

Dormancy and seed germination of *Sida Rhombifolia* L.

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Abstract

Experiments were conducted to evaluate the effects of seed scarification, temperature, light, salt and osmotic stress, and pH on seed germination, and also the effects of seed burial depth on seedling emergence of *Sida rhombifolia*. Scarification with sulphuric acid released seeds from dormancy and stimulated germination; though germination of scarified seeds was not influenced by light. Seeds treated with sulphuric acid for 120 min resulted in 65% germination compared with 5% for non-scarified seeds. The response to scarification indicates that a hard seed coat is the primary mechanism restricting germination. In two separate experiments, a concentration of 111 mM sodium chloride and an osmotic potential of -0.49 MPa reduced maximum germination (64 to 65%) of *S. rhombifolia* by 50%. Germination was not influenced by the pH of buffered solutions ranging from 5 to 9, and it varied from 60 to 65% over this range. Seedling emergence was greater than 60% at burial depths of 0.5 to 2 cm, but decreased thereafter, and no seedlings emerged from the seeds buried at 8 cm. The results of this study identify some of the factors enabling *S. rhombifolia* to be a widespread and problematic weed in the humid tropics and provide information that may contribute to its control.

Keywords: Effect, Treatment, Breaking dormancy, seeds

1. Introduction

Sida rhombifolia (*Sida orientalis*) is also a weed found in wastelands. The herb is erect, woody and easily recognized by its rhomboid lanceolate leaves. The plant is called Mahabala in Sanskrit. The stems are rich in mucilage and are employed internally as an emollient, diuretic, and as a febrifuge with pepper. The roots are bitter, cooling, and anthelmintic.

The awns on many fruits and high stem fibre content cause *S. rhombifolia* to be highly undesirable in pastures.

The awned mericarps that contaminate grain crops can also injure livestock when used in ration and young leaves may be poisonous (Holm *et al.*, 1997) [10]. In addition, the weed is an alternate host of nematodes and insects (Galinato *et al.*, 1999) [9]. These studies suggest that *S. rhombifolia* is a troublesome weed in the humid tropics as it infests a wide range of crops, it is poisonous to livestock, it is an alternate host for pests, and as such it can be a serious problem for farmers.

Several environmental factors, including temperature, light, soil salinity, pH and moisture, and seed burial depth influence weed seed germination. Some information about the germination requirements of *S. rhombifolia* is available for populations in the United States (Smith *et al.*, 1992) [12], but little is available for Asian populations. Our findings show that germination of *S. rhombifolia* is very low unless the seeds are scarified, but the previous study (Smith *et al.*, 1992) [12] did not mention whether the seeds used were scarified or not. Information on the environmental conditions necessary for weed germination and emergence is important for a better understanding of the nature of potential weed problems and development of weed control strategies. The objectives of this study, therefore, were to determine the effects of seed scarification, temperature, light, salt and osmotic stress, and pH on seed germination, and also the effects of seed burial depth on seedling emergence of *S. rhombifolia*.

2. Material and Methods

Seeds of *S. rhombifolia* were collected in May 2015 from many plants selected at random around the margins of several fields around Govt. Agriculture farm Kuthulia Rewa (M.P.). Seeds from many plants were bulked, cleaned and stored in a laboratory at room temperature (approximately 25 °C) until used in the experiments.

2.1 Germination Tests

Twenty-five seeds of *S. rhombifolia* were placed uniformly in each 9-cm-diameter petri dish containing 5 ml of distilled water or a treatment solution and double layer of filter papers (Whatman No. 1). Chemically scarified (120 min with sulphuric acid, H₂SO₄) seeds were used, unless otherwise specified. Seeds were washed in running tap water for 5 min before performing experiments with them. Seeds were incubated in light/dark (12 h/12 h) condition at 35/25 °C (day/night temperature), unless otherwise stated. Germination was determined after 14 days, at which time seeds with an emerged radicle were considered to have germinated.

2.2 Effect of Seed Scarification on Germination

Experiments were conducted to investigate whether germination in this species was inhibited by an impermeable seed coat. Seeds were scarified with concentrated H₂SO₄ at different time intervals (0, 5, 10, 30, 60, 120, and 180 min). Seeds were then incubated as described above and germination determined.

2.3 Effect of Temperature and Light on Germination

To determine the effect of temperature and light on germination, H₂SO₄ scarified seeds of *S. rhombifolia* were incubated under three different alternating day/night temperatures (35/25, 30/20 and 25/15 °C) in two light regimes [light/dark (12 h/12 h) and continuous dark (24 h)].

In the dark treatment, the dishes were wrapped in a double layer of aluminium foil.

2.4 Effect of Salt Stress on Germination

To determine the effect of salt stress on germination, H_2SO_4 scarified seeds of *S. rhombifolia* were incubated in 0, 25, 50, 100, 150, 200 and 250 mM sodium chloride (NaCl) solutions. Germination was determined as described previously.

2.5 Effect of Osmotic Potential on Germination

To evaluate the effect of osmotic stress, solutions with osmotic potentials of 0, 0.1, -0.2, -0.4, -0.6, -0.8 and -1.0 MPa were prepared by dissolving polyethylene glycol 8000 in distilled water as described by Michel (1983) [11]. Chemically scarified seeds were used in this experiment, and germination was determined as previously described.

2.6 Effect of pH on Germination

The influence of pH on the germination of *S. rhombifolia* seeds was determined by using buffer solutions of pH 5 to 9 (Chauhan and Johnson, 2008a) [4] together with a control treatment as distilled water of pH 6.2. Chemically scarified seeds were used in this experiment, and germination was determined as mentioned previously.

2.7 Effect of Seed Burial Depth on Seedling Emergence

The effect of seed burial depth on seedling emergence of *S. rhombifolia* was studied in a screenhouse (a chamber framed with 2-mm iron mesh and overhead transparent PVC cover to prevent rain damage) by placing 50 chemically scarified seeds in soil within 15-cm-diameter plastic pots. Seeds were placed on the soil surface or covered to depths of 0.5, 1, 2, 4, 6, 8 and 10 cm with soil. Soil with a pH of 6.2 and 1.3% organic carbon was passed through a 0.3- cm sieve and autoclaved before the pots were filled and commencing the experiment. Pots were subirrigated to maintain sufficient soil

moisture. Seedlings were considered emerged when a cotyledon could be seen, and the experiment was terminated when no further emergence was recorded for a continuous 7-day interval.

2.8 Statistical Analysis

All laboratory and screenhouse experiments were conducted in a randomized complete block design. Treatments of each experiment were replicated three times, each experiment was conducted twice, and the data were combined for analyses. Regression analysis (Sigma Plot 10.0) was used where appropriate; otherwise, means were separated using least significant difference (LSD) at $P=0.05$ (GENSTAT 8.0).

3. Results and Discussion

3.1 Effect of Seed Scarification on Germination

Chemical scarification with H_2SO_4 released *S. rhombifolia* seeds from dormancy and stimulated ($P<0.001$) germination. Germination increased with increased duration of scarification with H_2SO_4 up to 120 min (Table 1). Seeds treated with H_2SO_4 for 120 min resulted in 65% germination compared with 5% for nonscarified seeds.

Table 1: Effect of duration of scarification with concentrated sulphuric acid on germination of *Sida rhombifolia* seeds after 14 days of incubation in light/dark (12 h/12 h) regime at 35/25°C alternating day/night temperature

S. No.	Duration of scarification (min)	Germination (%)
1.	0	5.3
2.	5	7.3
3.	10	10.7
4.	30	16.0
5.	60	32.7
6.	120	65.3
7.	180	60.7
	lsd ($P = 0.05$)	11.5

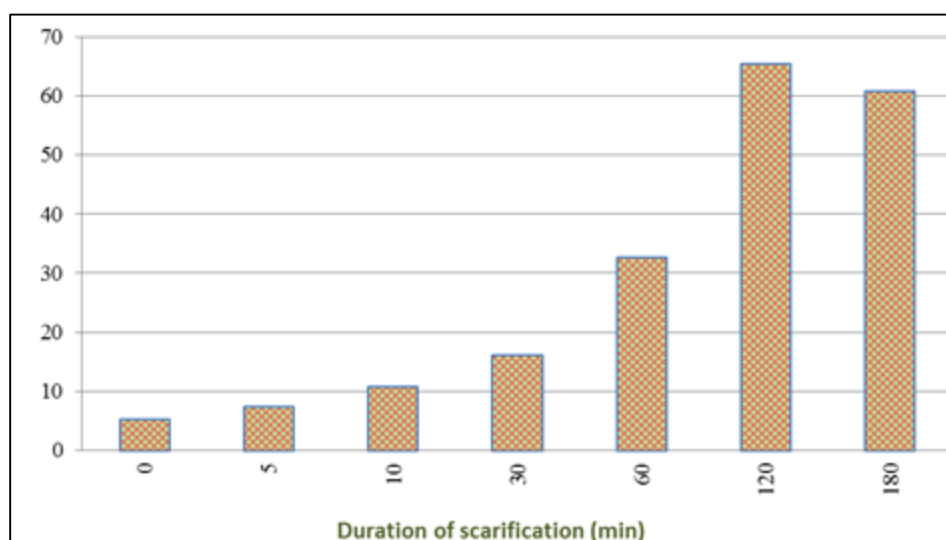


Fig 1: Effect of duration of scarification with concentrated sulphuric acid on germination of *Sida rhombifolia*

The increase in germination with scarification indicates that the hard seed coat is the primary mechanism of dormancy in *S. rhombifolia*. The stimulation in germination with scarification could be due to increased imbibition or the release from physical restriction of the seed coat. As an

alternative to acid treatment, seeds may be scarified by abrasion with sand paper, immersing briefly in boiling water, or cracking of the seed coat but these methods were not evaluated. The results suggest that seeds are unlikely to germinate in the field unless scarified and therefore seeds of

this species may persist in the soil for a long time, causing problem in future crops if scarification does not take place. Scarification in the field may occur due to temperature fluctuations, soil acids, incomplete predation by insects, fungi, bacteria and rodents, passage through the digestive tract of animals, abrasion by soil particles, and vegetation burning (Baskin and Baskin, 1998; Taylor, 2005) ^[1, 14]. Germination of *Malva parviflora* L. (little mallow), another species of Malvaceae family, was also stimulated by seed scarification (Chauhan *et al.*, 2006b) ^[7].

Table 2: Effect of alternating day/night temperatures (12 h/12 h) and light on germination of chemically scarified seeds of *Sida rhombifolia*

S. No.	Temperature	Germination (%)	
		Light	Dark
1.	25/15	2.75	8.7
2.	30/20	64.0	59.3
3.	35/25	63.3	58.7
LSD (p = 0.05)		10.9	

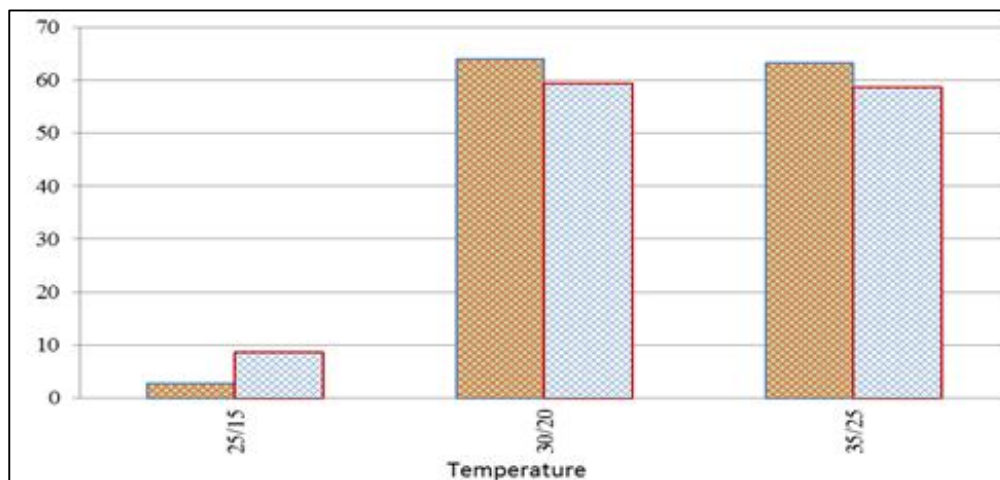


Fig 2: Effect of alternating day/night temperatures (12h/12h) and light on germination of chemically scarified seeds of *Sida rhombifolia*

3.2 Effect of Temperature and Light on Germination

Germination of chemically scarified seeds was influenced ($P < 0.001$) by the interaction between temperature and light (Table 2). At the higher temperatures, seed germination was not influenced by the light conditions. At the lowest temperature, however, germination was significantly lower in light/dark (3%) than in the dark (59%). This result indicates that germination of *S. rhombifolia* is inhibited by light at suboptimal temperatures. Similar results have been reported for other species (Chauhan *et al.*, 2006a) ^[6]. Similar germination response to light at higher temperatures suggests that seeds of *S. rhombifolia* could germinate whether buried in soil or exposed to light.

3.3 Effect of Salt Stress on Germination

A sigmoid response was observed in germination with increases in NaCl concentrations from 0 to 200 mM. Germination of chemically scarified seeds was greater than 55% upto the concentration of 50 mM NaCl; however, germination did not occur at 200 mM or greater concentration. As estimated from the fitted model, the concentration required for 50% inhibition of maximum germination was 111 mM NaCl. Soils with more than 100 mM NaCl (~electrical conductivity 10 mmhos/cm) are known with high level of salt content (Tanji and Kielen, 2002) ^[13], and our results indicate that seeds of *S. rhombifolia* may germinate in these saline soils. Crop cultivation, therefore, may be limited not only by salinity but also by weed competition.

3.4 Effect of Osmotic Potential on Germination

Germination of H₂SO₄ scarified seeds decreased linearly with decreasing osmotic potential from 0 to -1.0 MPa.

Germination decreased from 64 to 25% as osmotic potential decreased from 0 to -0.6 MPa, and germination did not occur at -1.0 MPa. The osmotic potential required for 50% inhibition of maximum germination was -0.49 MPa. In contrast to *S. rhombifolia*, germination in *M. parviflora* was completely inhibited at an osmotic potential of -0.6 MPa (Chauhan *et al.*, 2006b) ^[7]. Such comparisons suggest that *S. rhombifolia* could germinate under moderate waterstress conditions.

3.5 Effect of pH of Buffered Solution on Germination

Germination of H₂SO₄ scarified seeds of *S. rhombifolia* was not significantly affected ($P = 0.91$) by the tested range of pH solutions, and it varied from $60 \pm 3.7\%$ to $65 \pm 4.5\%$ over the pH range of 5 to 9. The results indicate that *S. rhombifolia* may germinate in many soil types used for growing most of the field crops in tropical countries. An optimum pH level is required during germination and growth of plants, but many weed species are tolerant of extreme pH levels (Evetts and Burnside, 1972) ^[8].

3.6 Effect of Seed Burial Depth on Seedling Emergence

Seed burial depth influenced ($P < 0.001$) seedling emergence of *S. rhombifolia*, though seedlings emerged from all burial depths ranging from 0 to 6 cm. Fifty-one per cent of the seedlings emerged from the seeds placed on the soil surface. Emergence was greater than 60% at burial depths of 0.5 to 2 cm, but decreased thereafter. Seedlings did not emerge from the seed burial depth of 8 cm or greater.

Decreased seedling emergence due to increased burial depth has been reported in several weed species (Chauhan *et al.*, 2006b; Chauhan and Johnson, 2008b) ^[7, 5], which could be linked to seed energy reserves. Larger seeds with greater

carbohydrate reserves can emerge from greater depths of burial (Baskin and Baskin, 1998) ^[1]. On the other hand, small-seeded species such as *S. rhombifolia* may have insufficient energy reserves to support hypocotyl elongation from deeper depths. Previous studies suggest that decreased germination at depth may be due to raised CO₂ derived from soil biological activity and slower gas diffusion, which is inversely correlated with burial depth (Benvenuti and Macchia, 1995) ^[2]. Similar results were reported for *Malva pusilla* Sm. (round-leaved mallow) and *M. parviflora*, with the greatest emergence occurring from 0.5 to 2 cm, with emergence declining from 3 to 6 cm, and no emergence occurred at 8 cm (Blackshaw, 1990; Chauhan *et al.*, 2006b) ^[3, 7].

In conclusion, germination of *S. rhombifolia* was stimulated by seed scarification, which indicates that the hard seed coat is the primary reason for inhibition of germination in this species. Results also suggest that seeds are unlikely to germinate in the field unless scarified and therefore seeds of this species may persist in the soil for a long time. Germination at the higher temperatures (30/20 to 35/25 °C) was not influenced by light conditions, suggesting that the introduction of no-till systems will make no difference from the aspect of light exposure. Seedling emergence was optimal at shallow burial depths, indicating that cultivation practices that achieve shallow burial of seeds may promote greater seedling emergence of *S. rhombifolia*. On the other hand, deep-tillage operations that bury the seeds below the maximum zone of emergence (i. e. 8 cm) would suppress emergence provided subsequent tillage is shallow to avoid the possibility of bringing back the seeds towards the soil surface.

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