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ORIGINAL ARTICLE

Effects of substratum, temperature, and treatments to overcome dormancy on the germination of *Fimbristylis dichotoma* seeds

Temperatura, substrato e tratamentos de superação de dormência na germinação de sementes de Fimbristylis dichotoma

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ABSTRACT: Tall fringe-rush (*Fimbristylis dichotoma*) is a common weed in wetlands. Knowledge of the physiology of its seed germination can significantly contribute to the development of management strategies. The objective of this study was to determine the best combination of temperature, substratum, and treatments leading to seed dormancy suppression thus enhancing seed germination. In a first phase, seeds were put to germinate under constant temperatures ranging from 15 to 40 °C at 5 °C intervals and alternating temperatures of 15-25, 15-35, and 20-30 °C (8 h of light). The effects were evaluated by means of a germination test carried out in filter paper. In another experiment, the seeds were submitted to the following treatments: 1. seeds immersion in H_2SO_4 for 3 min, 2. seeds immersed in H_2O for 15 min, 3. seeds immersed in a 1% KNO_3 solution for 15 min, 4. seeds dried at 50 °C for 12 h, and 5. non treated (check treatment) seeds. The effects in the second experiment were evaluated by means of a germination test carried out in the following substrata: 1. a ready-made substratum (bioplant prata™), 2. soil, and 3. sand. In both experiments, the evaluations were the following: total germination, germination first count (three days), speed of germination index, and mean time for seed germination. The experimental units were distributed in the germination chamber according to a completely random design with four repetitions. The results showed that the best germination result (94%) was attained when the tall fringe-rush seeds were dried for 12 h at 50 °C and put to germinate under either ready-made or soil substrata and under alternate temperatures of either 15-25 or 15-35 °C.

RESUMO: *O falso-alecrim-da-praia (Fimbristylis dichotoma) é uma planta daninha comum em áreas úmidas. O conhecimento da ecofisiologia da germinação dessa espécie pode contribuir significativamente para o desenvolvimento de estratégias de manejo. O objetivo deste trabalho foi identificar condições de temperatura, substrato e tratamentos de superação de dormência que favorecem a germinação de sementes dessa espécie. Numa primeira etapa da pesquisa, foi avaliada a germinação das sementes sob temperaturas constantes de 15 a 40 °C, com intervalos de 5 °C, e temperaturas alternadas de 15-25, 15-35 e 20-30 °C (8 h de luz)***.** *Numa segunda etapa, foram avaliados os tratamentos de imersão das sementes em* H_2SO_4 *por 3 min;* H_2O *por 15 min;* KNO_3 *(1%) por 15 min; secagem a 50°C por 12 h, e sementes sem tratamento (testemunha), avaliadas quanto à germinação em substrato industrializado para mudas, terra e areia. Determinaram-se a germinação; a primeira contagem de germinação (sete dias); o índice de velocidade de germinação, e o tempo médio de germinação até 40 dias, avaliado em um delineamento inteiramente casualizado com quatro repetições de 200 sementes. Concluiu-se que a germinação de sementes de falso-alecrim-da-praia deve ser realizada nas temperaturas alternadas de 15-25 e 15-35 °C, em substrato industrializado para mudas ou terra, e após secagem das sementes a 50 °C por 12 h, para a superação da dormência (94% de germinação).*

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1 Introduction

Fimbristylis dichotoma Vahl (Kranz) (Cyperaceae, Poales) is a member of the Cyperaceae family (order Poales) and in Brazil is known as "falso-alecrim-da-praia". In English its probably most used names are tall fringe-rush and two-leaf fimbristylis. It is a polymorphic plant, herbaceous, grows in thickets, erect, bearing short rhizomes, glabrous and triangular stems, 20-40 cm of height, originated from the south-west of Asia and being either annual or perennial. It is a frequent weed in the southern regions of Brazil. It is found both in wetlands and in drier soils. It is found infesting lawns, pastures, irrigated rice fields, cultivated plains, drainage channels, alongside roads, and in coastal plains where it flowers almost the whole year. It propagates by means of rhizomes and seeds (BARROS, 2009; ALBUQUERQUE et al., 2012; MARTINS; SCATENA, 2013).

The dispersion structure used as a seed in the asexual propagation of the species are fruits of the achene type which present in their interior a differentiated embryo with coleoptiles turned to the micropyle and the radicle laterally positioned. Germination starts to occur visibly between one and three days after sowing over wet paper, kept at 30 °C and it is characterized by the emergence of the coleoptile followed by the primary root (MARTINS; SCATENA, 2013).

Studies concerning germination physiology and dormancy of weed seeds are important for the understanding of the plants aggressive regeneration which is based on seed based propagation and may help in the development of control strategies, reduction of seed banks and aerial infestations (VIVIAN et al., 2008).

Temperature, water, and oxygen are the main external factors influencing seed germination (MARCOS FILHO, 2005). Three are the ways by which temperature affects seed germination: determining the capacity and the percentage of seed germination, removing primary and secondary dormancy or inducing secondary dormancy (BEWLEY; BLACK, 1985).

Seeds of some species germinate at an ample range of temperature. But, there is a temperature which is considered the optimum which is that that permits maximum germination in the shortest period of time. There are also the minimum and the maximum temperatures which are those which do not permit the seed to germinate; these are the cardinal temperatures. Thus, seed germinability is maximum within a variable range of temperature; below and above that range, germination tends to decrease (KISSMANN et al., 2008; NOGUEIRA et al., 2013).

The species display variable behavior in relation to temperature (MARCOS FILHO, 2005), although the range from 20 to 30 °C seems to be adequate for the germination of seeds of a large number of species both tropical and subtropical. Alternating temperatures favor seed dormancy breaking (BEWLEY; BLACK, 1985). So, it would be recommendable to include alternating temperatures in research works viewing to establish procedures for the analysis of seeds, alternating temperatures being necessary for non domesticated species (OLIVEIRA; PIÑA-RODRIGUES; FIGLIOLIA, 1989).

Such as temperature, substratum also has an influence on seed germination as determined by its structure, aeration, water holding capacity, etc, thus being capable of favoring or hampering seed germination. The substratum represents the physical support upon which the seed is placed with the function of offering and preserving the appropriate conditions for the seed germination and seedling growth (MARTINS; MACHADO; NAKAGAWA, 2008; NOGUEIRA et al., 2013). Some species are more demanding so as to show a good germination performance only in one type of substratum, such as "juca" (*Caesalpinia ferrea)* which should be sown in sand (LIMA et al., 2006) and "sabia" (*Mimosa caesalpineafolia*) which should be sown in towel paper (NOGUEIRA et al., 2013). The seeds of some other species though are more well adapted and are capable of germinating in various substrata, such as those of "tento vermelho" (*Adenanthera pavonina*) which are capable of germinating both over paper and in towel paper roll (KISSMANN et al., 2008) and "barbatimão" (*Stryphnodendron barbaditiman*) which germinates in vermiculite, towel paper roll, and soil (MARTINS; MACHADO; NAKAGAWA, 2008).

Other characteristics of the species may difficult or block the immediate germination of the seed, such as dormancy, common in seeds of weed species. In order to study either in the field or in greenhouse, researchers from either public or private institutions buy the weed seeds from special companies or they collect directly from the field. Those seeds must germinate prompt and uniformly in order to permit the testing of chemical control procedures or field management practicesOther characteristics of the species may difficult or block the immediate germination of the seed, such as dormancy, common in seeds of weed species. In order to study either in the field or in greenhouse, researchers from either public or private institutions buy the weed seeds from special companies or they collect directly from the field. Those seeds must germinate prompt and uniformly in order to permit the testing of chemical control procedures or field management practices (VIVIAN et al., 2008; PARREIRA et al., 2012). So, these seeds should be dormancy-free at the moment they are bought or that the dormancy might be easily suppressed before they are sown. Exposing the seeds of some species to temperatures between 40 and 80 °C in ovens has been reported as giving good results (ALMEIDA; SILVA, 2004; MARTINS; MARTINS, 2013), Treatments with KNO_3 and H_2SO_4 (ALMEIDA; SILVA, 2004; KISSMANN et al., 2008; BRASIL, 2009) or immersion in water (LACERDA et al., 2010) have also been reported to give good results.

The objective of this work was to verify which combinations of dormancy breaking factors would prompt and enhance *Fimbristylis dichotoma* seeds germination.

2 Materials and Methods

The plants from which the *Fimbristylis dichotoma* seeds were taken were herborized and identified by a specialist in herbarium plants of the Cyperaceae family (collector: Martins, D., no number. Registered as BOTU 027534). The racemes were manually harvested when they had one third of the grains shed and taken to a laboratory to complete the drying of the seeds under shade conditions where they remained for four days. After that period, the racemes were gently shaken over a paper sheet so as to release the seeds (MARTINS; MARTINS, 2013). The material that stayed on the rachis was discarded due to the fact that that material usually is composed of empty spikelets or immature seeds. The study was carried out at the Seed Analysis Laboratory of the College of Agrarian and Veterinary Sciences , a unit of the Paulista State University (UNESP), on its campus of Jaboticabal, state of São Paulo, Brazil.

Two experiments were carried out : in the first, temperatures for seed germination were evaluated; in the second, substrata and treatments for seed dormancy suppression were tested.

In the first experiment, the temperature regimens were the following: constant temperatures of 15, 20, 25, 30, 35, and 40 °C and alternate temperatures of 15-25, 15-35, and 20-30 °C with a photoperiod of 8 h. The seeds in a number of 200 per replication were placed over two sheets of filter paper wet according to instructions in (BRASIL, 2009) and these put inside plastic boxes (11.0 x 11.0 x 3.5 cm) and taken to a germination chamber.

In the second experiment, the seeds were submitted to the following treatments: 1. Immersed in H_2SO_4 for 3 min, 2. Immersed in water for 15 min, 3. Immersed in a 1% solution of KNO_3, and 4. Dried at 50 °C for 12 h, and 5. seeds not submitted to any treatment (check). The substrata were the following: 1. ready-made substratum (bioplant prata™), 2. soil, and 3. sand. The seeds were spread over these substrata and kept in a germination chamber at alternate temperatures of 15 and 35 °C. The ready-made, sand, and soil substrata were moistened, respectively, to 50, 50, and 60% of their field capacity (MARTINS; MACHADO; NAKAGAWA, 2008; BRASIL, 2009).

The results of both experiment consisted of daily germination counts of normal seedlings (BRASIL, 2009). The test was interrupted when seedling emergence was verified no longer to occur. Final results were expressed in percentage. First count of germination was carried out 3 days after sowing- the results being the percentage of normal seedlings found that day (MARTINS; SCATENA, 2013). Speed of germination index and the mean time for seed germination (SANTANA; RANAL, 2004). Were also determined making use of the number of normal seedlings emerged each day between 3 and 36 days after sowing.

As to the procedure for the statistical analysis of the data, the experiments had their experimental units distributed in the germination chamber according to a completely random design, with four repetitions. In the first experiment, the treatments consisted of nine temperature regimens under which the germination test was conducted. In the second experiment, the treatments consisted of the factorial 3 × 5 (substrata and treatments for overcoming seed dormancy). The data were submitted to the analysis of variance by the F test and the treatment means compared by the Tukey test ($p < 0,05$).

3 Results and Discussion

The first experiment showed that the constant temperatures of 15 and 40 °C did not permit the seeds to germinate. These consequently may be considered, respectively, as the minimum and the maximum temperatures for the germination of *F. dichotoma* seeds (Table 1). According to Marcos Filho (2005), the minimum, lower than 15 °C, and maximum, between 35 and 45 °C, are, respectively, those below and above which seed germination no longer occurs. Those extreme limits and the optimum temperatures are the so called cardinal temperatures (KISSMANN et al., 2008).

The temperature of 20 °C, although permitting the seeds a germination value within the maximum range, is seen to be characterized as a process slower and less uniform than that verified to occur when the temperatures were of 15-25 or 15-35 °C, as pointed by the results of all the vigor tests used in the experiment. The first count of germination was equal to zero, the speed of germination index (SGI) was below the average (8.685), the mean time for germination was very long - it demanded 20 days to complete whereas the more favorable temperatures permitted the germination process to complete in 4 to 7 days.

Therefore, the optimum temperatures for *F. dichotoma* seeds to germinate are the alternate ones of 15-25 and 15-35 °C - these were the temperatures allowing the highest germination, uniformity, and speed of germination according to the first count of germination, speed of germination index, and mean time for seed germination tests.

Alternating temperatures led to better results than constant temperatures for the following grass species: *Aristida gibbosa* (Nees) Kunth*, A. recurvata* (Nees) Kunth*, A. torta* (Nees) Kunth*, Axonopus barbigerus* (Kunth) Hitchc*, A. canescens* (Nees) Pilger*, Ctenium cirrhosum* (Nees) Kunth*, Hypogynium virgatum* (Desv.) Dandy*, Paspalum pectinatum* Nees*, P. reduncum* Nees ex Steud*, P. splendens* Hack, *Schizachyrium microstachyum* (Desv.) Roseng. Arr. & Isag (BEWLEY; BLACK, 1985); *Digitaria bicornis* (Lam.) Roem. & Schult., *Digitaria ciliaris* (Retz.) Koel., *Digitaria horizontalis* Willd, and *Digitaria insularis* (L.) Fedde (MONDO et al., 2010).

Table 1. *Fimbristylis dichotoma* seeds germination, first count of germination, speed of germination index (SGI), and mean time for germination as influenced by temperature regimens.

Treatment	Germination (%)	First count (%)	SGI	Mean time (days)
15 °C	0 d	0 b	0.00 e	-
20 °C	78 ab	0 b	8.69 cd	20 d
25 °C	61 c	0 b	13.45 c	10 c
30 °C	7 d	5 b	4.17 b	4 a
35 °C	5 d	0 b	1.53 de	8 bc
40 °C	0 d	0 b	0.00 e	-
20-30 °C	75 b	0 b	24.83 b	7 ab
15-25 °C	82 ab	33 a	42.68 a	5 a
15-35 °C	85 a	14 ab	39.19 a	4 a
C.V.%	7.6	129.5	15.0	15.8
F	418.24**	7.63**	179.44**	78.04**
d.m.s.	8.7	20.0	5.9	3.0

**Significant at the 1% level of probability. Means, in the same column, followed by the same letter, are not statistically different according to Tukey's test ($p < 0.05$).

Alternating temperatures favor the process of dormancy suppression and, consequently, the germination process of weed seeds. The higher germination of the seeds of some species under alternate temperatures is probably due to enzymatic mechanisms which are favored by the fluctuations in temperature under specific thermal amplitudes during the process catalyzed by those enzymes (VIVIAN et al., 2008; MONDO et al., 2010).

The alternate temperatures of 20-30, 15-25, 15-35, and the constant one of 30 °C enhanced germination speed so as to result in mean time for germination of seven, five, four, and four days, respectively. On the other hand, the constant temperatures of 30 °C or above it had a negative effect on total germination, giving origin to 7% or less of normal seedlings.

These results are in accordance with reports found in the literature since the optimal temperature for speed of germination is always higher than that for total germination. Temperatures above the optimum increase the speed of germination but the germination metabolism becomes increasingly disorganized so that the number of seeds capable of completing the germination process decreases rapidly thus increasing the number of dead seeds and abnormal seedlings (MARTINS et al., 2013; NOGUEIRA et al., 2013).

In the second experiment, the factors substratum and dormancy breaking treatments showed no significant interaction. Taken isolatedly though, these factors had significant effects on germination, first count of germination, and mean time for germination (Table 2).

Among the tested substrata, although all of them had similar effect on the speed of germination as evaluated by the first count of germination test and the mean time for germination, sand was unfavorable to total germination when compared to the other substrata. So, this substratum is not recommended for *F. dichotoma* seeds such as were the reported cases of *Adenanthera pavonina* L., *Stryphnodendron adstringens* (Mart.) Coville, and *Schizolobium parahyba* (Vell.) S.F. Blake (KISSMANN et al., 2008; MARTINS; MACHADO; NAKAGAWA, 2008; MARTINS et al., 2012). The influence exerted by the substratum on final seed germination is ascribed to the substratum structure, aeration, and water holding capacity (KISSMANN et al., 2008; MARTINS et al., 2012).

Submitting the seeds to immersion in H_2SO_4 for 3 min caused them to die, but all the other dormancy breaking treatments were effective in promoting seed germination in comparison with the check treatment (Table 2). The seeds immersed in H_2SO_4 not only did not germinate but they also were verified to be covered with fungi with an intense physical fragmentation of the pericarp so that totally coatless seeds and white achenes were observed. This was interpreted as a signal that the immersion in H_2SO_4 was too prolonged such as verified for other grass species (MARTINS; MARTINS, 2013).

Among the treatments showing efficiency in breaking dormancy of tall fringe-rush seeds, the drying of them at 50 °C for 12 h was verified to be the best since it not only resulted in the highest germination value (94%) but also gave the best results of the first count of germination test (8%) and the mean time for seed germination (seven days) test although the treatment of the seeds with the 1% KNO_3 solution gave statistically similar results.

The use of potassium nitrate, due to its oxidant action, is being recommended in the specialized literature as a routine method to overcome seed dormancy in the physiological tests with seeds of *Panicum maximum* and *Urochloa spp* (ALMEIDA; SILVA, 2004; VIVIAN et al., 2008; BRASIL, 2009; MARTINS; MARTINS, 2013). The effect of the temperature of 50 °C in breaking seed dormancy and promoting germination may be attributed to the alterations and shrinking of the coat structure cells which affect permeability to gases (ALMEIDA; SILVA, 2004; MARTINS; MARTINS, 2013).

The drying treatment (50 °C for 12 h) to which the *F. dichotoma* seeds were submitted was not only the more efficient but also the easiest, practical, safe and viable to apply having in mind the sowing of the seeds in the field. These are characteristics of interest for weed management studies in

Table 2. Germination (G), first count of germination (FC), and mean time for germination (MTG) of *F. dichotoma* seeds as influenced by substratum and seed dormancy breaking treatments.

Means		G(%)	FC(%)	MTG (days)
	Ready-made	68 a	4.8 a	9 a
Substratum (S)	Soil	70 a	6.1 a	10 a
	Sand	61 b	5.8 a	10 a
Seed dormancy	Control	71 d	6.0 b	14 c
Breaking	H_2SO_4/3´	0 e	0.0 c	-
treatment	H_2O/15´	82 c	6.2 b	10 b
(DBT)	KNO_3 (1%)/15´	90 b	7.5 ab	7 a
	50 °C/12 h	94 a	8.1 a	7 a
F S		4.8*	3.8*	3.8*
F DBT		3477.8**	43.2**	43.2**
F S × DBT		0.60 ^{ns}	1.67 ^{ns}	1.67 ^{ns}
d.m.s. S		1.7	1.3	1.9
d.m.s. DBT		2.6	1.9	2.5
C.V.%		3.7	30.7	23.7

*,**, ^{ns}Significant at 5%, 1% level of probability and not significant by F test, respectively. Means, in the same column, followed by the same letter, are not statistically different according to Tukey's test ($p < 0.05$).

which the sowing and infestation of the field with the weed species to be controlled by means of post-emergence herbicides is a research procedure (VIVIAN et al., 2008; MARTINS; MARTINS, 2013).

The treatment of the seeds by immersion in water for 15 min showed an intermediate efficiency in comparison with the check treatment and the best ones (KNO_3 and drying at 50 °C): compared to the check treatment (71%) it increased germination to 82% while the best treatments increased germination to 90 and 94% and reduced the mean time for germination from 14 to 10 days. On the other hand, it was less efficient in promoting germination and had a longer mean time for germination than the treatments of drying at 50 °C and immersion in the KNO_3 solution (1%).

The immersion of seeds in water at environmental or warmed temperature breaks seed dormancy due to the removal of soluble inhibitors found in the seed or fruit coat (BRASIL, 2009; LACERDA et al., 2010). In Nature, this is verified to occur due to the action of rains or in flooded areas (MARCOS FILHO, 2005), such as is the case of tall fringe-rush, a species which is found in irrigated rice areas or cultivated plains and drainage channels (BARROS, 2009; MARTINS; SCATENA, 2013).

4 Conclusions

It was concluded that the germination of tall fringe-rush seeds should be carried out under alternate temperatures of 15-25 or 15-35 °C in ready-made substratum or in soil after being dried at a temperature of 50 °C for 12 h in order to break seed dormancy.

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