




Environmental Factors on the Germination of Susceptible and Resistant Biotypes of *Cyperus iria* L. to ALS Enzyme Inhibiting Herbicides

Fatores Ambientais na Germinação de Biótipos Suscetíveis e Resistentes de *Cyperus iria* L. aos Herbicidas Inibidores da Enzima ALS

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Abstract

Herbicide resistance to acetolactate synthase (ALS) inhibitors has compromised the management of *Cyperus iria* L., one of the main weeds in irrigated rice cultivation. The objective of this study was to evaluate the germination and emergence of *C. iria* biotypes susceptible and resistant to ALS-inhibiting herbicides, analyzing the influence of different environmental factors. Laboratory and greenhouse experiments were conducted to assess the influence of light quality and osmotic potential on germination, as well as the effects of seed burial depth and ryegrass straw on *C. iria* emergence. All the experiments were carried out in a completely randomized design (CRD), with four replications. *C. iria* is classified as a positively photoblastic species, showing higher germination rates under white and red light spectra. The species exhibited moderate tolerance to water stress, being able to germinate across a wide range of osmotic potentials, which highlights its adaptability to different soil moisture conditions. The emergence of the resistant biotype was favored at shallower burial depths. Furthermore, the presence of ryegrass straw on the soil surface exerted a differential impact on seedling emergence, showing a suppressive effect at amounts close to 6.98 t ha⁻¹. These results indicate that adequate irrigation water management, combined with practices that prevent seed exposure on the soil surface and the maintenance of straw mulch, can reduce *C. iria* emergence and contribute to integrated weed management in irrigated rice fields.

Keywords: Irrigated Rice. Resistance. Germination. Emergence.

Resumo

A resistência aos herbicidas inibidores da enzima acetolactato sintase (ALS) tem comprometido o manejo de *Cyperus iria* L., uma das principais plantas daninhas da cultura de arroz irrigado. O objetivo deste estudo foi avaliar a germinação e a emergência de biótipos de *C. iria* suscetível e resistente aos herbicidas da ALS, analisando a influência de diferentes fatores ambientais. Foram conduzidos experimentos em laboratório e casa de vegetação para avaliar a influência da qualidade da luz e do potencial osmótico na germinação, bem como da profundidade de enterrio das sementes e de palha de azevém na emergência de *C. iria*. Todos os experimentos seguiram o delineamento inteiramente casualizado – DIC, com quatro repetições. *C. iria* é considerada fotoblástica positiva, apresentando maior germinação nos espectros de luz branca e vermelha. A espécie demonstrou tolerância moderada ao estresse hídrico, sendo capaz de germinar em uma ampla faixa de potenciais osmóticos, o que evidencia adaptabilidade a diferentes condições de umidade do solo. A emergência do biótipo resistente foi favorecida em profundidades mais superficiais. Além disso, a presença de palha de azevém na superfície do solo exerceu um impacto diferenciado sobre a emergência das plântulas, apresentando efeito supressor em quantidades próximas a 6,98 t ha⁻¹. Esses resultados indicam que o manejo adequado da água de irrigação, aliado a práticas que evitem a exposição das sementes na superfície do solo e à manutenção de palha como morta, pode reduzir a emergência de *C. iria* e contribuir para o manejo integrado de plantas daninhas em lavouras de arroz irrigado.

Palavras-chave: Arroz Irrigado. Resistência. Germinação. Emergência.

1 Introduction

Cyperus iria L., a species belonging to the Cyperaceae family, is an annual with a tufted growth habit. Originally from Asia, it has naturalized in Brazil, where it has become one of the main weeds in irrigated rice cultivation (Kissmann; Groth, 1997). The high competitiveness of *C. iria* results from its ability to adapt to humid and flooded environments, as well as from its C4 photosynthetic metabolism, which provides greater water-use efficiency and enhances light utilization, particularly in tropical environments characterized by intense solar radiation and high temperatures (Chauhan; Johnson, 2009; Larridon *et al.*, 2014).

At high densities, *C. iria* can cause rice lodging, which hinders harvest and may lead to yield losses of up to 64%, significantly impacting grain production (Dhammu; Sandhu, 2002). In addition, the species' reproductive biology favors rapid spread, as a single plant can produce approximately 5,000 seeds with low primary dormancy, enabling the establishment of a second generation within the same growing season (Galinato; Moody; Piggin, 1999).

Acetolactate synthase (ALS)-inhibiting herbicides have been widely used in the management of *C. iria* in irrigated rice systems, particularly after the introduction of the Clearfield® production system, which confers resistance to imidazolinones through induced mutation (Marchesan *et al.*, 2011). However, the intensive use of these herbicides has resulted in the selection of resistant populations (Riar *et al.*, 2015). High levels of resistance to several ALS inhibitors have already been reported in *C. iria*, including bispyribac-sodium, imazamox, imazethapyr, penoxsulam, halosulfuron-methyl, and pyrazosulfuron-ethyl, compromising the effectiveness of chemical control and increasing rice production costs (Chiapinotto *et al.*, 2017; Riar *et al.*, 2015).

Seed germination is a key process for weed establishment in agricultural environments and is influenced by several environmental factors (Marcos Filho, 2015). Water is essential at all stages of seed germination, and its scarcity is a limiting factor in the process. More negative water potentials reduce the seed imbibition rate and may completely inhibit germination, with the minimum water potential required to inhibit germination being an intrinsic trait of each species (Stefanello, 2008). Light incidence, often limited by vegetation cover and burial depth of seeds, is another determining factor for germination. Both factors restrict the availability of light and impose physical barriers that hinder the germination process (Guimarães; Souza; Pinho, 2002).

Several studies have examined differences in germination responses between herbicide-susceptible and herbicide-resistant weeds, such as *Lolium rigidum* Gaudin., *Polypogon fugax* Nees ex Steud., *Alopecurus japonicus* L., *Echinochloa colona* L., *Bassia scoparia* (L.) A. J. Scott, *Chloris virgata* Sw., and *Ageratum conyzoides* L. (Desai; Chauhan, 2021; Desai, Desai; Chauhan, 2024; Kumar *et al.*, 2018; Shrestha *et al.*, 2017; Tang *et al.*, 2015; Vila-Aiub *et al.*, 2005; Wu *et al.*, 2016). Differences in germination responses between susceptible and resistant weeds may be associated with resource allocation trade-offs in metabolism (Dhanda *et al.*, 2022). In this process, plants allocate resources strategically to cope with biotic stresses, such as pests and diseases, and abiotic stresses, such as herbicide application. This allocation ensures survival under adverse conditions, although it may reduce growth capacity or seed production (Dhanda *et al.*, 2022).

Previous studies have investigated the germination biology of *C. iria* by analyzing factors such as temperature, light, salt and osmotic stress, and seed burial depth. The effects of flooding depth and duration on germination, emergence, and growth have also been assessed (Chauhan; Johnson, 2009; Jiang *et al.*, 2024). However, few studies have addressed differences in germination between herbicide-resistant and herbicide-susceptible biotypes, particularly regarding environmental factors, representing an important knowledge gap.

Therefore, the objective of this study was to evaluate the influence of light quality, osmotic potential, seed burial depth, and ryegrass residue on the germination and emergence of *C. iria* biotypes susceptible and resistant to ALS-inhibiting herbicides.

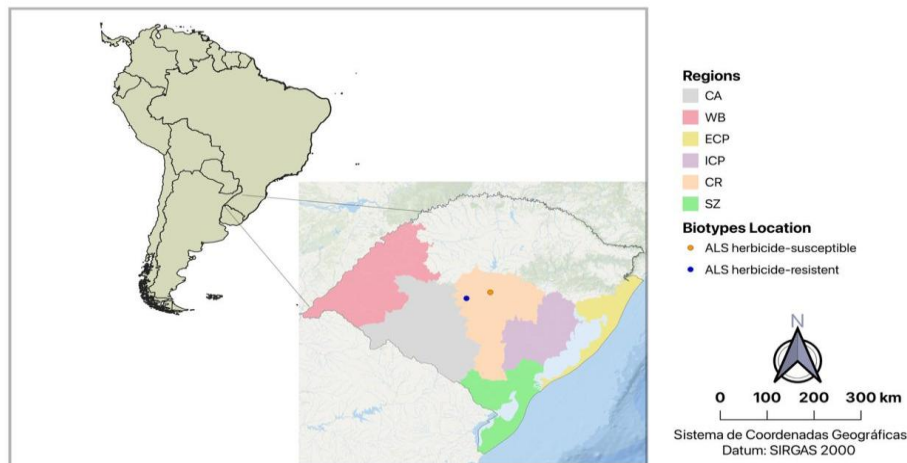
2 Material and Methods

2.1 Vegetal material

The *C. iria* seeds used in this study are part of the seed bank of the Weed Science Research Group at the Universidade Federal de Santa Maria (UFSM). These seeds were obtained from samples collected in irrigated rice fields in Rio Grande do Sul during the 2014/2015 growing season, as described by Ulguim *et al.* (2019). The biotypes susceptible and resistant to the herbicide

imazapyr+imazapic were collected in the municipalities of Agudo (29°40'28.62"W, 53°8'43.74"S) and Santa Maria (29°41'20.1"S, 53°38'45.5"W), respectively (Figure 1). The collection sites, located in the Central region of the state and approximately 32.39 km apart, exhibit similar climatic conditions, which helps reduce the potential environmental variability. Seeds were stored under controlled temperature conditions (10 ± 2 °C) until the multiplication stage, following the procedure adopted in this study.

Figure 1 – Collection sites of *C. iria* biotypes susceptible and resistant to acetolactate synthase (ALS)-inhibiting herbicides. CA (Campanha), FO (Fronteira Oeste), PCE (External Coastal Plain), PCI (Internal Coastal Plain), RC (Central Depression), and ZS (Southern Zone)



Source: the authors.

The seeds of the biotypes were multiplied in greenhouses located at the Departments of Biology (29°42'56.58"S, 53°43'13.53"W) and Crop Science (29°43'24.78"S, 53°43'13.16"W) of UFSM during 2021 and 2022. Seeds were considered mature when plants reached full senescence and were collected by gently shaking the inflorescences over paper bags. After collection, the seeds were manually cleaned, dried in the shade, and subjected to proper ventilation to prevent microbial contamination and physiological deterioration. The thousand-seed weight was 0.2 g for the susceptible biotype and 0.1 g for the resistant one. Subsequently, the seeds were labeled and stored in paper bags under refrigeration (± 8 °C) at the Teaching and Research Laboratory of Seeds, UFSM (29°43'22.05"S, 53°43'13.37"W), for further use.

2.2 Seed germination protocol

The experiments were conducted in a completely randomized design (CRD), with four replications. Factor A consisted of *C. iria* biotypes susceptible and resistant to ALS-inhibiting

herbicides, while Factor D consisted of the simulation of different environmental factors, including spectral light quality and osmotic potential.

For each replication, fifty seeds of each biotype were uniformly distributed in transparent acrylic boxes (gearboxes, 11 × 11 × 3.5 cm). Each gearbox contained three sheets of Germitest® paper moistened with distilled water or a specific solution, in a volume equivalent to 2.5 times the paper dry weight .

Due to primary dormancy, *C. iria* seeds did not require dormancy-breaking treatment (Chauhan; Johnson, 2009). The gearboxes were then placed in a germination chamber (Biochemical Oxygen Demand, BOD), set to alternating temperatures of 25/35 °C and a 12-hour light/dark photoperiod, for a period of 14 days (Chauhan; Johnson, 2009). To minimize evaporation, the gearboxes were placed inside plastic bags, and distilled water or specific solutions were added as needed.

The evaluated variables included germination, determined at 7 and 14 days after sowing (DAS), as well as shoot and root length, measured at 14 DAS. Shoot and root length were measured using a millimeter ruler, evaluating ten seedlings randomly selected in each replication (Brasil, 2009). The seeds showing primary root protrusion with a length ≥ 2 mm were considered germinated (Labouriau, 1983; Chauhan; Johnson, 2009).

2.3 Spectral Quality of Light in Seed Germination of *C. iria* Biotypes

Different spectral light qualities were used to evaluate their influence on the germination of susceptible and resistant *C. iria* biotypes. The treatments included blue light (450 nm), green light (500 nm), red light (700 nm), far-red light (760 nm), white light (380–760 nm), and darkness. To generate the spectral light qualities, the gearboxes were covered with two layers of cellophane paper in the colors corresponding to each treatment (blue, green, and red), following the methodology adapted from Roso *et al.* (2021). For the far-red light treatment, the boxes were covered with two layers of red cellophane and two layers of blue cellophane. White light was obtained without covering the boxes, while darkness was simulated using two layers of aluminum foil. Evaluations were conducted in a darkroom under green light to avoid external interference in the germination process (Yamashita; Alberguini, 2011).

2.4 Osmotic Potential in Seed Germination of *C. iria* Biotypes

Different water availability conditions were used to evaluate their influence on the germination of susceptible and resistant *C. iria* biotypes. Solutions were prepared with polyethylene glycol 6000 (PEG 6000), according to Villela, Doni-Filho, and Sequeira (1991), to achieve osmotic potentials of 0 (distilled water), -0.2, -0.4, -0.6, and -0.8 MPa. After preparation, the solutions were added to the

gearboxes to simulate osmotic stress conditions. The seeds remained in the PEG solutions throughout the 14-day incubation period in the BOD chamber, as described in the general protocol (Section 2.2).

2.5 General Seedling Emergence Protocol

The experiments were conducted in a completely randomized design (CRD) with four replications. Factor A consisted of *C. iria* biotypes susceptible and resistant to ALS-inhibiting herbicides, while Factor D involved the simulation of different seed burial depths and amounts of ryegrass residue.

For each replication, 25 seeds of each biotype were uniformly distributed in different containers. Expanded polystyrene trays (14 × 13 × 8.5 cm) were selected due to their large surface area, which facilitated uniform soil distribution. In addition, 500 mL plastic cups were used to ensure greater soil depth, favoring the seedlings' initial development.

The containers were filled with lowland soil classified as Typic Albaqualf (Planossolo Háplico eutrófico arênico), belonging to the Vacacaí mapping unit (Santos *et al.*, 2013). The soil was broken up, autoclaved, and subsequently sieved through a 0.3-cm mesh before use. The containers were placed in a greenhouse (29°43'24.78"S, 53°43'13.16"W), where thermal conditions were similar to the external environment. The experimental units were not subjected to supplemental lighting or direct rainfall during the experimental period. All the containers were placed in plastic trays, and irrigation was carried out on alternate days to maintain the soil constantly moist.

Among the evaluated variables, seedling emergence was determined at 28 days after sowing (DAS). Shoot and root length were measured using a millimeter ruler, evaluating 10 seedlings randomly selected in each replication (Brasil, 2009). Seedling emergence was characterized by the coleoptile appearance (Chauhan; Johnson, 2009).

2.6 Seed Burial Depth in Seedling Emergence of *C. iria* Biotypes

To evaluate the influence of burial depth on seedling emergence, seeds of susceptible and resistant *C. iria* biotypes were sown in plastic cups and covered with soil at depths of 0 (surface), 0.125, 0.25, 0.5, 1, 2, and 4 cm, using the soil described in the general emergence protocol. Emergence, as well as shoot and root length, were measured following the general protocol (Section 2.3).

2.7 Amount of Ryegrass Straw in Seedling Emergence of *C. iria* Biotypes

To evaluate the influence of residue amount on seedling emergence, seeds of susceptible and resistant *C. iria* biotypes were sown on the soil surface (0 cm) in polystyrene trays and covered with ryegrass (*Lolium multiflorum* L.) residue. The residue, obtained from ryegrass plants dried at 60 °C

and chopped into 2 cm fragments, was evenly distributed on the soil surface at rates of 0, 1, 3, 5, and 7 t ha⁻¹. Seedling emergence was evaluated following the general protocol (Section 2.3).

2.8 Statistical analysis

The data were initially analyzed for adherence to normal distribution and homogeneity of residual variances using the Shapiro-Wilk ($p < 0.05$) and Levene ($p < 0.05$) tests. When violations of statistical assumptions were detected, a square-root transformation was applied, adding a constant to zero values: $Y' = (Y + 0.5)^{0.5}$ (Yamamura, 1999). Subsequently, the data were subjected to analysis of variance (ANOVA) to determine the possible effects of treatments and interactions. When a significant effect was detected by the F-test ($p < 0.05$), appropriate post hoc analyses were conducted, applying Tukey's test ($p < 0.05$) for qualitative factors and regression analyses for quantitative factors. Data analyses and figure generation were performed using R software (R Core Team, 2023), with the packages ExpDes (Ferreira; Cavalcanti; Nogueira, 2021), MASS (Venables; Ripley, 2002), ggplot2 (Wickham, 2016), cowplot (Wilke, 2020), and metan (Olivoto; Lúcio, 2020).

3 Results and Discussion

3.1 Spectral Quality of Light in Seed Germination of *C. iria* Biotypes

According to the analysis of variance, there was no significant Biotype \times Light Quality interaction. However, when the main effects were analyzed, light quality was significant for all the variables studied.

When evaluating the germination responses of *C. iria* seeds under different light qualities, the highest germination percentages at 7 days after sowing were observed under white light (18%) and red light (17%). These treatments differed significantly from blue light (8%), far-red light (6%), and darkness (0%), which did not differ from each other (Table 1).

Table 1 – Germination (%) at 7 and 14 days after sowing (DAS), and root length (RL) and shoot length (SL), in centimeters (cm), of *C. iria* seedlings subjected to six light spectra

Light Quality	7 DAS (%)	14 DAS (%)	RL (cm)	SL (cm)
White Light	18a	29a	0.33a	1.42b
Red Light	17a	22a	0.29a	1.62ab
Green Light	13ab	17bc	0.36a	1.76ab
Blue Light	8bc	14bc	0.24a	1.66ab
Far-Red Light	6c	11c	0.28a	1.88a
Darkness	0c	0d	0.00b	0.00c

*Means followed by the same letter within a column do not differ significantly from each other according to Tukey's test at 5% probability.

Source: research data.

For germination at 14 DAS, the highest germination percentages were observed under white light (29%) and red light (22%), differing from the other treatments. In contrast, no germination was recorded in the absence of light, indicating that this condition did not stimulate the germination process.

The results demonstrate that *C. iria* can be classified as positively photoblastic, as seeds of this species germinate exclusively in the presence of light. This indicates that the absence of light does not stimulate germination, as evidenced by the lack of germination in darkness. These findings corroborate the results of Chauhan and Johnson (2009) and Jiang *et al.* (2024), who also observed the absolute requirement of light for *C. iria* seed germination, with inhibition occurring in the absence of light.

These results can be attributed to the activation of phytochrome, which occurs predominantly in the presence of red light (660 nm), converting the inactive phytochrome (Pr) into its active form (Pfr), which is essential for germination of photoblastic seeds. Borges and Rena (1993) reported that white light, due to its spectral composition, has an effect similar to red light because it includes wavelengths that activate phytochrome. In contrast, far-red light (730 nm) inactivates the active phytochrome (Pfr → Pr). These findings reinforce the importance of white and red light as germination stimuli for photoblastic seeds.

Previous studies highlight that light plays a crucial role in germination stimulation in various Cyperaceae species, including *Cyperus difformis* L., *Cyperus aromaticus* (Ridl.) Mattf. & Kük., *Fimbristylis littoralis* Gaudich, and *Fimbristylis miliacea* (L.) Vahl (Derakhshan; Gherekhloo, 2013; Chadha *et al.*, 2021; Chauhan; Johnson, 2009; Jiang *et al.*, 2024). This characteristic suggests an adaptive pattern related to light-dependent germination, favoring establishment in specific environments such as open areas with high sunlight exposure. This behavior may be associated with the wide diversity of the Cyperaceae family in tropical and subtropical regions, as highlighted by Goetghebeur (1998).

Control of *C. iria* should be carried out using an integrated approach, combining cultural methods with other management strategies. The application of dense layers of mulch from crops or winter cover crops reduces the germination capacity of this weed. This occurs due to the species' positive photoblastic germination requirement, as seeds need light to initiate the germination process. When *C. iria* seeds are covered by mulch, the lack of light stimulus prevents germination, avoiding seedling establishment in cultivated areas. Furthermore, seed viability tends to decrease over time in the soil due to microbial degradation, contributing to the reduction of the seed bank and lowering the probability of future reinfestations.

For root length, no significant differences were observed among treatments with white, red,

green, blue, and far-red light (Table 1). Only in the absence of light was root development absent, due to the complete lack of seed germination under this condition. Similar responses were observed for shoot length, in which the highest values occurred under far-red light, differing only from white light and darkness. As observed for root length, the absence of light resulted in zero shoot growth due to the complete absence of germinated seeds in this treatment (Table 1).

These results indicate that while germination strictly depends on specific light conditions, once initiated, root and shoot growth show lower sensitivity to variations in light quality. This reflects a differentiation in photosensitivity requirements during early plant development, where germination is the most restrictive stage regarding light availability, while subsequent growth occurs more independently of the light spectrum.

However, it is noteworthy that the greatest absolute shoot growth occurred under far-red light, with an average of 1.88 cm, without statistical difference compared to red, green, or blue light (Table 1). Germination, however, was not favored under this condition. Radiation with wavelengths between 730 and 740 nm, at the edge of the visible spectrum, is poorly absorbed and often reflected by plants, influencing processes such as hypocotyl elongation in response to light competition through phytochrome signaling. These photoreceptors perceive the altered red to far-red light ratio caused by shading from neighboring plants, triggering adaptive responses known as shade avoidance (Patterson, 1985).

In coexistence with rice, *C. iria* exhibited a height approximately 30% greater than the crop, highlighting shoot elongation as an adaptive strategy to ensure light access under shading conditions (Chauhan; Johnson, 2010). Increased shoot elongation can be associated with the plant's adaptive response to reduced light quality, a characteristic observed in environments with limited light availability, as demonstrated by Schmith and Wulff (1993) and Merotto Jr. *et al.* (2002). This increase in shoot length is an attempt to optimize light capture, considering that shading by neighboring plants may compromise photosynthesis and survival. However, this elongation may also entail physiological costs, such as reduced nutrient and water absorption capacity, in addition to compromising photosynthetic efficiency (Rajcan; Swanton, 2001).

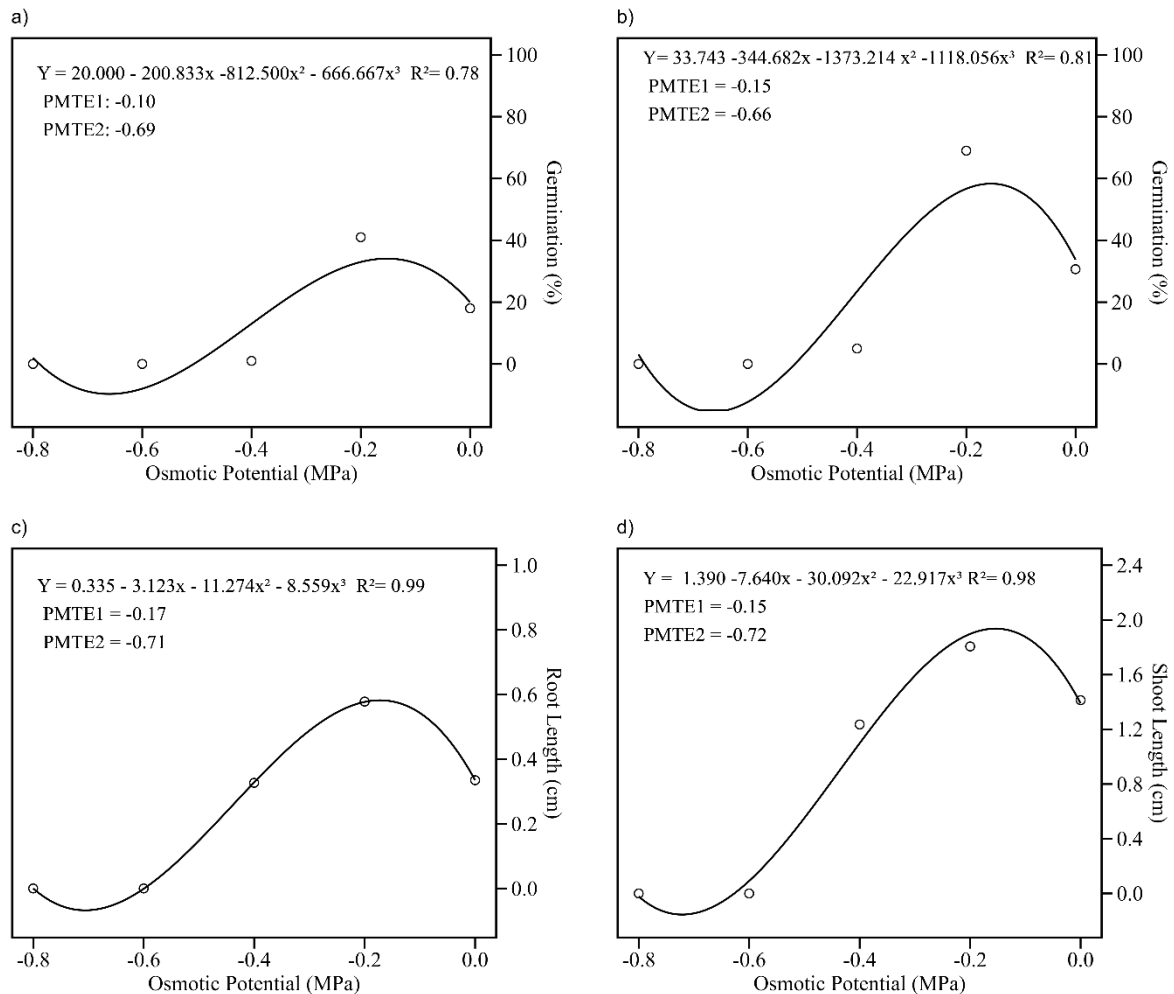
3.2 Osmotic Potential in Seed Germination of *C. iria* Biotypes

According to the analysis of variance, there was no significant Biotype \times Osmotic Potential interaction for all the variables studied. When the main effects were analyzed, a significant effect of osmotic potential was observed for all the variables.

The germination and growth responses of *C. iria* exhibited cubic responses to increasing levels of osmotic potential for all the variables (Figure 2). For germination at 7 DAS, osmotic potentials of

–0.69 and –0.10 MPa corresponded to the minimum and maximum points of the fitted cubic function, indicating the levels at which the process reached the minimum or maximum observed values (Figure 2a). Similar results were observed for germination at 14 DAS, with osmotic potentials of –0.66 and –0.15 MPa representing the minimum and maximum points, respectively (Figure 2b).

Figure 2 – Germination (%) at 7 (a) and 14 days (b) after sowing, and root length (c) and shoot length (d) of *C. iria*; PMTE1: minimum point; PMTE2: maximum point



Source : research data.

These results are consistent with those reported by Jiang *et al.* (2024) and Chauhan and Johnson (2009), who observed a 50% reduction in *C. iria* germination at osmotic potentials of –0.59 and –0.46 MPa, respectively. Although the cubic model used in the present study differs from the nonlinear models applied by these authors, the results converge in demonstrating that *C. iria* is sensitive to water stress, with germination decreasing as osmotic potential becomes more negative. These data reinforce the moderate tolerance of *C. iria* to water scarcity, evidenced by the species' ability to germinate across a broader range of osmotic potentials. This characteristic, together with the behavior

observed in this study, highlights the adaptation of *C. iria* to irrigated rice environments, where water potential near 0 MPa prevails during a large part of the crop cycle.

Germination of other *Cyperus* species tends to decline progressively as osmotic stress increases. *C. aromaticus* did not germinate at osmotic potentials equal to or lower than -0.8 MPa (Jiang *et al.*, 2024). In the case of *C. difformis*, responses varied depending on the population origin. Populations from the Philippines, Iran, and China required osmotic potentials of -0.12 , -0.47 , and -0.56 MPa, respectively, to reach a 50% reduction in germination (Chauhan; Johnson, 2009; Derakhshan; Gherekhloo, 2013; Jiang *et al.*, 2024). These results highlight inter- and intraspecific variability within the genus, indicating that tolerance to water stress may be associated with local adaptations, which may justify different management practices in distinct countries.

Root length and shoot length showed the lowest values at osmotic potentials of -0.71 and -0.72 MPa, respectively, whereas the highest values were recorded at osmotic potentials of -0.17 and -0.15 MPa (Figures 2c and 2d). These results indicate that *C. iria* exhibits more favorable root and shoot growth under conditions of higher water availability, reflecting the species' moderate sensitivity to water scarcity during early development.

Additionally, the difference between the minimum and maximum points for germination and growth variables suggests that *C. iria* employs distinct strategies throughout its life cycle. During germination, the species appears to prioritize conditions of higher water availability to ensure the activation of essential metabolic reactions, as water is fundamental for seed imbibition and the initiation of seed development (Bittencourt *et al.*, 2017; Fenner; Thompson, 2005). In contrast, during the growth phase, *C. iria* demonstrates greater resilience and developmental capacity even under more adverse conditions, as evidenced by the results obtained at more negative osmotic potentials.

The response of *C. iria* to water stress, observed under different osmotic potentials, reflects an adaptation that can be exploited in the management of this weed. The species shows moderate sensitivity to water scarcity, which directly influences germination and growth in environments with variable water availability.

Thus, management strategies involving water control, such as proper soil preparation and flood irrigation, are essential for managing *C. iria* in rice fields. Proper soil leveling, for example, is a critical practice as it ensures uniform water distribution, contributing to reduced infestation by *C. iria* (SOSBAI, 2022). Higher concentrations of this species are generally observed in the higher areas of fields, where irrigation deficiencies result in lower water availability.

In this context, flood irrigation emerges as one of the main management tools (Rao *et al.*, 2007). This method acts as a physical barrier, preventing seed germination and limiting seedling growth, particularly for seedlings that require higher oxygen levels for germination (SOSBAI, 2022). Studies

have shown that water layers with a depth of 5 cm or more completely suppress *C. iria* emergence, whereas shallower layers of 1–2 cm do not prevent emergence but significantly delay the process and limit the development of seedlings that do emerge (Ulguim *et al.*, 2019).

The importance of flood irrigation for controlling *C. iria* underscores the need for integrated management, which considers both proper soil preparation and early water application between the V2 and V4 stages of rice, when the plant has from 2 to 4 leaves (SOSBAI, 2022). This combination of practices can reduce the germination and growth of *C. iria* biotypes, enhancing management efficacy in irrigated rice fields.

3.4 Seed Burial Depth in Seedling Emergence of *C. iria* Biotypes

According to the analysis of variance, there was a significant Biotype × Depth interaction for the variable Emergence. When the main effects were analyzed, depth was significant for both root length and shoot length.

Considering the emergence of *C. iria* biotypes at different burial depths, the highest percentage of emerged seedlings was observed for the resistant biotype at depths of 0.125 cm (31%) and 0.25 cm (21%) (Table 2). No significant differences were observed for the other depths. These results indicate that the resistant biotype has an advantage under shallow burial conditions, which favor emergence.

Table 2 – Emerged seedlings (%) of *C. iria* biotypes (resistant and susceptible) subjected to different burial depths

Biotypes	Depths (cm)						
	0	0.125	0.25	0.5	1	2	4
Resistant	44a	31a	21a	5a	0a	0a	0a
Susceptible	37a	12b	8b	3a	1a	0a	0a

*Means followed by the same letter within a column do not differ significantly according to Tukey’s test at 5% probability.

Source : research data.

Regardless of biotype, the results indicate a marked reduction in seedling emergence with increasing seed burial depth. The highest emergence percentages were observed for seeds located on the soil surface (0 cm), whereas no seedlings emerged from depths greater than 2 cm. These results corroborate those obtained in the light quality study, in which the absence of light completely inhibited the biotypes’ germination.

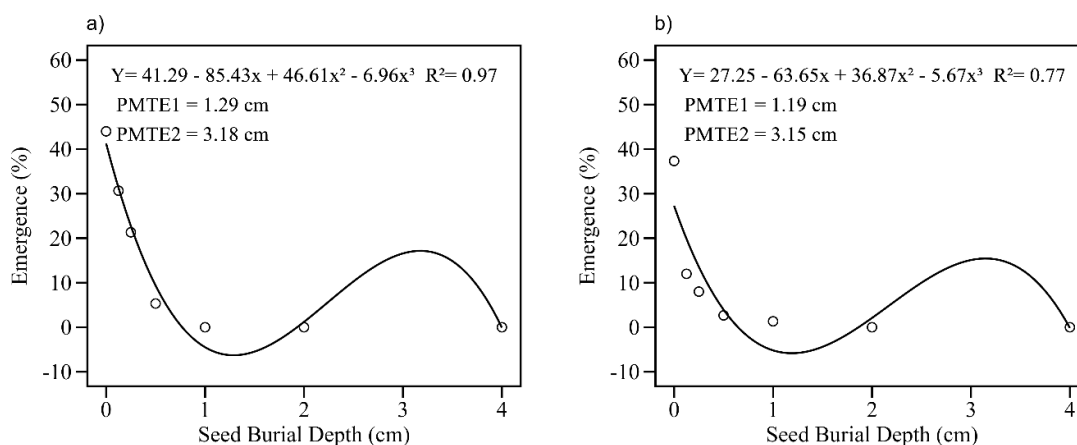
This behavior has also been observed in other *Cyperus* species, such as *C. difformis*, whose emergence did not occur at depths greater than 1 cm (Chauhan; Johnson, 2009; Derakhshan; Gherekhloo, 2013; Jiang *et al.*, 2024). Australian populations of *C. aromaticus* showed maximum germination when seeds were maintained on the soil surface (0 cm). However, emergence was

completely inhibited at a depth of 0.5 cm in one population and 2 cm in another (Chauhan, 2021).

Low emergence at greater depths can be explained by several environmental factors. Among them, light does not penetrate effectively beyond 0.2 cm of soil depth, reducing its availability and consequently germination. As reported by Woolley and Stoller (1978), less than 1% of incident light reaches the soil at these depths, which may explain the low or absent seedling emergence. In addition, soil temperature fluctuations are more intense near the surface and decrease with depth, which can negatively affect the germination. Lack of oxygen and reduced gas diffusion are also factors that can decrease emergence of seeds buried at greater depths.

When analyzing biotype emergence in relation to burial depth, a quadratic response was observed with increasing depth (Figure 6). Estimates indicated minimum emergence points at depths of 3.18 and 3.15 cm for the resistant and susceptible biotypes, respectively, corresponding to the depths where the fitted cubic function predicts minimum and maximum emergence (Figures 3a and 3b).

Figure 3 – Percentage of emerged seedlings of two *C. iria* biotypes subjected to seven burial depths (0; 0.125; 0.5; 1; 2; and 4 cm). Resistant biotype (a); Susceptible biotype (b); PMET1: minimum emergence point; PMET2: maximum emergence point



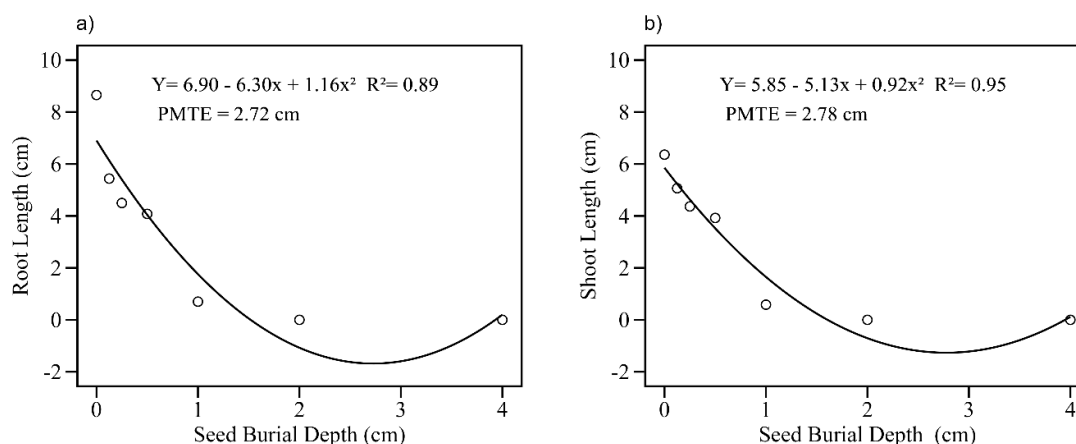
Source: research data.

The maximum emergence points were estimated at 1.29 cm for the resistant biotype and 1.19 cm for the susceptible biotype (Figures 3a and 3b). While previous analyses showed more pronounced differences in emergence rates between the biotypes, the differences in minimum and maximum emergence depths between the biotypes are small and practically negligible. Therefore, management strategies for both biotypes can be similar, as this variation is not sufficient to significantly impact emergence rates or the efficacy of control practices. In this context, *C. iria* emergence can be reduced

through practices such as harrowing and deep plowing, regardless of biotype (Derakhshan; Gherekhloo, 2013).

Regarding the growth characteristics of *C. iria* seedlings in relation to burial depth, a significant fit was observed for the quadratic equation, regardless of the biotype (Figure 4). As seed burial depth increases, reductions in both root length and shoot length are observed. These results indicate that seedling growth is favored at shallower depths, while greater depths may limit early development.

Figure 4 – Root length (a) and shoot length (b) of *C. iria* seedlings subjected to seven burial depths (0; 0.125; 0.5; 1; 2; and 4 cm). PMET: minimum emergence point.



Source : research data.

This limitation can be explained by the restriction of energy reserves, which compromises the growth needed to reach the light and initiate photosynthesis, making it difficult for seedlings from small seeds, such as *C. iria*, to emerge from greater depths (Mennan; Ngouajio, 2006). Therefore, seed size can influence seedling emergence, as larger seeds, due to greater energy reserves, are able to emerge from greater depths compared to smaller seeds (Humphries; Chauhan; Florentine, 2018).

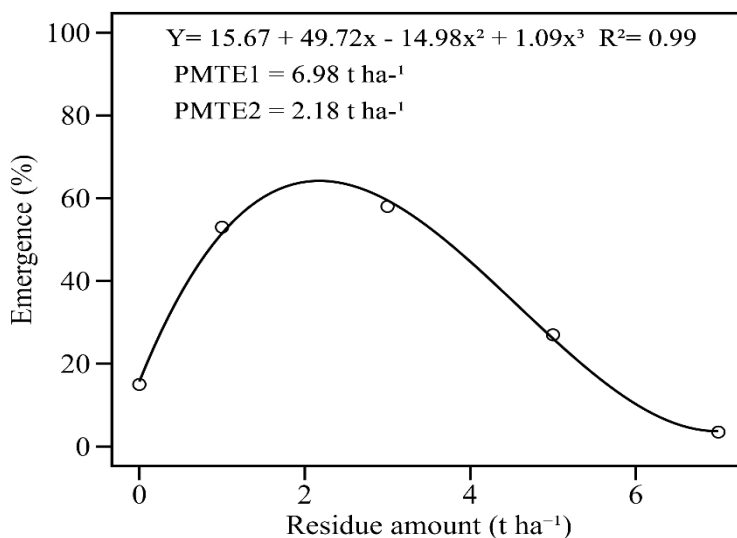
Cultivation systems based on reduced tillage and no-till favor the retention of seeds on the soil surface, facilitating germination and emergence when environmental conditions are favorable. This characteristic can enhance the effectiveness of chemical or mechanical control, contributing to the reduction of the soil seed bank. In this context, the use of pre-emergence herbicides emerges as a key control strategy to maintain low *C. iria* infestations under field conditions (Silva *et al.*, 2021).

3.5 Amount of Ryegrass Straw on Seedling Emergence of *C. iria* Biotypes

According to the analysis of variance, there was no significant Biotype × Amount of Ryegrass Straw interaction. For the main effects, a significant effect was observed for the amount of ryegrass straw.

When analyzing the emergence of *C. iria* seedlings in relation to the amounts of ryegrass straw on the soil surface, a significant cubic relationship was observed (Figure 5), indicating the levels at which seedling emergence reached the minimum or maximum observed values. Minimum emergence occurred at 6.98 t ha⁻¹, and maximum emergence at 2.18 t ha⁻¹ of straw (Figure 5). These results indicate that the presence of straw can influence seedling emergence depending on the amount applied on the soil surface. Maximum emergence may be related to the residual fraction of straw on the surface, which contributes to soil moisture retention, prevents desiccation, reduces thermal amplitude, and favors germination (Peres; Souza; Lavorenti, 2010).

Figure 5 – Percentage of emerged seedlings subjected to different amounts of ryegrass straw (0, 1, 3, 5, and 7 t ha⁻¹); PMET1: minimum point; PMET2: maximum point



Source : research data.

When applied in high amounts (6.98 t ha⁻¹), straw not only reduces light intensity but also alters light quality, a relevant effect for *C. iria*, a positively photoblastic species (Table 1). Simultaneously, straw acts as a physical barrier to emergence, making it difficult for seedlings to reach sufficient light at the surface for photosynthesis, thereby impairing germination, particularly in species with small seeds such as *C. iria* (Correia; Durigan, 2004; Pitelli; Durigan, 2001). This effect may be compounded when seeds are buried at depths greater than 2 cm (Table 2), further hindering germination.

Ryegrass straw suppressed *C. iria* emergence; however, its use should be carefully considered in certain production systems. Studies have shown that ryegrass cover was effective in suppressing weeds when straw amounts exceeded 3,000 kg ha⁻¹. Nevertheless, rice yield was reduced when straw reached 4,500 kg ha⁻¹ (Tomazetti *et al.*, 2021). This emphasizes the need for proper management of

straw quantity in the cultivation system, balancing weed suppression with the maintenance of rice productivity.

Thus, although straw mulch management presents positive results in controlling *C. iria*, it is essential that application is adjusted to the realities of production systems. Furthermore, effective control of *C. iria* requires an integrated approach that combines different management strategies to complement the use of straw and increase the efficiency of weed control.

The results indicate that the resistant biotype of *C. iria* exhibits higher emergence than the susceptible biotype under shallower burial conditions. This specific advantage suggests that, under these conditions, the resistant biotype may become relatively more prevalent in irrigated rice fields in the central region of the state, including the municipalities of Agudo and Santa Maria. It should be noted that this conclusion is limited to the conditions evaluated and cannot be generalized to all the *C. iria* populations, as local environmental and ecological factors may influence seed germination and subsequent seedling growth.

For future studies, it would be relevant to expand research to include geographically diverse populations of *C. iria* that are susceptible and resistant to ALS herbicides. Moreover, since resistant populations frequently coexist with susceptible populations, replacement series experiments could provide detailed information on the competitiveness between these populations. These results underscore the importance of integrated management strategies, in which proper irrigation management, combined with practices that prevent seed establishment near the soil surface and the use of straw mulch, reduces the emergence of *C. iria* biotypes and promotes effective weed control in irrigated rice fields.

4 Conclusion

The results of this study provide important insights into the germination and emergence biology of *C. iria*, with emphasis on ALS herbicide-resistant biotypes. Regarding germination and emergence, *C. iria* is considered positively photoblastic, with seeds germinating only in the presence of light. The species also demonstrated moderate tolerance to water stress, being able to germinate across a wide range of osmotic potentials, highlighting adaptability to different soil moisture conditions.

Concerning seed burial depth, the results indicate that the resistant biotype has an advantage at shallower depths, which favors emergence under these conditions. Additionally, the presence of ryegrass straw on the soil surface had differential impacts on *C. iria* seedling emergence, with amounts around 6.98 t ha⁻¹ capable of suppressing this process. These findings provide valuable information for adopting integrated management practices in irrigated rice fields.

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References

BITTENCOURT, H.V.H. *et al.* Seed germination ecology of *Eragrostis plana*, an invasive weed of South American pasture lands. *South Afric. J. Bot.*, v.109, p.246-252, 2017. doi: <http://dx.doi.org/10.1016/j.sajb.2017.01.009>

BORGES, E.E.L.; RENA, A.B. Germinação de sementes. In: AGUIAR, I.B.; PIÑA-RODRIGUES, F.C.M.; FIGLIOLIA, M.B. *Sementes florestais tropicais*. Brasília: ABRATES, 1993. p.83-135.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Regras para Análise de Sementes*. Brasília: MAPA, 2009b.

CHADHA, A. *et al.* Germination biology of three populations of Navua sedge (*Cyperus aromaticus*). *Weed Sci.*, v.9, p.69-81, 2021. doi: <https://doi.org/10.1017/wsc.2020.82>

CHAUHAN, B.S.; JOHNSON, D.E. Ecological studies on *Cyperus difformis*, *Cyperus iria* and *Fimbristylis miliacea*: three troublesome annual sedge weeds of rice. *Ann. Appl. Biol.*, v.155, n.1, p.103-112, 2009. doi: <https://doi.org/10.1111/j.1744-7348.2009.00325.x>

CHAUHAN, B.S.; JOHNSON, D.E. Responses of rice flatsedge (*Cyperus iria*) and barnyardgrass (*Echinochloa crus-galli*) to rice interference. *Weed Sci.*, v.58, n.3, p.204-208, 2010. doi: <http://dx.doi.org/10.1614/WS-D-09-00050.1>

CHAUHAN, B.S. Differential germination response of Navua sedge (*Cyperus aromaticus*) populations to environmental factors. *Weed Sci.*, v.69, n.6, 2021. doi: <http://dx.doi.org/10.1017/wsc.2021.48>

CHIAPINOTTO, D.M. *et al.* Cross-resistance of rice flatsedge to ALS-inhibiting herbicides. *Planta Daninha*, Viçosa, v. 35, n. 1, p. 1-12, 2017. doi: <https://doi.org/10.1590/S0100-83582017350100068>

CORREIA, N.M.; DURIGAN, J.C. Emergência de plantas daninhas em solo coberto com palha de cana-de-açúcar. *Planta Daninha*, v.22, n.1, p.11-17, 2004. doi: <https://doi.org/10.1590/S0100-83582004000100002>

DERAKHSHAN, A.; GHEREKHLOO, J. Factors affecting *Cyperus difformis* seed germination and seedling emergence. *Planta Daninha*, v.31, n.4, p.823-832, 2013. doi: <https://doi.org/10.1590/S0100-83582013000400008>

DESAI, H.S.; CHAUHAN, B.S. Differential germination characteristics of glyphosate-resistant and glyphosate-susceptible *Chloris virgata* populations under different temperature and moisture stress regimes. *Plos One*, v.17, n.6, 2021. doi: <https://doi.org/10.1371/journal.pone.0253346>

DESAI, D.H.; DESAI, H.S.; CHAUHAN, B.S. Germination attributes of metsulfuron-resistant and metsulfuron-susceptible tropical ageratum (*Ageratum conyzoides*) populations under various environmental conditions. *Weed Sci.*, v.72, n.4, p.360-367, 2024. doi: <https://doi.org/10.1017/wsc.2024.31>

DHAMMU, H.S.; SANDHU, K.S. Critical period of *Cyperus iria* L. competition in transplanted rice. IN: AUSTRALIAN WEEDS CONFERENCE: WEEDS THREATS NOW AND FOREVER. Plant

Protection Society of WA, Perth, 2002. p. 79–82.

DHANDA, S. *et al.* Characterization and management of metsulfuron-resistant *Rumex dentatus* biotypes in northwest India. *Agron. J.*, v.114, n.1, p.366-378, 2002. doi: <http://dx.doi.org/10.1002/agj2.20849>

SANTOS, H.G. *et al.* *Sistema Brasileiro de Classificação de Solos*. Rio de Janeiro: Embrapa Solos, 2013.

FENNER, M.; THOMPSON, K. *The Ecology of Seeds*. Cambridge: Cambridge University Press, 2005.

FERREIRA, E.B.; CAVALCANTI, P.P.; NOGUEIRA, D. ExpDes An R Package for ANOVA and Experimental Designs. *R Journal*, v.13, n.1, p.566-580, 2021. doi: <http://dx.doi.org/10.4236/am.2014.519280>

GALINATO, M. I.; MOODY, K.; PIGGIN, C.M. *Upland rice weeds of South and Southeast Asia*. Makati: International Rice Research Institute, 1999. 156 p.

GOETGHEBEUR, P. *Cyperaceae*. In: KUBITZKI, K. *et al.* *The families and genera of vascular plants*. Berlin: Sprinder-Verlag, 1998. p. 141-190.

GUIMARÃES, S.C.; SOUZA, I.F.; PINHO, E.V.R.V.; Emergência de *Tridax procumbens* em função de profundidade de semeadura, do conteúdo de argila no substrato e da incidência de luz na semente. *Planta Daninha*, v.20, n.3, p.413-419, 2002. doi: <https://doi.org/10.1590/S0100-83582002000300012>

HUMPHRIES, T.; CHAUHAN, B.S.; FLORENTINE, S.K. Environmental factors effecting the germination and seedling emergence of two populations of an aggressive agricultural weed: *Nassella trichotoma*. *Plos One*, v. 13, n. 7, 2018. doi: <https://doi.org/10.1371/journal.pone.0199491>

JENSEN, P.J.; HANGARTER, R.P.; ESTELLE, M. Auxin transport is required for hypocotyl elongation in light-growth but not dark-growth *Arabidopsis*. *Plant Physiol.*, v.116, p.455-462, 1998. doi: <https://doi.org/10.1104/pp.116.2.455>

JIANG, L. *et al.* Germination biology of three *Cyperaceae* weeds and their response to pre and post-emergence herbicides in paddy fields. *Agron.*, v.14, n.7, p.1592, 2024. doi: <https://doi.org/10.3390/agronomy14071592>

KISSMANN, K.G.; GROTH, D. *Plantas infestantes e nocivas*. Tomo I. São Paulo: BASF, 1997.

KUMAR, V. *et al.* Differential Germination Characteristics of Dicamba-Resistant *Kochia (Bassia scoparia)* Populations in Response to Temperature. *Weed Sci.*, v.66, n.6, p.721-728, 2018. doi: <https://doi.org/10.1017/wsc.2018.54>

LABOURIAU, L.G. *A germinação das sementes*. Washington: Secretaria Geral da Organização dos Estados Americanos. 1983.

LARRIDON, I. *et al.* Taxonomic changes in C4 *Cyperus* (*Cypereae*, *Cyperoideae*, *Cyperaceae*): combining the sedge genera *Ascolepis*, *Kyllinga* and *Pycneus* into *Cyperus* s.l. *Phytotaxa*, v.166, n.1, p.33-48, 2014. doi: <http://dx.doi.org/10.11646/phytotaxa.166.1.2>

MARCHESAN, E. *et al.* Produtividade, fitotoxicidade e controle de arroz-vermelho na sucessão de cultivo de arroz irrigado no Sistema Clearfield®. *Ciênc. Rural*, v.41, n.1, p.17-24, 2011. doi: <https://doi.org/10.1590/S0103-84782011000100004>

MARCOS FILHO, J. *Fisiologia de sementes de plantas cultivadas*. Londrina: ABRATES, 2015.

MENNAN, H.; NGOUAJIO, M. Seasonal cycles in germination and seedling emergence of summer and winter populations of catchweed bedstraw (*Galium aparine*) and wild mustard (*Brassica kaber*). *Weed Sci.*, v.54, n.1, p.114-120, 2006. doi: <http://dx.doi.org/10.1614/WS-05-107R1.1>

MEROTTO JR., A. *et al.* Interferência das plantas daninhas sobre o desenvolvimento inicial de plantas de soja e arroz através da qualidade da luz. *Planta Daninha*, v.20, n.1, p.9-16, 2002. doi: <https://doi.org/10.1590/S0100-83582002000100002>

OLIVOTO, T.; LÚCIO, A.D. metan: An R Package for Multi-Environment Trial Analysis. *Methods Ecol. Evol.*, v.11, n.6, p.783-789, 2020. doi: <https://doi.org/10.1111/2041-210X.13384>

PATTERSON, D.T. Comparative ecophysiology of weeds and crops. In: DUKE, S.O. *Weed physiology: Reproduction and ecophysiology*. Boca Raton: CRC Press, 1985. p. 102-129.

PERES, J.G.; SOUZA, C.F.; LAVORENTI, N.A. Avaliação dos efeitos da cobertura de palha de cana-de-açúcar na umidade e na perda de água do solo. *Eng. Agríc.*, v.30, p. 875-886, 2010. doi: <https://doi.org/10.1590/S0100-69162010000500010>

PITELLI, R.A.; DURIGAN, J.C. Ecologia das plantas daninhas no sistema plantio direto. In: ROSSELLO, R.D. *Siembra directa en el cono sur*. Montevideo: PROCISUR, 2001. p.203-210.

R CORE TEAM. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Viena, Áustria, 2023. Disponível em: <<https://www.R-project.org/>>. Acesso em: 6 jan. 2025.

RAJCAN, I.; SWANTON, C.J. Understanding maize-weed competition: resource competition, light quality and the whole plant. *Field Crops Res.*, v.71, n.1, p.139-150, 2001. doi: [https://doi.org/10.1016/S0378-4290\(01\)00159-9](https://doi.org/10.1016/S0378-4290(01)00159-9)

RAO, A.N. *et al.* Weed management in direct-seeded rice. *Adv. Agron.*, v.93, n.1, p.153-255, 2007. doi: [http://dx.doi.org/10.1016/S0065-2113\(06\)93004-1](http://dx.doi.org/10.1016/S0065-2113(06)93004-1)

RIAR, D.S. *et al.* Acetolactate synthase-inhibiting, herbicide-resistant rice flatsedge (*Cyperus iria*): cross-resistance and molecular mechanism of resistance. *Weed Sci.*, v.63, n. 1, p.748-757, 2015. doi: <http://dx.doi.org/10.1614/WS-D-15-00014.1>

ROSO, R. *et al.* Light quality and dormancy overcoming in seed germination of *Echium plantagineum* L. (Boraginaceae). *Bra. J. Biol.*, v.81, n.3, p.650-656, 2020. doi: <https://doi.org/10.1590/1519-6984.228777>

SCHMITH, J.; WULFF, R.D. Light spectral quality, phytochrome and plant competition. *Trends in Ecol. Evol.*, v.8, n.1, p.47-51, 1993. doi: [https://doi.org/10.1016/0169-5347\(93\)90157-k](https://doi.org/10.1016/0169-5347(93)90157-k)

SHRESTHA, A. *et al.* Differential Tolerance of Glyphosate-Susceptible and Glyphosate-Resistant

Biotypes of Junglerice (*Echinochloa colona*) to Environments during Germination, Growth, and Intraspecific Competition. *Weed Sci.*, v.66, n.3, p.340-346, 2018. doi: <https://doi.org/10.1017/wsc.2017.79>

SILVA, A. L. *et al.* Surveys of Weed Management on Flooded Rice Yields in Southern Brazil. *Weed Sci.*, v.70, n.2, p. 249-58, 2021. doi: <https://doi.org/10.1017/wsc.2021.77>

SOSBAI – Sociedade Sul-Brasileira de Arroz Irrigado. *Arroz irrigado: recomendações técnicas da pesquisa para o sul do Brasil*. Restinga Seca: SOSBAI, 2022.

STEFANELLO, R. Efeito do estresse hídrico na germinação e no vigor de sementes de anis (*Pimpinella anisum* L.), funcho (*Foeniculum vulgare* Miller) e endro (*Anethum graveolens* L.) *Rev Bras. Plantas Med.*, v.10, p.68-74, 2008.

TANG, W. *et al.* Effect of Environmental Factors on Germination and Emergence of Aryloxyphenoxy Propanoate Herbicide-Resistant and -Susceptible Asia Minor Bluegrass (*Polypogon fugax*). *Weed Sci.*, v.63, n.3, p.669-675, 2015. doi: <https://doi.org/10.1614/WS-D-14-00156.1>

TOMAZETTI, M.B. *et al.* Impact of ryegrass cover on lowland rice establishment. *Aust. J. Crop Sci.*, v.15, n.10, p.1217-1223, 2021. doi: <http://dx.doi.org/10.21475/ajcs.21.15.10.p2497>

ULGUIM, A.R. *et al.* Resistance Mapping of the Genus *Cyperus* in Rio Grande do Sul and Selection Pressure Analysis. *Planta Daninha*, n.37, p.1-8, 2019. doi: <https://doi.org/10.1590/S0100-83582019370100062>

ULGUIM, A.R. *et al.* *Manejo de Ciperáceas*. Pelotas: Rev. Cultivar, p.10-12, 2019.

VENABLES, W.N.; RIPLEY, B. D. *Random and mixed effects. Modern applied statistics with S*. New York: Springer-Verlag, 2002.

VILA-AIUB, M. *et al.* Ecological fitness of a multiple herbicide-resistant *Lolium rigidum* population: dynamics of seed germination and seedling emergence of resistant and susceptible phenotypes. *J. Appl. Ecol.*, v.42, n.2, p.288-298, 2005. doi: <https://doi.org/10.1111/j.1365-2664.2005.01017.x>

VILLELA, F.A.; DONI FILHO, L.; SEQUEIRA, E.L. Tabela de potencial osmótico em função da concentração de polietileno glicol 6.000 e da temperatura. *Pesq. Agropec. Bras.*, v.26, n.11/12, p.1957-1968, 1991. doi: <https://www.alice.cnptia.embrapa.br/alice/handle/doc/106202>

WICKHAM, H. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag, 2016.

WILKE, C.O. *cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'*. R package version 1.1.1, 2020.

WOOLLEY, J.T.; STOLLER, E.W. Light penetration and light-induced seed germination in soil. *Plant Physiol.*, v. 61, n.4, p.597-600, 1978. doi: <https://doi.org/10.1104/pp.61.4.597>

WU, X. *et al.* Germination Requirements Differ between Fenoxaprop-P-ethyl Resistant and Susceptible Japanese Foxtail (*Alopecurus japonicus*) Biotypes. *Weed Sci.*, v.64, n.4, p 653-663, 2016. doi: <http://dx.doi.org/10.1614/WS-D-16-00040.1>

YAMAMURA, K. Transformation using $(x+ 0.5)$ to stabilize the variance of populations. *Population*

Ecol., v.41, n.3, p.229-234, 1999. doi: <https://doi.org/10.1007/s101440050026>

YAMASHITA, O.M.; ALBERGUINI, A.L. Germination of *Vernonia ferruginea* as a function of breaking dormancy, light and temperature. *Comun. Sci.*, v.2, n.3, p.142-148, 2011. doi: <https://doi.org/10.14295/cs.v2i3.40>