



RESEARCH

Environmental Factors Influencing Seed Germination and Seedling Emergence of *Cyperus iria*

K. Nishanthan^{1*}, S. Dissanayaka², L. Pradheeban¹, T. Sivananthawerl² and B. Marambe²

¹Department of Agronomy, Faculty of Agriculture, University of Jaffna, Sri Lanka

²Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka

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Nishanthan, K. 
<https://orcid.org/0000-0001-8346-6559>



ABSTRACT

Weeds are often the principle biotic constraint to paddy crop production reducing paddy yield by about 15–21 % globally and by 20-40 % in Sri Lanka. The existing climatic factors are highly favourable for the growth of many weed species, which offer competition with paddy. Mature viable seeds of *Cyperus iria*, randomly collected from paddy fields in the five districts of the Northern Province of Sri Lanka, were used to assess the impact of selected environmental parameters and agronomic practices on their germination and seedling emergence. The weed seeds from different districts were subjected to four combinations of day/night temperatures coupled with three light durations in a Completely Randomized Designs as a two-factor factorial experiment. The temperature x light duration interaction with the highest seed germination was combined with different flooding heights and durations, followed by soil-burial depths. The frequencies of seed germination and seedling emergence were analyzed using the Chi-square test in SAS 9.1 software (P=0.05). *C. iria* seeds from Vavuniya recorded the highest seed germination at all combinations of day/night temperature and light durations, while the lowest was recorded from Jaffna. Seed germination was the highest at day/night temperature 35/30 °C and the lowest at 25/20 °C (P<0.05). The highest frequency of seed germination was observed in 24 h light and the lowest in 24 h dark, under all day/night temperatures. Flooding 5 days after sowing (DAS) at a height of 5 cm showed the lowest seedling survival measured at 28 DAS (P<0.05). The seedling emergence of *C. iria* was restricted (P<0.05) at soil burial depths of 2.5 cm and 5 cm. The results revealed that 25/20 °C day/night temperatures in 24 h darkness, seed burial >1 cm soil depth, and flooding at 5 cm height from 5 DAS suppressed *C. iria* seedling emergence and growth.

*Corresponding author- knishanthan81@gmail.com

INTRODUCTION

The success of a weed in any agro-ecosystem is determined by the rate of seed germination and seedling emergence, which are regulated by several environmental factors such as temperature, light, soil moisture, and pH (Koger, 2004). Photoperiodism influences the breaking of seed dormancy and enabling seeds to germinate readily under light than under dark conditions (Baskin and Baskin, 1998). Temperature plays a vital role in determining the periodicity of germinating seeds and the species distribution (Guan, 2009; Suriyasak et al., 2020). Seed germination rate usually increases linearly with increasing temperature, at least within a distinct range, and declines sharply at higher temperatures (Alvarado and Bradford, 2002). Light is an important ecological determinant for seed germination where the absence of light acts as an indicator that prevents germination of many seeds (Cristaudo et al., 2007).

Seed germination substantially varies with soil depth (Benvenuti et al., 2001a; Burmeier et al., 2010). Previous studies have demonstrated that seed germination and/or seedling emergence rates generally decline with seed burial depth (Benvenuti et al., 2001b; Traba et al., 2004; Burmeier et al., 2010), affecting the survival of the weed-seed bank in soils. When the seeds are buried in deep layers, the air permeability of the soil is reduced and the light is weakened, thereby inhibiting seed germination and seedling emergence (Chen and Maun, 1999; Li QY et al., 2006; Vleeshouwers, 1997). Furthermore, flooding can minimize weed incidence in agro-ecosystems; for example, in lowland paddy cultivation (Juraimi et al., 2011) mainly due to creation of an anaerobic condition (Caton et al., 2002; Ampong-Nyarko and De-Datta, 1991; Rao et al., 2007; Ismail et al., 2009, 2012; Mahajan and Chauhan, 2013). Further the timing, duration, and depth of flooding determine the extent of weed suppression and the weed-seed bank in soil (Mortimer et al., 2004).

Light is an important ecological indicator playing a crucial role in the rate and proportion of germination (Finch-Savage and Leubner-Metzger, 2006). The absence of light

prevents seed germination (Crisaudo et al., 2007), which can be induced again once transferred to complete light. Response of seed germination to light varies from species to species where sedge weeds such as *C. difformis*, *C. iria* and *Fimbristylis miliacea* require absolute light for germination (Chauhan and Johnson, 2009a).

The seed germination rate of plants usually increases linearly within the optimum temperature range but declines sharply at higher temperatures (Alvarado and Bradford, 2002). Day/night temperatures of 30/20 °C and 35/25 °C enhances seed germination of some weed species such as synedrella (*Synedrella nodiflora*) (Chauhan and Johnson, 2009b) and rice flatsedge (*C. iria*) (Chauhan and Johnson, 2009a). Chozin and Nakagawa (1988) showed that seed germination rate of *C. iria* is higher at a constant temperature of 35 °C (69%) than at 15 °C (17%). Similar results were reported for many other plant species (Vidal et al., 2007; Rizzardi et al., 2009; Ghaderi et al., 2010).

Cyperus iria is a troublesome sedge weed in agricultural fields, particularly in lowland paddy cultivation, due to its competitive nature and ability to reduce crop yields significantly. Environmental factors including temperature, light, flooding, and burial depth can greatly influence the germination, growth, and reproductive success of aggressive agricultural weeds such as *Echinochloa crus-galli* (Marambe et al., 1997), *Rumex obtusifolius* (Benvenuti, 2001b), *Ludwigia hyssopifolia* (Chauhan and Johnson, 2009a), *C. difformis* (Derakshan and Gherekhloo, 2013) and *Nasella dichotoma* (Humphries et al., 2018). Investigating the effects of these environmental conditions and agronomic practices such as ploughing depth and flooding height and duration on *C. iria* can provide insights into its adaptive capacity and help predicting its distribution and abundance in different regions in Sri Lanka and devise control measures

Therefore, the objective of this study was to elucidate the response of *C. iria* to changes in the environmental conditions such as temperature, light duration, seed burial depth

and flooded conditions during seed germination and seedling growth.

METHODOLOGY

The study consisted of three experiments conducted sequentially at the Faculty of Agriculture, University of Jaffna at Kilinochchi, Sri Lanka. Twenty-five mature *C. iria* inflorescences were randomly collected from paddy fields heavily populated with this sedge weed from each of the five districts, namely, (a) Jaffna: (9.6930° N, 80.1652° E), (b) Mannar (8.9810° N, 79.9044° E), (c) Mullaitivu (9.2675° N, 80.8128° E), (d) Kilinochchi (9.3803° N, 80.3770° E), and (e) Vavuniya (8.7542° N, 80.4982° E) in the Northern Province of Sri Lanka. The collected weed inflorescences were pooled separately and kept in separate paper bags. The inflorescences were subsequently mixed with sand to facilitate rubbing and to extract mature seeds. The extracted seeds were cleaned manually and preserved in a plastic container under refrigeration (4 °C) until further use. The seed viability of the collected seed lots were tested before their use. Fifty (50) randomly selected seeds were placed in Petri dishes laid with a Whatman No. 1 filter paper, initially moistened with 5 ml of distilled water. The filter papers with seeds were kept moistened continuously during the study at room temperature. The number of germinated seeds was counted after 3 to 4 days and a germination percentage >80% was considered as viable seeds.

Experiment 1. Effect of day/night temperature and duration of light on seed germination and seedling emergence

Germination of freshly harvested seed samples collected from five districts was determined in a multi-thermo incubator (MTI 202, Japan) containing five chambers, under four levels of day/night temperature combinations coupled with three levels of light duration. Two incubators were used for the experiment: one provided the light treatment while the other provided the dark treatment. The experiment was conducted for different treatment combinations in a sequence due to the limited space available.

The experiment was conducted as a three-factor factorial in a Completely Randomized Design. The day and night temperatures were set to represent the annual variation of those parameters in the Northern Province, *i.e.*, the maximum temperature from 28 °C to 37 °C and minimum from 22 °C to 28 °C (Northern Province Statistical Information, 2020). The day and night temperatures were set as greater, within, and lower than the average temperature in the Northern Province of Sri Lanka. Thus, the four levels of day/night temperature regimes used in the study were (i) 25/20 °C, (ii) 30/25 °C, (iii) 35/30 °C, and (iv) 40/35 °C while the three levels of light duration were (i) continuous light (11 klux – Specific thermal incubator light intensity), (ii) 12 h light / 12 h dark, and (iii) continuous dark periods. In each day/night temperature combinations, the diurnal difference was maintained at 5 °C to represent the diurnal temperature difference of the districts in the Northern Province (<https://www.weather-forecast.com/>). Fifty seeds of *C. iria* from each composite sample from the five districts of the Northern Province were placed evenly in Petri dishes (9 cm diameter) containing two Whatman No. 1 filter papers. Four replicates were used per treatment. Each Petri dish was initially moistened with 5 ml of distilled water and the filter paper was kept moistened continuously. For germination in complete darkness, Petri dishes were wrapped in two layers of 0.024 mm thick aluminum foil and for 12 hours darkness, all Petri dishes were covered with a sheet of aluminum foil when the dark treatment was imposed. Daily germination count was taken until 15 days and the germination percentage was calculated.

Experiment 2. Effect of flooding on seed germination and seedling emergence

The combination of day/night temperature x light duration that provided the highest germination percentages in experiment 1 were selected for the experiment 2. Plastic containers with flat and non-perforated bottom (25 cm length x 22.5 cm width x 19 cm height) were filled with paddy soil collected from a farmer's field to a height of 8 cm (6.25 kg soil). The soil was not sterilized prior to use to represent the field conditions, but preliminary studies were conducted to ensure

C. iria seeds were not present by ensuring that no seed germination was observed for at least 14 days. Fifty randomly selected *C. iria* seeds from each district of the Northern Province were row-planted in separate containers. The seeds were planted on the soil surface, and then covered loosely with a 1 cm soil layer, moistened continuously using a hand sprayer for the first 4 days. Paddy soil was kept moistened at these early stages to represent paddy field conditions as it affects the germination of seed paddy. Water was impounded gradually to heights of 0 (no flooding), 2.5 cm and 5 cm from the soil surface at 5, 10, 15 and 20 days after sowing (DAS) and the water levels were maintained up to 28 DAS. The treatment was imposed ensuring that the seeds below the soil surface will not float. The experiment was conducted in a rain shelter as a two-factor factorial (Factor 1 = height of the water level, and Factor 2 = flooding duration) in a CRD with three replicates (180 containers), each replicate containing 50 seeds. Seedling emergence was defined as the appearance of the coleoptile. The seedlings were counted at 3-day intervals until 28 DAS for all treatments.

Experiment 3. Effect of seed burial depth on seed germination and seedling emergence

Paddy soil collected from the Northern Province of Sri Lanka was filled into bottom-perforated plastic pots (30 cm diameter x 20.5 cm height; 0.0144 m³ volume). The soil was kept moistened to the field capacity and kept for 3-4 days until the weed seed bank in the soil germinated. After germination, all weed seedlings were removed. For each burial treatment, the soil to the respective depth was taken out from the pot, and *C. iria*

seeds were spread evenly, covered with the same volume of removed soil to ensure all the treatments were sown to the correct depth. Fifty seeds of *C. iria* were randomly selected from five composite seed samples, each representing the five different districts in the experiment. The treatment structure used in the experiment is presented in Table 1. The maximum burial depth was selected from our unpublished data that showed *C. iria* seeds did not germinate if buried deeper than 5 cm in paddy soil.

The surface broadcasted seeds (burial depth = 0 cm) was used as the control to compare the germination behavior of seeds placed in different burial depths. Each burial depth for a district had four replicates (80 pots) laid down in a CRD. Soil in the pots was moistened initially using a hand sprayer and irrigated when required to keep it moistened continuously. Seedling emergence (visibility of the coleoptile) was measured. The experiment was terminated when no further emergence was recorded for 20 continuous days.

Statistical analysis

Germination percentage data were analyzed using PROC CATMOD procedure in a log linear model with maximum likelihood analysis of variance using the SAS (9.1) software. The frequencies of seed germination and seedling emergency were analyzed using the Chi-square test in SAS software test at $p=0.05$. The seedling count at 28 DAS was analyzed using the PROC CATMOD procedure in a log linear model with maximum likelihood analysis of variance in the SAS software. Initially, the effects of flooding height, flooding date, and districts were evaluated in the model.

Table 1. Treatments and seed burial depths

Treatments	Seed burial depth from the soil surface
T ₁	0 cm
T ₂	1 cm
T ₃	2.5 cm
T ₄	5 cm

RESULTS AND DISCUSSION

Effect of temperature and light

The maximum likelihood analysis was conducted for the first-order effects where the model was saturated, and the likelihood ratio (G^2) was found to be non-significant ($p=1.000$). Therefore, the interaction effects (second order) were not evaluated for the germination data. Results showed that the germination percentage of *C. iria* was significantly ($p<0.0001$) affected by increasing the day/night temperature combinations, duration of light, and the district where seeds were collected (Table 2).

The results indicated a significant association ($P<0.001$) between the district and the observed germination frequencies. The highest germination was reported in *C. iria* seeds collected from the Vavuniya district and the least was reported from the Jaffna district (Figure 1). This geographic variation observed in the germination chamber may be due to the

seed source or genetic variation, microclimate differences, and localized environmental influences. Furthermore, seed properties such as seed size (Dunlap and Barnett, 1983), seed coat hardness or permeability (Rolston, 1978), and seed reserves and hormones (Kucera et al., 2005; Lv et al., 2018) determine seed germination, which were not analyzed in the present study.

The germination of *C. iria* seeds had a significant association with the day/night temperature with the lowest recorded at 25/20 °C, where a 22.7% reduction was observed compared to the highest recorded at 35/30 °C, and 17.23% than the temperature combination of 40/35 °C (Figure 1). Temperature is an important and critical environmental factor determining the timing of germination (Koger et al., 2004; Fenner and Thompson, 2005; Vicente et al., 2020), affecting both the germination power and germination speed (Yilmaz, 2008).

Table 2. Maximum Likelihood Analysis of Variance at level 1 for temperature, district, and light

Source	Degrees of Freedom	Chi-Square	P>Chi-Square
Temperature	3	47.08	<0.0001
District	4	26.37	<0.0001
Light	2	465.38	<0.0001
Likelihood Ratio	50	16.16	1.0000

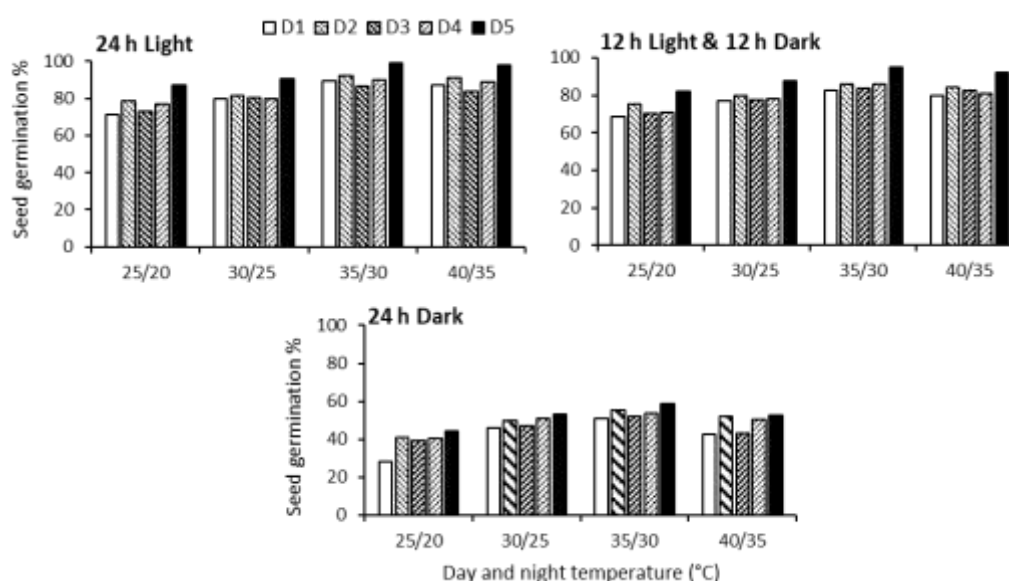


Figure 1. Effect of temperature and light on *C. iria* seed germination in five districts of Northern Province. D₁ = Jaffna, D₂ = Mannar, D₃ = Mullaitivu, D₄ = Kilinochchi, and D₅ = Vavuniya.

Temperature has a substantial impact on both biochemical and physiological processes in viable seeds. In the present study, *C. iria* seeds showed an increase in germination up to the temperature 35/30° C and a decrease in temperature beyond 40/35° C. The day/night temperatures 35/30 °C may be the optimal for *C. iria* seed germination. Marambe et al., (1992) also reported that the rate of seed germination is faster under optimal conditions due to rapid water absorption by seeds. A similar pattern was observed in *C. difformis* seeds, which showed greater germination at day/night temperatures 30/20 °C (91.5%), 35/25 °C (93%) and 40/30 °C (88%) than at 25/15 °C (Derakhshan and Gherekhloo, 2013). Some studies have shown that the number of germinated seeds increases linearly as the temperature rises to an optimal level, and decreases linearly at higher temperatures (Steinmaus, 2000; Bradford, 2002).

The different light durations (24 h light, 10 klux), 12 h light/12 h dark and 24 h dark periods) had significant associations ($P < 0.001$) with seed germination of *C. iria*. The highest frequency of seed germination was observed with 24 h light while the lowest was under 24 h dark conditions (Figure 2). In the 24 h light duration, the seed germination

percentage of *C. iria* was increased by 5.1% and 44.2% than at 12 h light/12 h dark and 24 h dark light period, respectively. The results provide evidence to confirm that the day and night temperature of 25/20 °C in the 24 h dark period resulted in the lowest seed germination of *C. iria* than other treatment combinations.

Light is an environmental signal that plays a crucial role in determining the proportion and rate of germination (Finch-Savage and Leubner-Metzger, 2006), where germination that is inhibited by darkness is reversed when the same seeds are transferred to complete light. Seed germination response to light varies considerably depending on the plant species where some species require light to germinate (Chauhan and Johnson, 2009b) while others germinate equally in light and dark (Teuton et al., 2004). Chauhan and Johnson (2009b) reported that three sedge weed species, *C. difformis*, *C. iria*, and *Fimbristylis miliacea* had an absolute light requirement for germination. The results of the present study (Figure 2C) clearly showed only the higher day/night temperature regimes imposed were able to support seed germination of *C. iria* even to levels marginally above 50% under complete darkness.

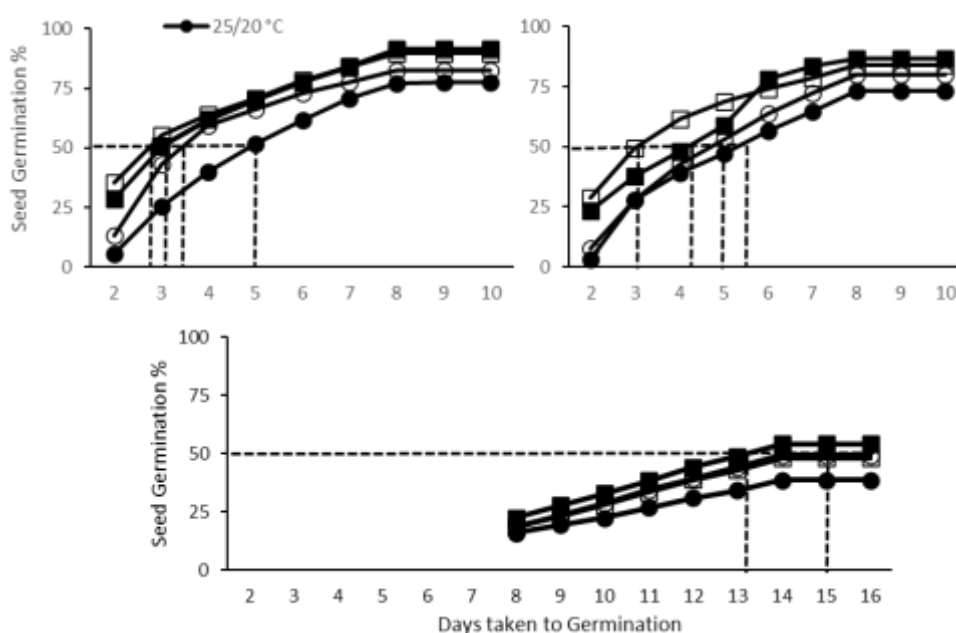


Figure 2. Effect of light and day/night temperature on time taken to seed germination. (A) 24 h light, (B) 12 h light and 12 dark h, and (C) 24 h dark. The dotted lines indicate the time taken for 50% germination.

Environmental factors such as oxygen, light, temperature, moisture and soil chemistry influence seed germination and seedling emergence of weed species (Chauhan, 2012; Shu et al., 2015). Temperature relates directly to water absorption and biochemical reactions in the seeds and regulates the metabolism facilitating the germination process (Paiva et al., 2016). Light and temperature control the germination process through affecting the levels of Absciscic acid (ABA) and Gibberellic Acid (GA) in lettuce seeds, and phytochrome is reported to regulate the metabolism of ABA in light-induced germination processes (Yoshioka et al., 1998; Sawada et al., 2008; Dong et al., 2012). Jain (2021) reported that full sunlight conditions at 30-35 °C increase germination indices of *Asparagus racemosus* and *Andrographis paniculata*.

The regulation of seed germination by light involves complex physiological and signal transduction processes and gene expression pathways, which is controlled by phytochromes and affected by various environmental factors such as temperature and moisture (Mollard and Insausti, 2009; Franklin and Quail, 2010 ; Chen et al., 2014). Although phytochrome is the basic cycle for the germination of seeds requiring light, the embryo can also germinate without light (Ravan et al., 2003). The seeds of *A. racemosus* and *A. paniculata* have demonstrated positive photosensitivity (Jain, 2021). With these supporting studies, results of the present study also suggested that light and temperature interact to influence the time to reach 50% germination (Figure 2). Higher day/night temperatures (40/35 °C) and continuous light promoted early and rapid seed germination, while lower day/night temperatures (25/20 °C) and complete darkness delayed the germination rate of *C. iria* significantly ($P < 0.05$). Accordingly, the number of days to reach 50% germination varied depending on the higher day/night temperatures and continuous light conditions as illustrated in Figure 2.

Effect of flooding

According to the maximum likelihood analysis for the first-order effects, the log linear model was not saturated where the likelihood ratio (G^2) was found to be significant ($P < 0.001$). Therefore, the interaction effects (second-order effects) were evaluated for the data on the survival of seedlings. The maximum likelihood analysis was performed for the second-order effects where the likelihood ratio (G^2) was found to be non-significant ($P = 0.9943$). Results showed that seedling count at 28 DAS was significantly affected ($P < 0.0001$) by flooding height, flooding duration, and flooding height x flooding duration, but the district, and the interactions, namely, flooding height x district and flooding duration x district, had no significant impact on seed germination ($P < 0.05$; Table 3).

Flooding height had a significant association ($P < 0.001$) with seedling survival estimated at 28 DAS. Seeds planted without flooding had the highest seedling survival, indicating the favorable conditions for seedling development. Increasing flooding height had a negative impact on seedling survival (Figure 3) indicating the sensitivity of seedlings to excessive water saturation. The chi-square analysis showed that the highest frequency of seedlings at 28 DAS was demonstrated when flooding was imposed late (20 DAS) irrespective of the flooding height while the lowest at early flooding on 5th DAS. Late flooding increased the survival of *C. iria* seedlings by 6.6 % with flooding at 10 DAS, by 35.9% at 15 DAS and 74.5% at 20 DAS compared to early flooding at 5 DAS across all heights.

The results revealed that the seedling survival of *C. iria* decreased by 77.8 % at 2.5 cm flooding height and by 87.6% at 5 cm height at 5 DAS compared to that of no flooding. Flooding can restrict oxygen availability, hinder gas exchange and create an unfavourable environment for seedling survival (Sasidharan and Voesenek, 2015). It may be due to younger seedling not being able to survive in the 2.5 -5 cm flooding height highlighting the importance of water management at early stages to control *C. iria* in paddy fields. Mature seedling require

maintenance of a higher water level as explained by Chauhan and Johnson (2009a) who reported the need for deep flooding to suppress growth of *C. difformis* seedlings already emerged at 21 DAS.

Effect of seed burial depth

The seedling emergence rate of *C. iria* seeds, buried at different soil depths, did not show a significant variation based on the district of seed collection. This indicates that the geographical location of seed collection may

not be a strong predictor of seedling emergence from those found in different soil layers. However, in general, soil-burial of seeds of *C. iria* had a significant association ($P < 0.001$) with seedling germination (Figure 4). The surface-sowing of seeds (0 cm depth) resulted in a seed germination rate of 84.4%, which decreased by 52.1% when the seeds were buried at 1 cm, and by 98.1% when buried at 5 cm soil depth. The study revealed that deeper soil-burial depths (2.5-5 cm from soil surface) significantly reduce the seed germination of *C. iria*.

Table 3. Maximum Likelihood Analysis of Variance of second order effects for flooding height, flooding duration and district

Source	DF	Chi-Square	Pr > ChiSq
Flooding Height	2	1272.41	<.0001
Flooding duration	3	332.32	<.0001
Flooding height x duration	6	339.83	<.0001
Districts	4	1.23	0.8731
Flooding height x district	8	10.16	0.2542
Flooding duration x district	12	1.74	0.9997
Likelihood Ratio	20	10.6	0.9943

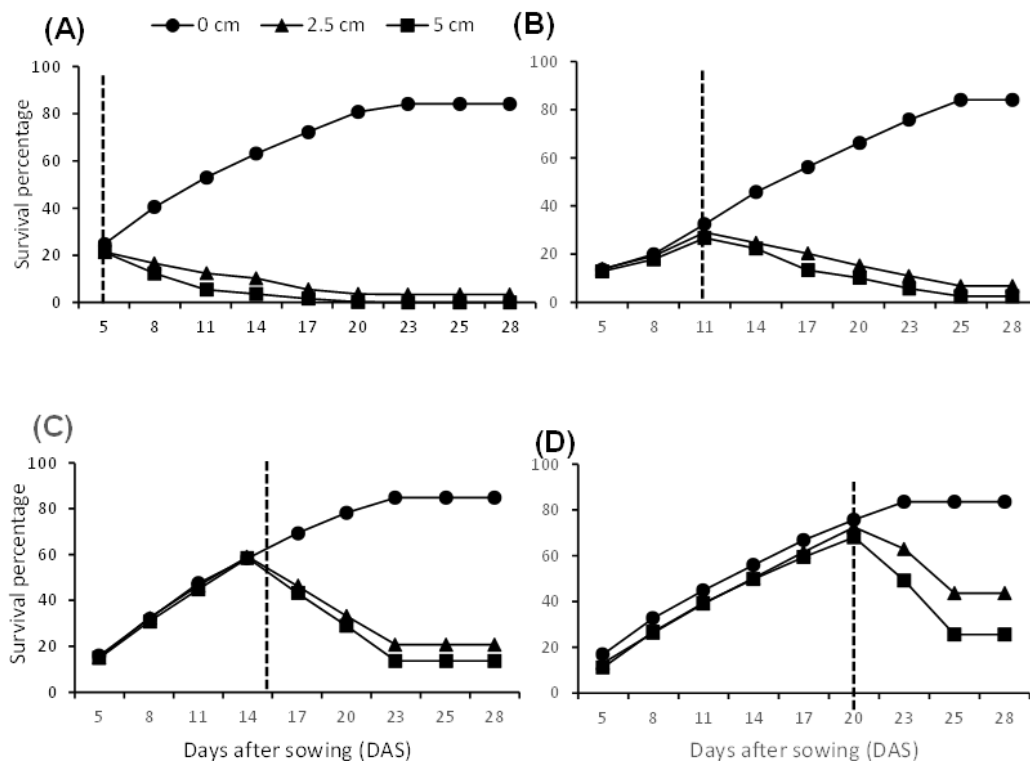


Figure 3. Effect of flooding height and duration on seedling survival percentage of *C. iria*. Vertical dotted line represents the time of imposition of flooding; (A): Flooding imposed at 5 DAS, (B): Flooding imposed at 10 DAS, (C): Flooding imposed at 15 DAS, and (D): Flooding imposed at 20 DAS.

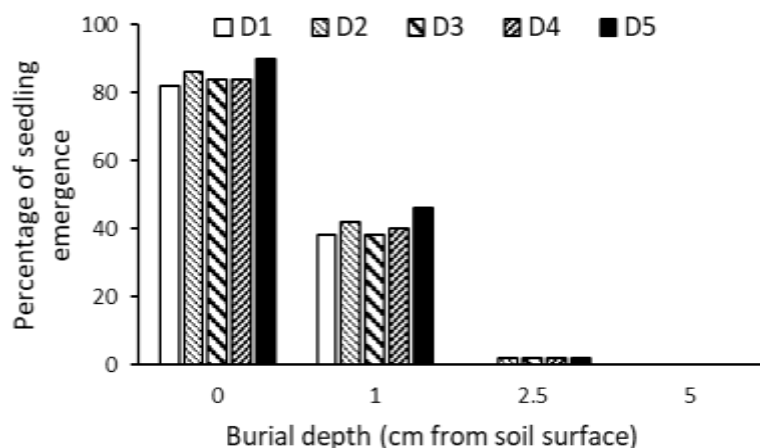


Figure 4. Emergence of *Cyperus iria* seedling from seeds buried at different soil depths. D_1 = Jaffna, D_2 = Mannar, D_3 = Mullaitivu, D_4 = Kilinochchi, D_5 = Vavuniya.

The deeper soil-burial depth could cause lower seed germination and seedling emergence due to reduced light penetration (Benvenuti, 1995), limited oxygen availability (Benvenuti, 2003), and moisture stress, and temperature fluctuations (Rubin and Benjamin, 1984). Previous studies have reported that sedge weeds such as *C. iria*, *C. difformis*, *F. miliacea*, and grasses such as *Leptochloa chinensis* were unable to emerge from a soil depth greater than 1 cm (Chauhan and Johnson, 2009a, 2010). As reported in the present study for *C. iria*, seeds of *C. difformis* sown on the soil surface have shown the highest percentage of seedling emergence, with no seedlings emergence from seeds buried in soil at depths of 1 cm (Derakhshan and Gharekhloo, 2013). Furthermore, the proportion of germinated seeds of *Echinochloa crus-galli* (Martinkova and Honek, 2013; Marambe et al., 1995; Begum et al., 2006a; Chauhan and Johnson, 2011), and *L. hyssopifolia* and *C. difformis* (Chauhan and Johnson, 2009b,c) decreased with burial depth. Begum et al. (2006b) reported that deeper seed burial (1 cm sowing depth), flooding depths of ≥ 5 cm within 14 DAS were effective in suppressing the emergence and growth of *F. miliacea*. Ismail et al. (1995) also indicated that emergence of *E. crus-galli*, *E. colona*, *C. iria*, *L. hyssopifolia*, and *Rhynchospora corymbosa* was lower in soils flooded up to 4 cm water depth compared to seeds sown at all sowing depths in saturated soil.

CONCLUSION

Temperature, light, flooding depth and duration, and soil-burial depth significantly influenced seed germination and seedling emergence of *C. iria*. The day/night temperatures of 25/20 °C, keeping the seeds in continuous darkness, maintaining a continuous flooding height at 2.5 - 5 cm at early stages of weed growth (from 5 day after sowing) at least for 28 days and burying *C. iria* seeds in soil at depths >1 cm significantly reduced the seed germination and seedling emergence of this troublesome sedge weed in paddy cultivation. Land preparation (e.g., deep ploughing) and water management (e.g., impounding standing water at early stages) would help to manage *C. iria* effectively in paddy fields. The management strategy should be designed based on the ecology of the weed coupled with other available technologies.

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