaking germination experiments during 2010 at department of Agronomy, University of Agriculture, Faisalabad, Pakistan.

Experiment I: Effect of dry heat seed treatment on dormancy release and germination of *R. capitata*

Seeds of *R. capitata* were placed in shallow containers in a preheated oven according to prescribed temperature and duration, i.e. 70 °C for 1, 2 and 4 hours; 70 °C for 1, 2, 3 and 4 days; 200 °C for 5, 10, 15, 30 and 45 min. After each treatment, the seeds were cooled immediately and sown.

Experiment II: Effect of hot water seed treatment on dormancy release and germination of *R. capitata*

Seeds were placed in boiling water for 5, 15, 30, 60, 90, 120 and 150 min then immediately removed from the boiling water and kept at room temperature to cool before sowing.

Experiment III: Effect of stratification on dormancy release and germination of *R. capitata*

The seeds were placed in a tightly glass jar, and stored in the refrigerator at a temperature of 2-5 ºC for 5, 10, 30 and 60 min; 3, 6 and 12 hours; 1, 2, 4, 8, 15 and 30 days.

Experiment IV: Effect of seed scarification with HCl + H_2SO_4 on dormancy release and germination of *R. capitata.*

The seeds were treated with HCl (36%) for 20, 40, 60, 80, 100 and 120 min separately and then with H_2SO_4 (96%) for 30 min at 30 °C. The seeds were then rinsed several times in distilled water after treating with acids. Untreated seeds were used as a control.

Experiment V: Effect of seed scarification with HNO_3 + H_2SO_4 on dormancy release and germination of *R. capitata.*

The seeds were treated with HNO_3 (64%) for 20, 40, 60, 80, 100 and 120 min separately and then with H_2SO_4 for 30 min at 30 °C. The seeds were then rinsed several times in distilled water after treating with acids. Untreated seeds were used as a control.

Germination test

After cooling or rinsing, seeds were allowed to dry on blotter paper at the laboratory temperature (30 °C) before being placed in Petri dishes in the above mentioned experiments. Seeds were surface sterilized by soaking in a 5% sodium hypochlorite (NaOCl) solution for 5 min and subsequently rinsed five times with distilled water. Seeds were placed on double-layered What man No 10 filter paper moistened

with 10 mL of distilled water in 15 cm diameter Petri dishes. All the dishes were sealed with a strip of Para film to reduce water loss.

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:

$$\mathrm{GI} = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \text{------} + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

Time needed for 50% germination of seedlings (T_{50}) was calculated according to the following formula 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:

$$\mathrm{MGT} = \Sigma\,(D_n)/\Sigma\,n$$

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.

Each experiment had a randomized complete block design with four replicates and with 25 seeds per Petri dish. Data were recorded for up to 21 d and then analyzed statistically by 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

Dry heat, hot water and stratification seed treatment

Various seed treatments (dry heat, hot water and stratification) of *R. capitata* showed no response in breaking dormancy (Table-1, 2, 3). The non-germinated seeds were still hard and viable, except dry heat treatments in oven at 200 ^{o}C and germinated successfully when scarified with sand paper.

In leguminous weed species, dry heat breaks physical seed dormancy by modification of the seed coat mechanically. After this, the fractured seed coat allows further imbibitions and hence germination takes place (Bradstock and Auld, 1995). Rigorous heat applied to

seeds may rupture their hard seed coats or may soften waxy coverings present over the seeds (Tarrega *et al.,* 1992). However, in our study a range of dry heat treatment had no effect on breaking seed dormancy of *R. capitata.* Seed treatments with hot water had been described to improve germination of hard seed coat species by uplifting water and O_2 permeability of the testa of seed coat (Teketay, 1998; Aydın and Uzun, 2001). However, in this study, various hot water seed treatments failed to encourage *R. capitata* seed germination. The response of *R. capitata* seeds to a range of stratification treatments (Table-3) is similar to that repotted by Susko *et al.* (2001) in kudzu (*Pueraria lobata* (Willd.) Ohwi). He reported that keeping kuzdu seeds at 5°C for 0-6 weeks did not influence seed germination.

Scarification with HCl + H_2SO_4

When non-germinated seeds of HCl treatments (20, 40, 60, 80, 100 and 120 min) were re-treated with H_2SO_4 for 30 min, the seeds germination significantly ($p < 0.05$) increased over control (Table-4), but did not differ statistically in HCl treatments for different times. Seeds treated with HCl for 80, 100 and 120 min. + H_2SO_4 for 30 min. had the minimum response to T_{50} and recorded 0.94, 0.78, and 0.90 days, respectively. Minimum MGT (2.15) was detected in seeds treated with HCl for 100 min + H_2SO_4 for 30 min, followed by HCl for 120 min + H_2SO_4 for 30 min (2.17). Both were statistically at par with each other. Seeds treated with HCl for 20, 40 and 60 min + H_2SO_4 for 30 min took significantly more MGT than other treatments, but remained at par with one another. Maximum GI (5.70) was recorded in seeds treated with HCl for 100 and 120 min + H_2SO_4 for 30 min However, there was no germination in control treatment.

Scarification with HNO_3 + H_2SO_4

Soaking of *R. capitata* seeds in HNO_3 for 20, 40, 60, 80, 100 and 120 min + H_2SO_4 for 30 min resulted in significant increase in germination of the seeds (Table-5). Maximum germination percentage (77.50 %) was recorded when seeds were treated with HNO_3 for 120 min + H_2SO_4 for 30 min, but did not differ significantly from seeds treated with HNO_3 for 60, 80 and 100 min+ H_2SO_4 for 30 min. Minimum T_{50} and MGT was recorded in seeds treated with HNO_3 for 120 min + H_2SO_4 for 30 min while maximum values were recorded in seeds treated with HNO_3 for 20 min + H_2SO_4 for 30 min. Germination index was increased with increase in soaking time of seeds in HNO_3+ H_2SO_4 for 30 min. The control treatment had no effect on germination.

Different acids (HCl, HNO_3 and H_2SO_4) had been widely used for breaking dormancy of many hard seed coat species like European milkvetch (*Astragalus hamosus* L.), blackdisk medick [*Medicago orbicularis* (L.) Bartal.] (Patane and Gresta, 2006) and *Albizia* spp. (Tigabu and Oden, 2001).

Table-1. Effect of dry heat seed treatment on breaking dormancy and germination of *Rhynchosia capitata*

Treatment	Time	Result
70 °C	1 hour	No germination
70 °C	2 hour	No germination
70 °C	4 hour	No germination
70 °C	1 day	No germination
70 °C	2 days	No germination
70 °C	3 days	No germination
70 °C	4 days	No germination
200 °C	5 min	No germination
200 °C	10 min	No germination
200 °C	15 min	No germination
200 °C	30 min	No germination
200 °C	45 min	No germination

Table-2. Effect of hot water seed treatment on breaking dormancy and germination of *Rhynchosia capitata*

Treatment	Time	Result
Boiling water	5 min	No germination
Boiling water	15 min	No germination
Boiling water	30 min	No germination
Boiling water	60 min	No germination
Boiling water	90 min	No germination
Boiling water	120 min	No germination
Boiling water	150 min	No germination

Table-3. Effect of stratification seed treatment on breaking dormancy and germination of *Rhynchosia capitata*

Treatment	Time	Result
2-5 °C	5 min	No germination
2-5 °C	10 min	No germination
2-5 °C	30 min	No germination
2-5 °C	60 min	No germination
2-5 °C	3 hour	No germination
2-5 °C	6 hour	No germination
2-5 °C	12 hour	No germination
2-5 °C	2 days	No germination
2-5 °C	4 days	No germination
2-5 °C	8 days	No germination
2-5 °C	15 days	No germination
2-5 °C	30 days	No germination

Table-4. Effect of seed scarification with HCl+H_2SO_4 on dormancy release and germination of *R. capitata*

Treatments	Germination (%)	T_{50} (days)	MGT (days)	GI
Control	0.00 b	0.00 c	0.00 d	0.00 d
20 min HCl + H_2SO_4 30 min	75.00 a	1.33 a	2.28 a	4.87 bc
40 min HCl + H_2SO_4 30 min	72.50 a	1.28 a	2.29 a	4.66 c
60 min HCl + H_2SO_4 30 min	70.00 a	1.24 a	2.27 a	4.62 c
80 min HCl + H_2SO_4 30 min	72.50 a	0.94 b	2.20 b	5.20 ab
100 min HCl + H_2SO_4 30 min	72.50 a	0.78 b	2.15 c	5.70 a
120 min HCl + H_2SO_4 30 min	75.00 a	0.90 b	2.17 bc	5.70 a
LSD (P<0.05)	6.6153	0.1836	0.0414	0.5342

Means sharing the same letter in a column did not differ significantly at 5 % probability level
T_{50} = Time taken to 50% germination, MGT = Mean Germination time, GI = Germination Index, LSD = Least Significance Difference.

Table-5. Effect of seed scarification with HNO_3+H_2SO_4 on dormancy release and germination of *R. capitata*

Treatments	Germination (%)	T_{50} (days)	MGT(days)	GI
Control	0.00 d	0.00 e	0.00 e	0.00 f
20 min HNO_3 + H_2SO_4 30 min	65.00 c	2.56 a	3.16 a	2.83 e
40 min HNO_3 + H_2SO_4 30 min	70.00 bc	1.60 b	2.61 b	4.25 d
60 min HNO_3 + H_2SO_4 30 min	72.50 ab	1.18 c	2.25 cd	4.95 c
80 min HNO_3 + H_2SO_4 30 min	72.50 ab	0.90 cd	2.03 d	5.37 b
100 min HNO_3 + H_2SO_4 30 min	75.00 ab	1.00 cd	2.48 bc	5.33 bc
120 min HNO_3 + H_2SO_4 30 min	77.50 a	0.70 d	2.14 d	6.20 a
LSD (P<0.05)	6.6153	0.3919	0.2940	0.4099

Means sharing the same letter in a column did not differ significantly at 5 % probability level
T_{50} = Time taken to 50% germination, MGT = Mean Germination time, GI = Germination Index, LSD = Least Significance Difference.

Germination percentage of various treatments of seeds with HCl (20, 40, 60, 80, 100 and 120 min) + H_2SO_4 for 30 min was statistically similar which suggest that the HCl alone is completely insufficient to scratch the seed coat. Gradual increase in germination percentage and GI; and decrease in MGT and T_{50} with increase in soaking time of seeds in HNO_3 from 20 to 120 min and then treatment with H_2SO_4 for 30 min revealed that H_2SO_4 was the only acid that ruptured the hard seed coat of *R. capitata* seeds to induce germination. The mechanism of possible seed germination influenced by H_2SO_4 is due to its capability to rupture seed coat, hence leading to water absorption and thus imbibitions of seeds.

Decrease in T_{50} and MGT and increase in GI of *R. capitata* seed beyond 60 min soaking in HCl + 30 min in H_2SO_4 and after 20 min soaking in HNO_3+ 30 min in H_2SO_4, respectively in the second experiment could be due to effect of acids which speed up germination. Similar results were obtained in experiments with african locust bean (*Parkia biglobosa* [Jacq.] Benth.) seeds (Aliero, 2004) in which seeds were soaked in 50 to 100 % concentrated H_2SO_4 for 1 to 5

min; european milkvetch (*Astragalus hamosus* L.) and blackdisk medick [*Medicago orbicularis* (L.) Bartal.] (Patane and Gresta, 2006) and morong seed [*Enterolobium contortisiliquum* (Vell.)] (Malavasi and Malavasi, 2004) seeds in which seed dormancy was broken by soaking seeds in H_2SO_4 for 30, 60, 120 and 180 min.

CONCLUSION

The results of various treatments in our study confirmed that seeds of *R. capitata* exhibit dormancy due to hard seed coat. Various seed scarification methods break down *R. capitata* seed coat impermeability that resulted in a considerable increase in germination percentage. 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 soil and thus escape the effects of post germination weed control measure.

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