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# ABSTRACT

Experiments were conducted to evaluate the effects of seasonal variations, seed treatments, temperature and light, and also the influence of different field condition, method of propagation and depth of seed burial on the germination, and development behavior of Sacciolepis interrupta. The weed possesses a high level of innate dormancy, and seeds were found to be dormant from September to April. The peak period of germination was found to be the 3<sup>rd</sup> and 4<sup>th</sup> weeks of June. Different seed treatments failed to break the dormancy in the off-season period, while in June, treatment with concentrated sulphuric acid resulted in highest germination percentage. Higher temperature of 38°C and continuous light for a period of 14 days resulted in highest germination percentage of 68%. Seeds, culm cuttings and root clumps had equal regeneration capacity. Continuous submergence was found to be ideal for seed germination. The emergence from surface sown seeds in pots was >70% and the emergence declined with increase in seeding depth. The present study, together with information on weed seed bank dynamics could be exploited for developing strategies for control and management of this weed.

#### Keywords: Dormancy, propagation and seed treatment

Seed germination is a complex physiological process affected by factors like temperature, moisture, light, seeding depth and others (Bewley and Black, 1994; Baskin and Baskin, 1998). Seed dormancy and survival are governed by fluctuations in soil temperature that vary with soil depth and the seed appears to have seasonsensing and burial-depth detection processes caused by changes in temperature. Seed dormancy plays a significant role in the survival of weeds owing to differential germination patterns that are controlled by light (seeding depth) and other environmental conditions (temperature and moisture) in the seed surroundings. Since light generally does not penetrate the soil for more than a few centimeters, the seed dormancy cycle is mostly determined by changes in temperature (Baskin and Baskin, 1994) which differs with soil depth (vanAssche and vanlerberghe, 1989) and moisture (Richard and Street, 1984).

Weed infestation in rice is one-of the major yield barriers responsible for low productivity, especially under direct seeded rice. *Sacciolepis interrupta* is a tropical grass weed, which mimics rice, belonging to the family Poaceae and commonly called as '*Pollakkala*' in Malayalam, meaning a weed that is hollow. It is an annual or perennial herb and is reported to flower and fruit year round when vegetatively propagated and the flowers are pollinated by insects. It can be distinguished from other species of *Sacciolepis* by its robust, spongy, floating culms and large spikelets. Its rhizomatous structure, and habit of rooting from lower nodes, makes it a perennial plant and it further grows and multiplies as separate individuals simultaneously with rice, owing to mimicry with rice seedlings (Clayton *et al.*, 2006). Renu (1999) conducted primary laboratory studies for testing the germination of *S. interrupta* and found that the seed was dormant and the germination rate of seeds under laboratory conditions was very poor.

A study on the germination ecology of the weed will give information on the germination, emergence, regeneration and development behavior, which can be exploited for effective weed management. In India, no systematic work on the germination ecology of this weed has been done. So the present study was undertaken to study the germination behavior of *S. interrupta* under laboratory conditions as well as in pot culture.

# MATERIALS AND METHODS

Seeds of *S. interrupta* were collected from areas of severe infestation from different rice growing tracts of Kerala, in the months of August and September. A composite sample was made by pooling the seeds of many plants, and stored in air tight containers for further studies. Seeds were collected in three consecutive years *i.e.* 2016, 2017 and 2018, to compare the viability of newly shed (fresh), and seeds collected earlier (six month old seeds) as well as full matured, and physiologically matured seeds.

#### Germination studies

Laboratory experiments were conducted during 2017 and 2018 in Department of Agronomy, College of Horticulture, Vellanikkara, Kerala Agricultural

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University. To understand the germination behaviour of *S. interrupta*, soil was collected from areas of major weed infestation, and solarized for one month for conducting pot culture studies. Completely randomized experimental design was adopted for these experiments. The details of materials and methodology adopted for the following studies were as follows:

#### Seed viability

Fresh and six month old seeds of *S. interrupta* were soaked in water for 12 h for full imbibition and crushed with pestle and mortar to expose the embryo for easy staining. 25 crushed seeds of both types were placed separately in petri plates lined with moist filter paper in three replicates and later 0.5% tetrazolium solution (Tz) was added. Embryos were tested for pink coloured staining.

### Seasonal variations in germination

Non-scarified fresh and six month old seeds, twentyfive of each type were placed in petri dishes lined with filter paper and soaked in distilled water at room temperature and tested for germination at weekly intervals upto four weeks. Subsequently the test was repeated at monthly intervals for 12 months. Seeds with 1 mm emerged radical were considered as germinated.

#### Effect of seed treatments on germination

Seeds of fresh and six month old (twenty-five per type, in three replicates) were subjected to different seed treatments like mechanical scarification *i.e.*, breaking seed coat by rubbing with sand paper, and acid scarification using diluted sulphuric acid (10% and 50%), concentrated sulphuric acid, and 1% solution each of nitric acid, per chloric acid, potassium hydroxide, for varying periods (5 min to 1 h). Then seeds were washed thrice in running water and allowed to germinate in petri dishes at room temperature. Scarification by placing seeds in boiling water for short interval (3-10 min) was also tested.

# Effect of temperature and light

Fifty seeds of *S. interrupta*, of both fresh and one year old seeds were taken, soaked for 24 h in water and one set was placed in petri dishes filled with sterilized moist sand, and another in petri dishes lined with moist filter paper, incubated at 28°, 33° and 38°c in an incubator under continuous light, continuous dark and alternate light and dark conditions for 14 days each and rate of germination was determined.

#### Ideal conditions for germination

Fifty fresh seeds of *S. interrupta* were sown in pots simulating three field situations, T<sub>1</sub>-upland (moist

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conditions maintained by irrigating on alternate days for a period of one month),  $T_2$ - lowland with continuous flooding- (puddled soil flooded throughout for a period of one month), and  $T_3$ - lowland conditions with alternate flooding and draining (puddled soil flooded for 10 days alternated with five days without flooding for a period of one month). Factorial combinations of the three treatments in the presence or absence of rice, seeds were maintained in five replicates, with 4 pots in each treatment per replication for a period of one month, and emergence and establishment of *S. interrupta* were recorded.

# Methods of propagation

To assess the regeneration capacity of *S. interrupta*, different plant parts like seeds, culm cuttings with nodal roots and root clumps were grown in pots in three replicates and germination of seeds, and regeneration capacity of other plant parts was assessed in the most suited condition of growth.

# Effect of depth of burial

Twenty five seeds of *S. interrupta* were placed at 0 (surface), 2, 5, 10 and 15 cm depths in soil in pots and germination and emergence were observed at weekly intervals. Each treatment was replicated three times.

#### Study on seed longevity

Seeds of both rice and *S. interrupta*, and *S. interrupta* alone were placed in plastic net bags (25 seeds per bag) and buried in pots at depths of 0, 2, 4 and 8cm from the surface and filled with soil for one year. The treatments consisted of 8 factorial combinations, with four sowing depths in the presence or absence of rice in three replicates. Germination was recorded at biweekly intervals for a period of one year, by taking out the seeds from net bags and sowing them in pots.

### **RESULTS AND DISCUSSION**

#### Seed viability

Both six month old as well as fresh seeds, showed pink colour staining. Among both lots, fresh seeds were more viable and showed higher proportion of stained embryos compared to the six month old seeds (Plate 1).

### Seasonal variations in germination

Non-scarified fresh and six month old seeds of *S. interrupta* when allowed to germinate monthly, showed 0% germination in initial three months *i.e.*, from January to March, as well as in the last four months *i.e.*, September to December. The germination improved from the 3<sup>rd</sup> week of April onwards in fresh seeds and from 1<sup>st</sup> week of May in case of old seeds. Germination percentage was highest in the 3<sup>rd</sup> and 4<sup>th</sup> weeks of June



Plate 1: Seed viability in S. interrupta

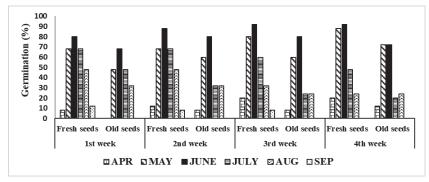


Fig.1: Seasonal variations in germination of S. interrupta

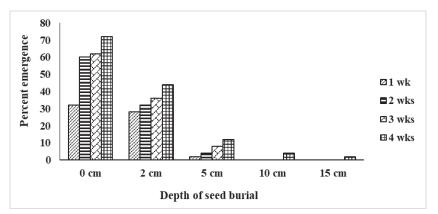


Fig. 2: Effect of burial depth on S. interrupta emergence

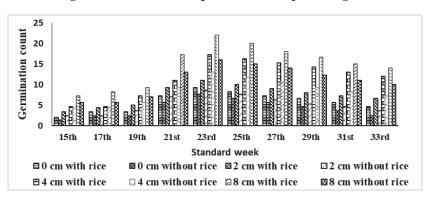


Fig. 3: Effect of depth of seed burial with and without rice seeds on germination of S. interrupta

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Treatments	Germination of new seeds (%)		Germination of six months old seeds (%)	
	September	May	September	May
T <sub>1</sub> Breaking seed coat	0	68	0	48
$T_2$ Sulphuric acid (10%)	0	72	0	60
$T_{3}$ Sulphuric acid (50%)	0	72	0	60
T <sub>4</sub> Sulphuric acid (Conc)	0	80	0	60
$T_{5}$ Nitric acid (1%)	0	64	0	52
$T_{6}$ Per chloric acid (1%)	0	64	0	64
$T_7$ Potassium hydroxide (1%)	0	64	0	48
$T_{8}$ Boiling water (100°C)	0	72	0	52
T <sub>o</sub> Control	0	64	0	36

Table 1: Effect of seed scarification treatments on germination of S. interrupta

for both fresh and old seeds (92 and 80% respectively) (Fig. 1). Fresh seeds showed a higher rate of germination compared to six month old seeds, in the months when germination occurred, probably due to the non-dormancy of freshly harvested seeds compared to old seeds. Similar results was confined in nine different *Rumex* species by vanAssche *et al.* (2002). Abraham and Abraham (2005) studied the seasonal variations in *Mikania* species and found that peak period of germination was during May to June *i.e.*, on first receipt of rains, and fresh seeds had higher germination rate.

# Effect of seed treatments on germination

Both fresh and six month old seeds showed a high rate of dormancy when experiments were conducted from September to April, and all the scarification treatments including hot water treatment, failed to induce germination. But when the experiments were conducted during May to August, it resulted in very good germination. The response of old seeds to scarification treatments was higher compared to fresh seeds. Dhawan (2009) reported that both mechanical scarification and chemical scarification resulted in 100% germination in one, two and three year old seeds of Melilotus species and six month old seeds had higher germination rate than fresh seeds. Treatment with concentrated sulphuric acid for 5 to 30 min resulted in 80% germination in fresh seeds, and perchloric acid (1%) recorded highest germination percentage of 64% in six month old seeds compared to control, which had lowest rate of germination in both the cases. Tigabu and Oden (2001) reported that concentrated H<sub>2</sub>SO<sub>4</sub> caused modification or scarification of the hull or seed coat membranes, and also supplied additional oxygen to the seed and thereby caused breaking of seed dormancy of many species with impermeable seed coat. In general, the fresh seeds had high rate of germination percentage compared to older seeds even after scarification (Table 1).

#### Effect of temperature and light

Germination of *Sacciolepis* seeds was influenced by the interaction between temperature and light. Light strongly stimulated germination, while darkness completely inhibited germination of *S. interrupta*. Maximum germination (68%) occurred when seeds were exposed to higher temperature of 38°C continuous light for a period of 14 days. With the same temperature under alternate light and dark conditions, a germination of 54.7% was recorded. At low temperatures, there was no influence of light on germination and germination was 0% (Table 2).

 Table 2: Effect of temperature and light on germination of S. interrupta

Temperatu (°C)	re M	ean germina (%)	ion	
-	Continuous light		Alternate light and dark	
28	0.0	0.0	0.0	
33	46.7	0.0	0.0	
38	68.0	0.0	54.7	

Temperature and light are considered as important factors influencing seed germination of several weed species (Benvenuti *et al.*, 2004; Burke *et al.*, 2003). Seeds of some weed species like tropical signal grass (*Urochloa subquadripara*) germinate equally in light and dark conditions (Teuton *et al.*, 2004) and seeds of other species like Chinese sprangletop (*Leptochloa chinensis* (L.) Nees) and barnyard grass (*Echinochloa crus-galli* (L.) Beauv.) require light to stimulate germination (Chauhan and Johnson, 2008; Boyd and Acker, 2004). *S. interrupta* seeds germinated at higher temperatures both under continuous light as well as alternate light and dark conditions, showing that the weed was positively photoblastic under high temperature regimes.

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Treatments	7 DAS	14 DAS	<b>30 DAS</b>
Main factors			
T <sub>1</sub> - Upland	3	4	4
$T_2$ - Lowland with continuous flooding	21	31	29
$T_3^2$ - Lowland with alternate flooding and draining	12	21	18
LSD (0.01)	2.18	1.70	1.91
Sub factors			
S <sub>1</sub> - Sacciolepis with rice seeds	9	14	13
$S_2^2$ - Sacciolepis without rice seeds	16	23	21
LSD (0.01)	1.78	1.38	1.62
Interaction			
T <sub>1</sub> S <sub>1</sub>	4	6	6
	2	2	2
$\mathbf{T}_{1}\mathbf{S}_{2}$ $\mathbf{T}_{2}\mathbf{S}_{1}$	27	37	35
T,S,	15	24	22
$\mathbf{T}_{2}\mathbf{S}_{2}$ $\mathbf{T}_{3}\mathbf{S}_{1}$	15	26	23
$\mathbf{T}_{3}\mathbf{S}_{2}$	9	16	14
LSD (0.01)	2.26	2.40	2.81

Table 3: Effect of soil moisture status on emergence of S. interrupta

Table 4: Effect of methods of	propagation on morpholog	ical characters of S. interrupta

Sl. No.	Characters	Seeds	Culm cuttings	Root clumps
1	Plant height (cm)	112-127	68-72	83-87
2	No. of tillers (No's)	2-3	4-6	4-6
3	Leaf length (cm)	24-27	29-32	34-37
4	Leaf width (cm)	0.7-0.9	1.4-1.6	1.3-1.4

### Ideal condition for germination

S. interrupta seed, under continuous submergence showed higher germination and emergence compared to upland condition. In the treatment lowland with continuous flooding highest germination percentage was recorded at 7, 14 and 30 days after sowing, followed by lowland conditions with alternate flooding and drying, and the emergence was lowest under upland conditions. Kaur et al. (2008) reported that under high moisture conditions emergence and growth of rice weeds, especially grassy weeds, was more compared to dry conditions. S. interrupta responded well to flooding, which was the ideal condition for its germination, indicating that flooding in rice may not help in decreasing the emergence of the weed. Burial along with rice was found to significantly reduce the germination of S. interrupta at all dates of observation and this may be due to the competition exerted by rice seeds on S. interrupta, inhibiting its germination, Jose et al. (2013) also found that the germination percentage of weedy rice was 56% in presence of rice and it was 67% in absence of rice. The interaction effect was significant and highest

emergence rate of 27, 37 and 35 was observed at 7, 14 and 30 DAS respectively in lowland condition with continuous flooding, in the absence of rice seeds. The rate of germination was low when *S. interrupta* was sown along with rice seeds and it was higher in absence of rice seeds (Table 3).

#### Methods of propagation

*S. interrupta* showed different methods of propagation. Seeds, culm cuttings and root clumps were on par in germination and regeneration capacity. Morphological characters like branching, tillering, leaf length, and leaf width was found to be improved in vegetatively propagated plants compared to seed propagated ones, whereas plant height was greater in seedlings (Table 4).

# Effect of depth of burial

The emergence of *Sacciolepis* decreased as the depth of seed placement in soil increased (Fig. 2) which is quite remarkable as the seed of many species when placed on surface had little or no emergence (Singh *et* 

Treatments	Seeds germinated (number)		
	23 <sup>rd</sup> Std wk	25 <sup>th</sup> Std wk	27thStd wk
Main factors			
$T_1$ - 0 cm depth	9	8	7
$T_2 - 2 \text{ cm depth}$	10	9	8
$T_3 - 4$ cm depth	15	14	13
$T_4^-$ 8 cm depth	19	18	16
LSD (0.01)	1.51	1.39	1.43
Sub factors			
S <sub>1</sub> - <i>Sacciolepis</i> with rice seeds	15	14	12
$S_2^-$ Sacciolepis without rice seeds	11	10	9
LSD (0.01)	1.07	0.98	1.01
Interaction			
$T_1S_1$ - Sacciolepis with rice seeds at 0 cm depth	9	8	6
$T_1S_2$ - Sacciolepis without rice seeds at 0 cm depth	8	7	7
$T_{2}S_{1}$ - <i>Sacciolepis</i> with rice seeds at 2 cm depth	11	10	9
$T_{2}S_{2}$ - <i>Sacciolepis</i> without rice seeds at 2 cm depth	9	8	6
$T_3S_1$ - <i>Sacciolepis</i> with rice seeds at 4 cm depth	17	16	15
$T_3S_2$ - Sacciolepis without rice seeds at 4 cm depth	13	12	11
$T_{4}S_{1}$ - <i>Sacciolepis</i> with rice seeds at 8 cm depth	22	20	18
$T_4 S_2^{-}$ - Sacciolepis without rice seeds at 8 cm depth	16	15	14
LSD (0.01)	2.14	1.97	2.02

Table 5: Effect of depth of burial with and without rice seeds on germination of S. interrupta

al., 2007). The per cent of seedling emergence decreased drastically beyond the placement depth of 5 cm and it was only 12% at placement depth of 5cm. About 72% of seeds germinated when seeds were placed at the surface (0 cm) and it was 44% at 2 cm depth by end of 4 weeks after sowing. It was thus clear that Sacciolepis emerged mostly from shallow depths of the soil and seeds located deep in the soil beyond 5cm, had less chance of emergence. The findings were confirmed by Benvenuti et al. (2004) who reported progressive decline in seedling emergence of grass weeds like Leptochloa chinensis with increasing depth of seeding. Tillage, thus has a profound influence on the emergence of S. interrupta, when new lots of buried seed are brought to the surface, coinciding with the receipt of rains and development of submerged conditions which favour germination.

#### Seed longevity

Weed seed persistence in the soil is a common trait. Seeds of many weed species can remain viable in the soil longer than 10 years (Burnside, 1996). Seed longevity is the prime contributor for weed seed persistence. When the effect of seed longevity on germination percentage of *S. interrupta* seeds was tested through the year, it was found to be highest in the month of June (23<sup>rd</sup> to 25<sup>th</sup> standard week) compared to other months.

Davis et al. (2005) reported that environmental factors like seed depth placement, tillage and abiotic environmental factors affect seed persistence. Tillage systems influence vertical weed seed distribution in the soil profile, and this differential distribution could affect weed seedling emergence (Chauhan et al., 2006). Seed longevity studies showed that shallow burial depth had a higher rate of viability loss than burial at deeper depth. Highest germination in S. interrupta was recorded in the month of June irrespective of the depth of burial. The rate of germination was found to be increased in presence of rice, and this could be correlated with the findings of Takeuchi et al. (2001) who reported that germination of submerged Monochoria seeds was accelerated by the presence of rice seeds, and rice seeds play a critical role in promoting germination of Monochoria species (Xuan et al., 2016). In S. interrupta germination rate was highest in 23rd standard week in the treatment T<sub>4</sub>*i.e.*, burial at 8cm depth along with rice seeds (Table 5). Weed seed persistence in the soil seed bank was proportional to burial depth (Harrison et al., 2007).

Abeyesekara et al. (2010) found that germination of seeds buried at 20 cm depth was 68% four weeks after burial and decreased to 33% at 80 weeks after burial from seed burial studies conducted at Sri Lanka. The germination in S. interrupta was found to increase from 15th standard week *i.e.*, 1st week of May, and was highest during 23rd standard week *i.e.*, June, and started to decline from there and reached zero by end of 33rd standard week *i.e.*, 4<sup>th</sup> week of August (Fig. 3). Significantly higher germination percentage was observed in seeds buried at 8 and 4 cm depth, as compared to 2 cm and surface buried seeds. The presence of rice seeds significantly improved germination of Sacciolepis, and was highest in the 23rd standard week. Interaction effects also revealed significantly higher germination when buried at deeper depths with rice seeds. At both 23rd and 25th standard weeks, highest germination was observed when buried at 8cm depth along with rice seeds.

Seeds of *S. interrupta* are characterized by small, dormant seeds which are able to germinate only under favorable environmental conditions. There are two possiblelocations of seed dormancy mechanisms among grass species *i.e.*, innate dormancy (primary dormancy), which occurs in the embryo covering structures, or inside the embryo, and induced dormancy (secondary dormancy) which are due to environmental conditions (Simpson, 1990; Adkins *et al.*, 2002).

Embryos of *S. interrupta* seeds were found to be viable, but failed to germinate even after scarification treatments in the off season, but almost all of them were able to germinate during the period from May to August, indicating a high level of innate dormancy which required a biochemical trigger, which in *S. interrupta* could be related to the season.

The results obtained from the study reveal that the seeds of *S. interrupta* are dispersed with high levels of induced dormancy and are able to germinate in both upland as well as flooded conditions. This species is one of the very few which are able to germinate even under complete oxygen depletion. This may be the reason of the shift of the weed from dry seeded rice to wet seeded rice too. Different methods of propagation and regeneration capacity of different plant parts, promote its survival on the field bunds even after tillage operations, leading to increase in soil seed bank during off season also.

*S. interrupta* seed germination is strongly influenced both by temperature and light. Higher temperatures with continuous light triggered the germination. Seeds placed on the soil surface and at 2 cm depth had higher rate of germination and emergence, showing the absolute requirement of light for germination as one of the reasons for response to burial depths. Seed longevity studies, revealed that prolonged seed persistence of *S. interrupta* seeds could be expected, and a persistent seed bank due to secondary dormancy would favour the periodic reappearance of species and increase in the soil seed bank.

The present study, together with other information on weed seed bank dynamics could improve knowledge required for more efficient weed control and management. At shallow burial depths, seedling emergence was optimal indicating that cultivation practices that achieve shallow burial of seeds may promote greater seedling emergence of *S. interrupta*. On the other hand, deep-tillage operations that buries the seeds below the maximum zone of emergence (*i.e.*, 8 cm) would suppress emergence but increase the soil seed bank in the long term. The survival mechanisms of *S. interrupta* are indicative of the strong possibility of its emergence as a major threat in rice production in the humid tropics.

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