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Ecophysiological aspects of seed germination in *Sagittaria montevidensis* biotypes resistant and susceptible to herbicides

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Abstract – The objective of this work was to evaluate the ecophysiological aspects of seed germination in California arrowhead (*Sagittaria montevidensis*) biotypes resistant and susceptible to herbicides. The experimental design was completely randomized. In paddy rice fields, seeds were collected from two biotypes that are resistant (SAGMO 10 and SAGMO 32) and from one that is susceptible (SAGMO 35) to acetolactate synthase and photosystem II inhibiting herbicides. Seed dormancy release was performed with 2.0% potassium nitrate, chemical (1.0% H_2SO_4 for 30 s) and mechanical (sandpaper) scarification, 10 ppm gibberellic acid, water imbibition for 24 hours, water bath at 60°C, and a control. Germination was evaluated at temperatures from 10 to 40°C and seedling emergence at 0.0, 0.5, 1.0, 2.0, 4.0, and 5.0 cm burial depths. All biotypes showed a germination higher than 70% after mechanical scarification. The highest germination rate occurs at 25.9°C for SAGMO 35, the susceptible biotype, and at 26.2 and 26.5°C, respectively, for SAGMO 10 and SAGMO 32, the resistant biotypes. For all biotypes, the highest seedling emergence occurs in seeds positioned at the 0.5 and 1.0 cm burial depths.

Index terms: *Oryza sativa*, ALS- and PSII-inhibiting herbicides, California arrowhead, pre-germinated system, seed dormancy.

Aspectos ecofisiológicos da germinação de sementes em biótipos de *Sagittaria montevidensis* resistentes e suscetível a herbicidas

Resumo – O objetivo deste trabalho foi avaliar aspectos ecofisiológicos da germinação de sementes em biótipos de sagitária (*Sagittaria montevidensis*) resistentes e suscetível a herbicidas. O delineamento experimental foi o inteiramente casualizado. Em campos de arroz, foram colhidas sementes de dois biótipos resistentes (SAGMO 10 e SAGMO 32) e de um suscetível (SAGMO 35) aos herbicidas inibidores da acetolactato sintase e do fotossistema II. A liberação da dormência foi realizada com 2,0% de nitrato de potássio, escarificação química (1,0% de H_2SO_4 por 30 s) e mecânica (lixa), 10 ppm de ácido giberélico, embebição em água por 24 horas, banho-maria a 60°C e um controle. Avaliaram-se a germinação às temperaturas de 10 a 40°C e a emergência de plântulas aos 0,0, 0,5, 1,0, 2,0, 4,0 e 5,0 cm de profundidade de enterrio. Todos os biótipos apresentaram germinação superior a 70% após escarificação mecânica. A maior taxa de germinação ocorre a 25,9°C para SAGMO 35, o biótipo suscetível, e a 26,2 e 26,5°C, respectivamente, para SAGMO 10 e SAGMO 32, biótipos resistentes. Para todos os biótipos, a maior emergência de plântulas ocorre em sementes posicionadas a 0,5 e 1,0 cm de profundidade de enterrio.

Termos para indexação: *Oryza sativa*, herbicidas inibidores da ALS e FSII, sagitária, sistema pré-germinado, dormência de sementes.

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Introduction

Rice (*Oryza sativa* L.) is an important agricultural crop around the world, consumed daily by billions of people, especially in Asian countries (Ziska et al., 2015). Brazil is the largest rice producer outside of the Asian continent, with an average annual production of 11.2 million tons (Acompanhamento…, 2020). The states of Rio Grande do Sul and Santa Catarina account for more than 80% of the Brazilian rice production (Acompanhamento…, 2020).

Many lowland fields used for paddy rice production have hydromorphic soils, in which flood inundation occurs during the full crop cycle, an important characteristic for management weed and assuring a high grain yield (Mentges et al., 2013). Under a pre-germinated system, also known as water-seeded rice, soil is prepared in a systematized area for soil leveling, followed by the distribution of pre-germinated seeds in the flooded area (Machado et al., 2006). However, flooding conditions are also a favorable environment for the development of aquatic weeds such as California arrowhead (*Sagittaria montevidensis* Cham. & Schltdl.), a broadleaf weed commonly found in water-seeded rice that can reduce grain yield up to 10% depending on the plant population (Gibson et al., 2001).

Initially, the chemical control of California arrowhead in water-seeded rice included the application of acetolactate synthase (ALS)-inhibiting herbicides, especially of pyrazosulfuron-ethyl, metsulfuron-methyl, and bispyribac-sodium at postemergence (Merotto Junior et al., 2010). However, due their continuous use, the control efficacy of these herbicides declined, leading rice farmers to change to photosystem II (PSII)-inhibiting herbicides such as bentazon that is widely applied for the management of the weed (Moura et al., 2015). Currently, the resistance to ALS- and PSII-inhibiting herbicides represents a major challenge in several paddy rice areas, with populations showing cross- and multiple resistance to these modes of action, reducing the chemical options for California arrowhead control (Moura et al., 2016).

California arrowhead usually grows from seeds located at the end of the stem, containing unisexual inflorescences with achenes and seeds, which are easily dispersed, floating on water surface (Kissmann, 1997). However, weed population dynamics and fluctuations can change due to herbicide resistance, especially if the soil seed bank increases in such a way as to trigger future infestations. Other traits directly involved in the temporal regulation of the germination and longevity of soil seed banks include endogenous and exogenous seed attributes, such as primary dormancy, seed longevity, and abscisic (ABA) and gibberellic acid (GA_3) balance, as well as environmental conditions (Evans et al., 2016; Née et al., 2017).

Seed germination requires favorable environmental conditions to promote embryo growth, which is strongly regulated by seed dormancy, an adaptive cost for weeds (Graeber et al., 2012; Née et al., 2017). Seed dormancy is a natural process based on the physiological and physical factors that occur during the ripening of seeds from the mother plant, regulated by environmental conditions such as air temperature, light radiation, gaseous exchanges, and soil moisture (Kendall et al., 2011; He et al., 2014), affecting the dynamics of the population in the soil.

Efficient strategies to reduce soil seed banks and prevent weed interference in agricultural systems include seed germination stimulation and an increased depletion (Evans et al., 2016). In this case, breaking dormancy is a critical step for seed germination after exposure to favorable conditions, and the selection of an effective method for this depends on the type of dormancy (Finch-Savage & Footitt, 2017). Temperature and seed position in the soil profile regulate seed dormancy and germination, contributing to the light signal perception by phytochromes, hormonal balance, gene expression, and transcription factors (Kendall et al., 2011). Pleiotropic genes, for example, can regulate ABA and flavonoid synthesis, increasing seed dormancy (Née et al., 2017), whereas temperature and soil moisture can affect the germination of weed seeds (Fogliato et al., 2010).

Understanding the relationship between weed biology and germination will enhance integrated weed management strategies aiming to decrease herbicide resistance, also assuring a greater sustainability of the current technologies that are becoming restricted in several paddy rice fields (Westwood et al., 2018). Furthermore, studies on the ecophysiological aspects of California arrowhead – related to methods for seed dormancy release and to evaluations of environmental conditions for germination – are still necessary and could provide practical information, helping to reduce the soil seed bank and prevent the evolution of weed resistance.

The objective of this work was to evaluate the ecophysiological aspects of seed germination in California arrowhead biotypes resistant and susceptible to herbicides.

Materials and Methods

Two experiments were carried out between September 2017 and October 2018 to evaluate seed-dormancy release methods and seed germination temperature, being repeated in time throughout the summer growing season at the Seed Laboratory of Faculdade de Agronomia Eliseu Maciel of Universidade Federal de Pelotas, located in the municipality of Pelotas, in the state of Rio Grande do Sul, Brazil (31°80'15"S, 52°41'48"W). To assess seed emergence depth, another experiment was performed in a greenhouse at the Terras Baixas experimental station belonging to Embrapa Clima Temperado, located in the municipality of Capão do Leão, also in the state of Rio Grande do Sul (31°80'32"S, 52°40'22"W). The experimental design was completely randomized, with four replicates, totaling 200 seeds.

The used seeds were hand harvested from California arrowhead biotypes grown in three paddy rice fields in different municipalities of the state of Santa Catarina, Brazil, which were managed under a pre-germinated system, with a repeated use of ALS- and PSII-inhibiting herbicides by the rice growers. The seeds were obtained from mother plants of the following biotypes: SAGMO 35, susceptible to the herbicides, in the municipality of Bombinhas (27°08’45’’S, 48°30’23’’W); SAGMO 10, cross-resistant to ALS-inhibiting herbicides, in Itajaí (26°56’39’’S, 48°45’38’’W); and SAGMO 32, multiple-resistant to ALS- and PSII-inhibiting herbicides, in Ilhota (26°51’59’’S, 48°46’40’’W). The susceptibility and resistance of the biotypes were confirmed by the dose-response curve (Moura et al., 2015). After harvested, these seeds were self-pollinated throughout the two experimental years to guarantee seed viability and stored at 5°C until suitable for use in trials to evaluate seed dormancy, temperature germination, and seedling emergence out of the soil. Previously, 200 seeds of each biotype were assessed to determine seed viability using a 1.0% tetrazolium salt solution (2,3,5-triphenyltetrazolium chloride) and were kept in dark imbibition for 24 hours, at 30°C, to develop the embryo red color of viable seeds. Seeds were longitudinally cut through the embryo, for which solution concentration and exposure time were pre-determined due to the lack of reference values available in the literature for this weed species. The SAGMO 35, SAGMO 10, and SAGMO 32 biotypes showed 95, 92, and 94% seed viability, respectively.

The seed-dormancy release methods evaluated were: 0.2% potassium nitrate (KNO_3), chemical scarification with sulfuric acid (H_2SO_4), mechanical scarification with sandpaper, 10 ppm GA_3, water imbibition for 24 hours, water bath at 60°C, and a control treatment. Fifty seeds from each California arrowhead biotype were used per replicate, being placed in petri dishes containing 25 mL water/treatment solution. For chemical scarification, seeds were immersed in a 1.0% H_2SO_4 solution and shaken for 30 s, then washed for 5 min and dried on absorbent paper for 20 min at room temperature. For mechanical scarification, seeds were covered by the rough surface of sandpaper sheets (grit size 150), which had a weight of 750 g put on top of them to produce the same work power and were rubbed counter-clockwise four times (α=1440°) for an even scarification of the seed tegument. For the water imbibition and water bath methods, seeds were imbibed in distilled water for 24 hours at room temperature and in a water bath for 5 min at 60°C, respectively. The seeds were maintained in petri dishes with 25 mL distilled water and placed in a biological oxygen demand (BOD) chamber, at 25°C, with a 12 hour light/dark photoperiod.

The germinated seedlings – with protrusion of the primary root – were counted daily over a 14 day period shortly after the beginning of the test, with the accumulated values being expressed in percentage. Data normality was analyzed by the Shapiro-Wilk test and subjected to the analysis of variance ($p<0.05$). The percentages of accumulated seed germination for the biotypes under different seed-dormancy release methods were adjusted by the nonlinear exponential regression with three parameters, using the SigmaPlot, version 12.0, software (TIBCO Software Inc., Palo Alto, CA, USA). The mathematical expression relating the response was given by: $Y = a / 1 + e^{-((x - x0) / b)}$, where Y is the accumulated germination percentage, e is the exponential function, x is the days after the beginning of the germination test, x_0 is the days required to promote 50% germination, a is the upper limit, and b is the curve slope.

To assess germination temperature, initially, mechanical scarification – considered the best method to break dormancy – was performed on seeds of all biotypes. A total of 200 seeds, 50 from each biotype, were placed in petri dishes containing 25 mL distilled water. Germination was evaluated in a BOD chamber at 10, 15, 20, 25, 30, 35, and 40ºC, under a 12 hour light/dark photoperiod. Data normality was analyzed by the Shapiro-Wilk test and subjected to the analysis of variance ($p<0.05$). The regression analysis was performed for the temperature independent variable, with adjustment for the nonlinear model of the quadratic-type curve, given by: $Y= a + bx + cx^2$, where Y is the germination response; x is the germination temperature; and a, b, and c are the estimated equation parameters.

Mechanical scarification was used to break the dormancy of 50 seeds from each biotype, which were then placed in 5 L plastic pots containing samples of a soil classified as a Planossolo Háplico Eutrófico solódico (Santos et al., 2013), equivalent to an Albaqualf (Soil Survey Staff, 2014). The seeds were buried at 0.0, 0.5, 1.0, 2.0, 4.0, and 5.0 cm depth and maintained under flooding (±1.0 cm) throughout the field experiment.

For seedling emergence in the field, data normality was analyzed by the Shapiro-Wilk test and subjected to the analysis of variance ($p<0.05$). The regression analysis was performed using the Sigma Plot, version 12.0, software (Systat Software Inc., San Jose, CA, EUA), adjusted to the Gauss nonlinear model, given by $Y = y_0 + a \times e^{[-0.5 \times ((x - x0) / b)^2]}$, where Y is the germination response, y_0 is the intercept value when $x = 0$, a is the difference between the maximum and minimum values of the response variable, e is the exponential function, x is burial depth, x_0 is the upper limit, and b is the slope of the trend line.

Results and Discussion

The seeds of the different California arrowhead biotypes were not significantly affected by treatments, only by the dormancy release methods (Figure 1 and Table 1). The evaluated seeds showed high dormancy levels, with a germination lower than 18% for the control. Mechanical scarification increased seed germination to 67% and was considered the most efficient method to break dormancy, whereas water imbibition for 24 hours and water bath at 60°C raised germination rates from 18 to 31%. However, the solutions of 0.2% KNO_3, 1.0% H_2SO_4 for 30 s, and 10 ppm GA_3 did not enhance significantly seed germination.

Seed dormancy is an important evolutive trait for several weed species that ensures a long-term survival for seedlings to germinate under favorable development conditions (Pipatpongpinyo et al., 2020). For weed seeds present in the soil, temperature and soil water status are important signals that indicate the temporal window to activate embryo activity and promote seed germination (Graeber et al., 2012). However, inadequate conditions can induce secondary dormancy and delay seedling establishment and development (Footitt et al., 2011).

Seed germination increased shortly after mechanical scarification, showing values greater than 70% regardless of the evaluated biotype, which is attributed to primary dormancy due to the impermeable tegument (Figure 1). In contrast, the accumulated germination was lower than 30% for the other methods used for dormancy release and of 18% for the control. Different results were obtained for other weed species. In maple-leaved goosefoot (*Chenopodium hybridum* L.), mechanical scarification was considered the best method to break seed dormancy, with thresholds greater than 80% (Hu et al., 2017). In giant ragweed (*Ambrosia trifida* L.) seeds, however, chemical scarification with 0.1% KNO_3 enhanced germination up to 34%, whereas 0.02% GA_3 solution was not considered an efficient method to release dormancy (Page & Nurse, 2015).

Dormancy release strongly affects the activation of the signals for germination, which trigger changes in the turgor and mechanical properties of the cell wall, enhancing water uptake and promoting germination (Finch-Savage & Footitt, 2017). For *Arabidopsis thaliana* Schur seeds shed from mother plants, there was a gradual dormancy release with a dependent endogenous hormonal regulation between ABA and GA, which can repress the specific dormancy genes, homologous repressors, and chromatin modifications involved in the germination process (Née et al., 2017). Therefore, higher levels of ABA act as negative regulators, whereas GAs can promote germination (Graeber et al., 2012). In contrast, the seeds from different California arrowhead biotypes treated with

10 ppm GA_3 showed a germination lower than 20% that was quite similar to that of the control (Figure 1). In rigid ryegrass (*Lolium rigidum* Gaudin), 10 µm GA_3 did not increase seed germination due to the absence of changes in the ABA:GA balance, which is an indicative that other factors can play an essential role in germination induction (Goggin et al., 2009). Therefore, the strong dormancy of the tegument of California arrowhead seeds shows the importance of rupturing this outer covering with sandpaper to enhance water or oxygen permeability, activating directly basal metabolism and promoting seed germination.

The germination of seeds from the different California arrowhead biotypes was affected by temperature.

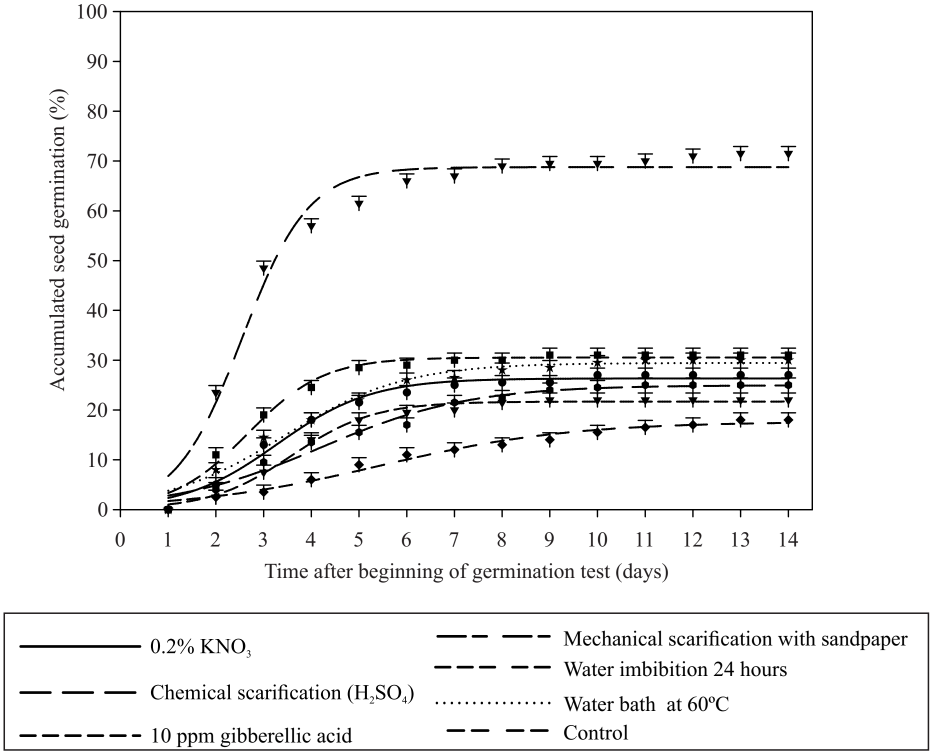

Figure 1. Accumulated germination of California arrowhead (*Sagittaria montevidensis*) seeds over time (days) under different seed-dormancy release methods. Points represent the average of the treatment at each day evaluated. Bars represent the standard deviation.

Table 1. Estimation parameters used for the nonlinear exponential regression of the accumulated germination percentage of California arrowhead (*Sagittaria montevidensis*) seeds over time (days) under different seed-dormancy release methods.

Seed-dormancy release method	Regression parameter[(1)]			
	a	x_0	b	R^2
Potassium nitrate (0.2% KNO_3)	26.01	3.25	0.96	0.98
Chemical scarification (1.0% H_2SO_4)	24.95	4.20	1.55	0.98
Gibberellic acid (10 ppm)	21.68	3.59	0.86	0.99
Mechanical scarification with sandpaper	68.26	2.53	0.68	0.98
Water imbibition for 24 hours	30.52	2.67	0.79	0.98
Wash bath at 60ºC	29.44	3.42	1.26	0.98
Control	17.63	5.44	1.99	0.98

(1)Nonlinear exponential regression with three parameters: $Y = a / 1+e^{-(x - x0 / b)}$, where Y is the accumulated germination percentage, e is the exponential function, x is the days after the beginning of the germination test, x_0 is the days required to promote 50% germination, a is the upper limit, and b is the curve slope. R^2, coefficient of determination.

Lower percentages were found for seeds from the SAGMO 32 and SAGMO 10, with multiple resistance to ALS- and PSII-inhibiting herbicides and with cross-resistance to ALS-inhibiting herbicide, respectively, in comparison with SAGMO 35, a susceptible biotype, especially at temperatures between 15 and 35ºC (Figure 2). The maximum seed germination occurred at 25.9°C for seeds of the SAGMO 35 biotype and at 26.2 and 26.5°C, respectively, for those of SAGMO 10 and SAGMO 32, reaching values near 70%. Although both resistant and susceptible biotypes showed a high seed viability – measured previously in the tetrazolium solution –, the germination of resistant biotypes at greater temperatures may be an indicative that these biotypes require a major thermal sum, leading to a delay in germination in paddy rice fields. Several studies have shown the role of temperature in promoting seed germination due to its involvement in enzymatic and biochemical processes that degrade the seed reserves and redox reactions, which are essential for embryo growth and radicle protrusion (Graeber et al., 2012; Rosental et al., 2014; Née et al., 2017).

At 10ºC, regardless of the California arrowhead biotype, no seeds germinated, whereas at temperatures greater than 30°C, germination was strongly reduced (Figure 2). At 15°C, approximately 38% of the seeds from the resistant biotypes germinated, causing earlier infestations under lower soil temperatures. In the literature, irrigated rice showed an optimal germination at 32.1°C (Ali et al., 2006) and weedy rice, between 20 and 25°C (Young-Son, 2010). For the SAGMO 10 and SAGMO 32 herbicide-resistant biotypes, the optimal temperature ranged from 26.2 to 26.5°C, based on the maximum slope of the quadratic curve, showing that the seeds from these biotypes germinated at a lower temperature than rice. In smallflower umbrella sedge (*Cyperus difformis* L.) with ALS herbicide resistance, maximum seed germination occurred at 33ºC, with a lower thermal time than rice (Pedroso et al., 2019). Seeds of three-leaf arrowhead (*Sagittaria trifolia* L.) populations from different paddy rice fields in Japan

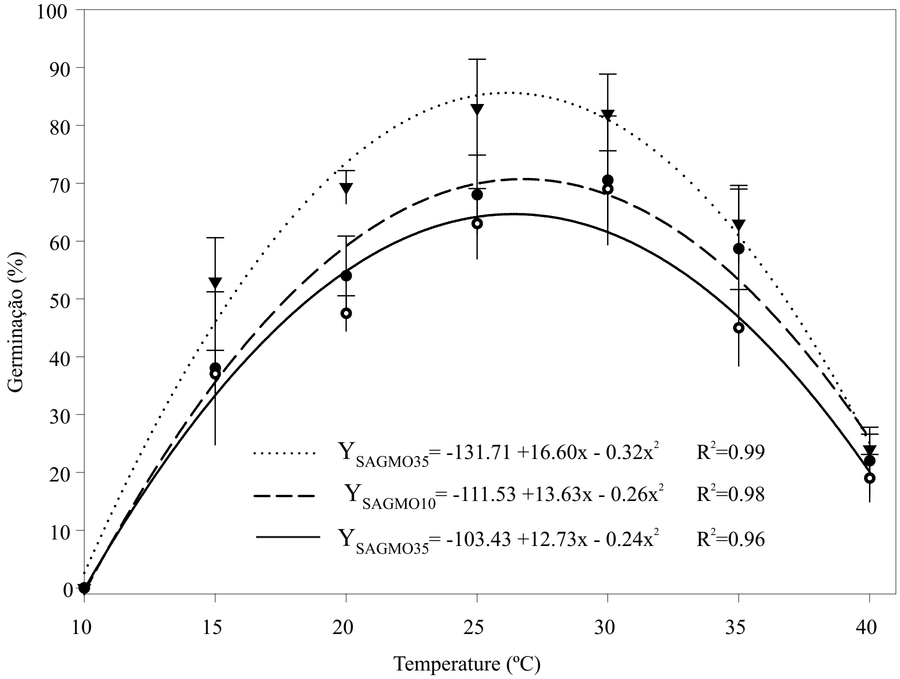

Figure 2. Seed germination of different California arrowhead (*Sagittaria montevidensis*) biotypes at 14 days after the beginning of the germination test under different temperatures. Bars represent the standard deviation. Biotypes: SAGMO 35, susceptible; SAGMO 10, cross-resistant to acetolactate synthase-inhibiting herbicide; and SAGMO 32, resistant to acetolactate synthase- and photosystem II-inhibiting herbicides.

required light radiation and temperatures above 30/20°C for germination, which reached values greater than 60% (Ozaki et al., 2018).

In cropping systems, temperature is an environmental signal that regulates the germination of dormant and quiescent seeds, although it can also induce secondary dormancy under unfavorable conditions and increase seed longevity in the soil seed bank (Bittencourt et al., 2016). However, the competitive ability of the weed depends on how rapidly its seedling establishes itself to explore the space. For example, the emergence of seedlings from the three-leaf arrowhead under an optimal temperature favored either biomass or rhizome accumulation (Daimon et al., 2014), being affected by the position of the seed in the soil.

The burial depth of California arrowhead seeds reduced seedling emergence rates, with no significant differences between the evaluated biotypes (Figure 3).

A greater germination above 80% was observed for seeds buried at 0.5 and 1.0 cm. The germination rates were 58% for seeds positioned on soil surface, but lower than 4.0% for those buried at 4.0 and 5.0 cm throughout the 14 day period after the beginning of the experiment. The three-leaf arrowhead showed a similar germination when seeds were positioned up to 10 cm depth (Xiao et al., 2010) and persisted for at least nearly one year when the seeds were positioned at 3.0 cm depth (Ozaki et al., 2018). In rice flatsedge (*Cyperus iria* L.) and globe fringerush [*Fimbristylis miliacea* (L.) Vahl], the seeds that were not buried showed the greatest germination and those that were buried at 2.0 cm or deeper did not germinate (Chauhan & Johnson, 2009).

When smaller weed seeds are buried at greater depths, their germination can be reduced due to the lower soil temperature and light perception (Finch-Savage & Footitt, 2017; Zhao et al., 2018). Furthermore, weed emergence and development in paddy rice fields can be affected by a continuous flood management, with a water blade greater than 4.0 cm (Chauhan & Johnson, 2009). For California arrowhead, maximum germination rates for the seeds located on the soil

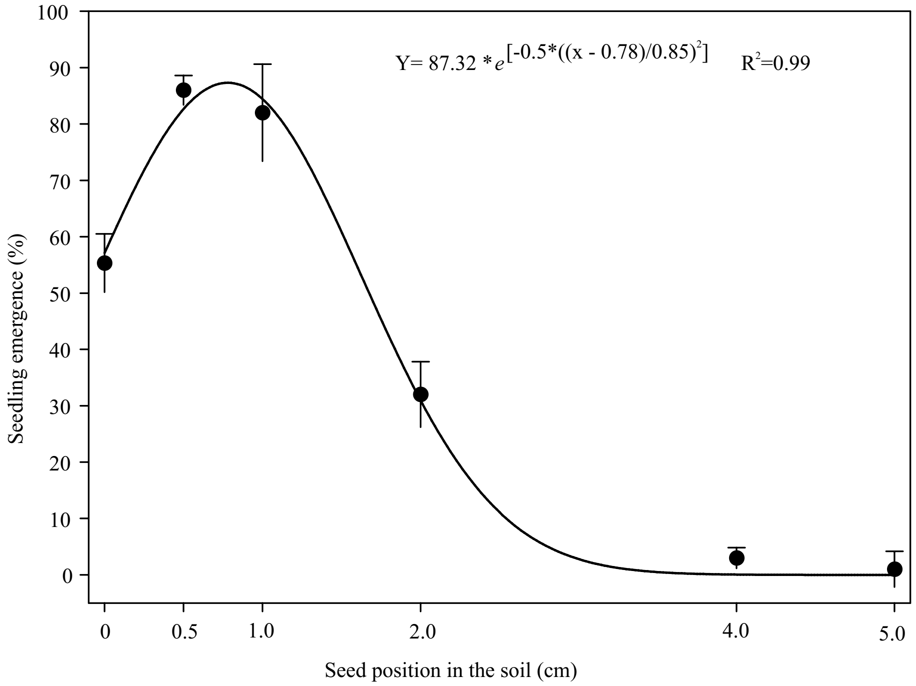

Figure 3. Seedling emergence of different California arrowhead (*Sagittaria montevidensis*) biotypes 14 days after the seeds were buried in different positions in the soil. Bars represent the standard deviation. Biotypes: SAGMO 35, susceptible; SAGMO 10, cross-resistant to acetolactate synthase-inhibiting herbicide; and SAGMO 32, resistant to acetolactate synthase- and photosystem II-inhibiting herbicides.

surface are essential to optimize seedling emergence. The obtained results confirm that there is a need to break seed dormancy, germination occurs between 25 and 30°C, and seeds near soil surface show high emergence rates and a better establishment, traits that should be considered to reduce the damage caused by California arrowhead and to delay the evolution of herbicide resistance in paddy rice fields.

Conclusions

1. Mechanical scarification with sandpaper enhances the germination rates of California arrowhead seeds, with values greater than 70% regardless of the biotype.
2. The highest germination rate occurs at 25.9°C for SAGMO 35, the susceptible biotype of California arrowhead (*Sagittaria montevidensis*), and at 26.2 and 26.5°C, respectively, for SAGMO 10 and SAGMO 32, the resistant ones.
3. The highest emergence of California arrowhead seedlings occurs at the burial depths of 0.5 and 1.0 cm.

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