SEED DORMANCY AND GERMINATION IN *Hymenachne amplexicaulis* (POACEAE)

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ABSTRACT

Hymenachne amplexicaulis (Rudge) Nees is a grass of fast growth and high production of seeds, native to Central and South America, may cause ecological damage, which is introduced. This study aimed to identify the mechanisms of seed dormancy of *H. amplexicaulis*. Was evaluated the efficiency of gibberellic acid (0.05%) and potassium nitrate (0.2%) in two lots of seeds stored for 6 and 18 months. The periods of hydration of 0 (control), 3, 6, 9, 12, 24, 36 and 48 hours at a temperature of 23± 1°C and removal of the glumes, were evaluated in the lot with six months of storage. Seed germination was higher when exposed to potassium nitrate, while gibberellic acid did not promote germination. Seed hydration and removal of the glumes caused an increase in percentage and germination speed index.

Keywords: Gibberellic acid, Glumes removal, Hydration, Nitrate potassium.

DORMÊNCIA E GERMINAÇÃO DE *Hymenachne amplexicaulis* (POACEAE)

RESUMO

Hymenachne amplexicaulis (Rudge) Nees é uma gramínea de rápido crescimento e elevada produção de sementes, nativa das Américas Central e do Sul, podendo causar danos ecológicos onde é introduzida. Este trabalho teve por objetivo identificar os mecanismos da dormência das sementes de *H. amplexicaulis*. Assim

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foi avaliada a eficiência do ácido giberélico (0,05%) e do nitrato de potássio (0,2%) em dois lotes de sementes, armazenados por seis e 18 meses. Os períodos de hidratação de zero (controle), 3, 6, 9, 12, 24, 36 e 48 horas, na temperatura de 23±1°C e a remoção das glumas foram avaliados no lote com seis meses de armazenamento. A germinação das sementes foi maior quando expostas ao nitrato de potássio, enquanto que o ácido giberélico não promoveu a germinação. A hidratação das sementes e a remoção das glumas causaram um aumento na porcentagem e no índice de velocidade de germinação.

Palavras-chave: Ácido giberélico, Remoção das glumas, Hidratação, Nitrato de potássio.

INTRODUCTION

Native to the Central and South America, the *Hymenachne amplexicaulis* (Rudge) Nees, is a grass found in environments with intermittent periods of flooding (Enriquez-Quiroz et al., 2006), occurring in wetlands, riversides and temporarily flooded areas (Silveira and Weiss, 2014), being considered weed in cultivated areas, rangelands and water reservoirs. Sturza et al. (2011) reported its occurrence as well as the increase of its population in rice fields in the state of Rio Grande do Sul (RS), Brazil.

Efficient nitrogen metabolism, promoting the vigorous growth of new leaves and tillers, combined with the large number of seeds and the large accumulation of reserves in its rhizomes (Wearne et al., 2010) explain the significant rise in this plant's population in humid areas of Central and South America, some countries in African (Clayton et al., 2006), Mexico (Enriquez-Quiroz et al., 2006), Indonesia (Holm et al., 1979), United States and Australia (Csurches et al., 1999). In Queensland and Northern Territory in Australia, where it was introduced as forage, the proliferation of this plant is associated with serious ecological and economic damage, with substantial costs regarding its control (Land and Water Australia 2008). Although it also spreads vegetatively by stolons and rhizomes fragments, it is the seeds that are responsible for the plant's wide spread because of its panicles, 20-40 cm in length, which can produce more than four thousand high viability seed (Charleston, 2006), dispersed by water flow, mammals, fishes and birds (Hunter et al., 2010).

In the case of *H. amplexicaulis* and many other species, seed dormancy is an important natural defense, survival and perpetuation strategy in environments with different stress conditions that hinder seedling survival (Reynolds et al., 2013). It is an intrinsic phenomenon of the seed and can manifest in the form of dormancy imposed by seed coat, embryo dormancy and dormancy due to the imbalance between germination promoters and inhibitors, making viable seeds not germinate even when external factors necessary to the germination process such as the availability of light, water and oxygen are adequate (Finch-Savage and Leubner-Metzger, 2006).

Thus, dormancy is manifested as a factor that hinders the achievement of research strategies on weed management and control (Vivian et al., 2008). Being aware of its mechanisms and methods are of great relevance to understanding the plant's preferred forms of propagation, germination of the soil seed bank and its relationship with environmental conditions. The study of *Hymenachne amplexicaulis* germinative characteristics can be an important tool for suggesting rational management methods,

reducing the problems caused by species. In view of this, this study aimed to determine the effectiveness of pre-germination treatments to overcome seed dormancy, evaluating the effect of germination promoters, storage periods and seed hydration, as well as removal of the glumes, in the percentage of germination.

MATERIALS AND METHODS

The seeds used in the experiment were collected in February 2012 and 2013 in naturally established populations in the adjacent rice fields located in the town of Formigueiro (30°04'13.6''S, 53°33'00.6''W), in Rio Grande do Sul state, in southern Brazil. The panicles were randomly collected throughout the canopy of the plant population, were placed inside paper bags and gently agitated in order to select the caryopsis at the beginning of natural abscission, ie, with the same level of physiological maturity. The collected seeds, approximately 500 g each season, were dried in the shade for a period of 15 days and subsequently stored in paper bags at room temperature (22±3°C) and humidity (60±10%), with similar conditions for both lots, until the beginning of the tests, in August 2013. The monthly climatic data for the years 2012 and 2013 are shown in tab. 1.

Table 1 - Total solar insolation, total precipitation, maximum and minimum temperatures and average relative humidity in the years 2012 and 2013. Meteorological station of Santa Maria, RS (29°43'29.4" S, 53°43'13.7" W). INMET 2014

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
	Solar insolation (hours)											
2012	251.6	204.8	-	218.9	153.6	158.1	152.7	179.9	123.1	221.3	118.0	236.0
2013	211.5	187.4	213.9	179.5	108.6	126.6	175.1	146.1	165.9	221.9	244.1	285.2
	Total precipitation (mm)											
2012	162.1	165.4	131.7	25.6	96.5	76.8	91.4	164.5	345.3	108.7	480.9	305.7
2013	405.9	124.7	25.1	116.8	117.7	128.9	238.3	109.4	244.9	49.3	71.3	157.9
	Middle maximum temperature (°C)											
2012	29.8	30.3	29.2	27.2	23.8	18.5	17.1	23.2	20.8	25.4	28.3	29.8
2013	30.1	31.7	30.1	26.1	21.0	19.8	19.5	19.6	22.	24.4	27.8	30.4
	Middle minimum temperature (°C)											
2012	18.1	19.6	18.3	13.9	12.1	7.3	6.2	11.4	12.5	13.5	19.0	19.7
2013	20.6	22.0	19.2	14.2	12.7	10.3	9.0	9.4	13.1	12.7	14.9	18.2
	Middle relative humidity (%)											
2012	75.3	78.7	83.3	80.1	83.2	83.8	83.3	79.6	85.2	75.3	82.4	76.0
2013	79.1	77.1	77.5	77.4	87.4	85.2	82.7	82.4	83.4	71.3	66.5	67.1

Experiments were conducted in Didactic and Research Seeds Laboratory, Department of Phytotechny, Federal University of Santa Maria (UFSM), Brazil. The tests were performed according to the rules of seed analyzes, established by the Ministry of Agriculture, Livestock and Supply (Brasil, 2009). For all the tests of germination and germination speed index (GSI), completely randomized design with four replications of 50 seeds was used, except for the determination of weight, where four replicates of 100 seeds per lot were used. The seeds were sown on four filter paper sheets in acrylic box,

moistened with treatments (gibberellic acid, potassium nitrate or distilled water in the experiment 1, distilled water in the experiment 2 and potassium nitrate or distilled water in the experiment 3) in the amount of 2.5 times the weight of the paper and placed in a germination chamber, alternating cycles of 8 hours of light at 30°C and 16 hours dark at 20°C. Counts of germinated seeds were taken every 24 hours after installation of the tests and were concluded with the germination of all seeds of the one replications (Brasil, 2009), at 11, 8 and 5 days after implantation of tests for experiments 1, 2 and 3, respectively.

For evaluation purposes, it was considered germinated the seed that gave rise to normal seedlings. It were evaluated the GSI, percentage, and in some cases, the cumulative percentage of germination. To calculate the GSI, was used the formula proposed by Maguire (1962), according to equation 1,

$$GSI = (G1/1 + G2/2 + G3/3 + ... + Gn / n), \quad (1)$$

where G1, G2, G3 and Gn correspond the percentages of germinated seeds in the first, second, third and nth day after sowing.

The number of germinated seeds was used to calculate the germination percentage. The mean and standard deviation were calculated for germination percentage, GSI and weight of 100 seeds. Prior to analyses, data were checked for normality (Shapiro-Wilk test) and homoscedasticity (Levene test). Only the data of germination exhibited a non-normal distribution and were transformed using the expression $\arcsen\sqrt{(x/100)}$, to ensure normality. The main effects and interaction of storage time and promoters of germination (experiment 1) and removal of glumes and promoters of germination (experiment 3) on germination percentage and GSI were analyzed by two-way ANOVA. The effects of soaking times (experiment 2) were analyzed by one-way ANOVA. The means were separated by Tukey test ($p \leq 0.05$), using the statistical software SISVAR (Ferreira, 2011). The specific methodology used in each test is described below.

Storage time and overcoming seed dormancy

The treatments consisted of combinations of seed lots with different storage time (6 and 18 months) and the promoters of germination, gibberellic acid (AG_3) at 0.05%, potassium nitrate (KNO_3) 0.2% and distilled water as a control. To characterize the lots with six and 18 months of storage, four replicates of 100 seeds of each lot were weighed, since the size of the seed (the differentiation stage of the embryo and nutrients supply) may influence in the germination (Larcher, 2004). The percentage of germination and the GSI were evaluated.

Soaking time and seed germination

The *H. amplexicaulis* seeds of were soaked in distilled water for a period of 3, 6, 9, 12, 24 and 48 hours at room temperature 23±1°C. In the control treatment seeds were not soaked. Subsequently, the seeds were germinated. The percentage of germination and the GSI were evaluated.

Removal of glumes

The treatments were composed of a combination of factors naked seeds or seeds protected by their glumes and the seed germination promoter potassium nitrate (0.2%) or distilled water (control). The percent germination, GSI and cumulative germination were evaluated.

RESULTS

For both germination percentage and GSI, there was interaction between the factors storage time and the germination promoters potassium nitrate, gibberellic acid and the control water (Tab. 2). When the seeds were stored for six months, potassium nitrate promoted a higher percentage of germination, differing statistically to the gibberellic acid and the control. However, in the lot of seeds stored for 18 months, the results did not differ among treatments. The gibberellic acid and the control showed statistically similar percentage of germination, regardless of the time of seed storage. On the other hand, the potassium nitrate promoted different percentage germination between the two lots. The GSI showed similar behavior to the variable germination.

Table 2 - Germination (radicle emergence), germination speed index and weight of *Hymenachne amplexicaulis* seeds as a function of storage time and germination promoters. Means followed by the same capital letters in the columns, and small letters in the lines, are not significantly different by Tukey test ($p \leq 0.05$). The standard deviation is in parentheses.

Storage time	Germination promoter		
	Potassium nitrate	Gibberellic acid	Control
	Germination (%)		
6 months	95(±3.83)Aa	17(±1.91)Ab	21(±5.00)Ab
18 months	29(±6.22) Ba	21(±1.91) Aa	29(±11.0)A^{ns}
	Germination speed index		
6 months	15.97(±1.32)Aa	2.95(±0.63)Ab	3.26(±1.10)Ab
18 months	4.64(±0.99)Ba	3.19(±0.32)Aa	4.03(±1.61)A^{ns}
	Weight of 100 seeds (g)		
6 months	0,042(±0.004)A		
18 months	0,039(±0.003)A		

After being soaked in water for different periods of time at room temperature (23±1°C), seeds of *H. amplexicaulis* had an increased percentage of germination statistically significant when compared to the control (Tab. 3). The periods of 3, 6, 9, 12, 24 and 36 soaking hours had higher germination percentages, but similar results between them. When hydrated for 48 hours, the seeds had a higher germination percentage, significantly different from the other treatments.

Table 3 - Germination (radicle emergence), germination speed index of *Hymenachne amplexicaulis* seeds, exposed to different periods of soaking seeds in distilled water. Means within columns followed by the same letter are not significantly different at Tukey test ($p \leq 0.05$). The standard deviation is in parentheses.

Treatment	Germination (%)		GSI	
48 hours	88 a	(±10.38)	13.93 a	(±2.12)
36 hours	69 b	(±6.81)	10.27 ab	(±1.27)
24 hours	63 b	(±11.01)	8.96 bc	(±1.46)
12 hours	50 b	(±6.93)	6.78 c	(±1.03)
9 hours	67 b	(±8.87)	9.67 bc	(±1.27)
6 hours	47 b	(±13.10)	6.73 c	(±1.78)
3 hours	50 b	(±2.83)	6.78 c	(±0.35)
Not soak	2 c	(±3.00)	0.35 d	(±0.43)
CV (%)	13.52		8.16	

The removal seed glumes significantly increased germination percentages (Tab. 4). For the variables analyzed, there was a significant interaction between factors seed with or without glumes and potassium nitrate germination promoter or water. When glumes were removed, the percentage of seed germination was similar for potassium nitrate and control; however, in the presence of seed coats, potassium nitrate promoted greater number of germinated seeds, confirming the previous results. In seeds of *H. amplexicaulis* there was also an anticipation of germination in the treatments of seeds without glumes, reaching 91% germination on the fifth day after sowing (Fig. 1).

Table 4 - Germination (radicle emergence), germination speed index (GSI) of *Hymenachne amplexicaulis* seeds according to presence or absence of glumes and germination promoter used. Means followed by the same capital letter in the line or small letter in the column are not significantly different at Tukey test ($p \leq 0.05$). The standard deviation is in parentheses.

Seeds	Germination (%)				GSI			
	Potassium nitrate		Water		Potassium nitrate		Water	
Without glumes	89Aa	(±4.12)	91Aa	(±5.74)	28.95Aa	(±2.14)	28.05Aa	(±1.89)
with glumes	59Ab	(±14.46)	0Bb	(±0.00)	13.15Ab	(±3.42)	0.00Bb	(±0.00)
CV (%)	11.57				7.08			

DISCUSSION

To study the dormancy mechanisms of *Hymenachne amplexicaulis* in the experiment 1, the seeds were treated with the germination promoters gibberellic acid and potassium nitrate, using water as control and exposed to favorable conditions for germination. The use of gibberellic acid as a germination promoter is recommended when the overcoming dormancy involves hormonal changes in the embryo (Pawlowski, 2009). In this case, the low percentage of germination and GSI promoted by the

treatments with gibberellic acid, similar to the control treatments, possibly indicate that this is not the cause of *H. amplexicaulis* seed dormancy.

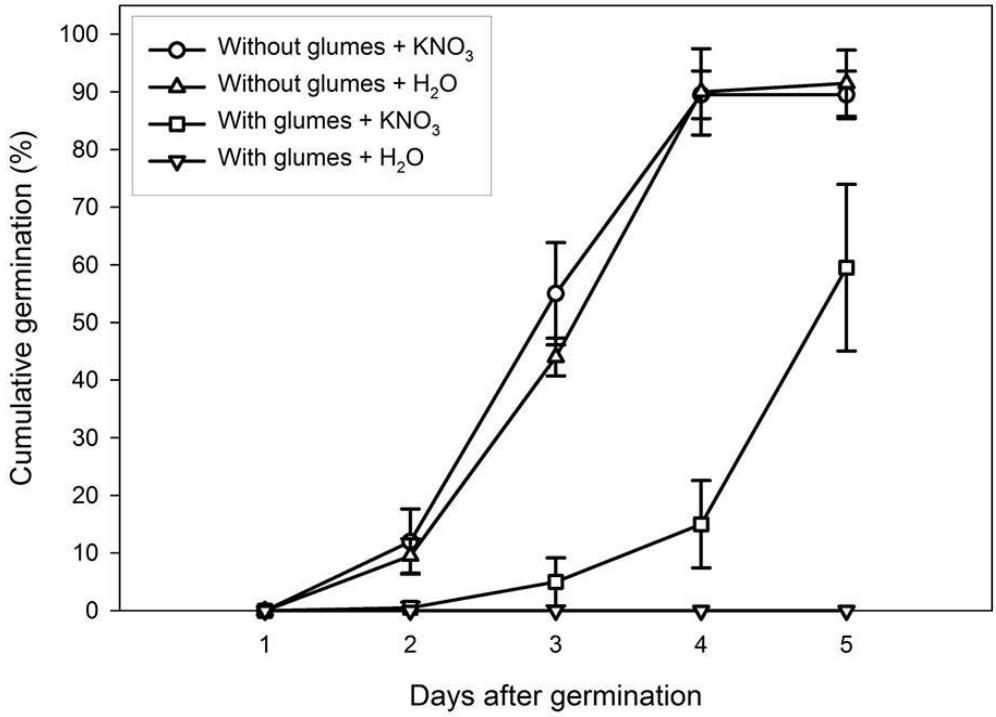

Figure 1 - Cumulative germination (mean ± s.d.) of *Hymenachne amplexicaulis* seeds with or without glumes.

The highest percentage of germination promoted by potassium nitrate on the six months storage lot may be explained by the efficiency of such treatment in the overcoming dormancy, combined with high seed viability. The largest GSI for seeds stored for six months and exposed to potassium nitrate confirms the effectiveness of this treatment to overcome dormancy, thus promoting a reduction of germination time. The efficiency of potassium nitrate is concentrated in the increase of availability of oxygen level by decreasing the oxygen available for citric acid cycle (Bewley and Black, 2013), thus it is recommended for seeds with coats impermeable to gases (Franke and Nabinger, 1996) or the presence of chemicals that alter the balance of gases in the embryo. Furthermore, the potassium nitrate is related to the dormancy overcome, especially in species that have light-sensitive seeds (Shanmugavalli et al., 2007), as is the case of *H. amplexicaulis* (Campbell et al., 2009).

On the other hand, the potassium nitrate was not efficient in promoting germination of seeds stored for 18 months, which may indicate a reduction in the viability of seeds. However, environmental stimuli that occurred during the seed maturation and immediately after your dispersion can influence the complex process of dormancy, requiring specific stimuli to overcome it (Vivian et al., 2008). Thus, the considerable difference in precipitation and total solar insolation values (Tab. 1), between the years 2012 and 2013, in the months of occurrence of seed maturation, may have influenced the dormancy requirements in two lots, making the seeds do not

respond or respond partially to the same stimulus. The weight of 100 seeds did not differ significantly among the lots and the remaining tests were conducted with the seed stored for six months.

The prior hydration of seeds favored the germination. During the germination, water has the function of stimulating and controlling of physiological and biochemical processes, such as promotes the softening of the seed coat, favoring the penetration of oxygen, increasing the volume of the embryo and reserve tissue, stimulating the basic metabolic activities and favoring the growth of the embryonic axis (Marcos Filho, 2005). Thus, within the evaluated periods, the highest seed germination in the 48h-soak treatment can be explained by the absorption of water needed to initiate the germination and/or by overcoming dormancy process, with smaller periods being insufficient to trigger completely such processes. This fact is also demonstrated by GSI, where occurred an increase in the variable values in all treatments with soaking, wherein the 48h-soak treatment, was 40 times higher than the control, where seeds were not soaked prior to the test.

In *H. amplexicaulis* seeds, it is possible that the soaking water is responsible for the removal or dilution of chemical dormancy promoters. Similar example occurs in *Onopordum acanthium* L. seeds, which have high concentrations of soluble inhibitors, already dispersed in a dormant state and to germinate, the seeds need to be washed to reduce the concentration of inhibitors (Cavers et al., 2011). The optimal hydration time is conditioned by factors intrinsic to the seed as a species, contact area, size and shape of pore, chemical composition and permeability of the seed coat, amount of wax in the epidermis and also by environmental factors such as water availability and temperature (Carvalho and Nakagawa, 2012; Beckert and Silva, 2002), and the level of hydration of the seeds, although linked to factors such as temperature and light, is the second most important factor in inducing and overcoming dormancy (Vivian et al., 2008).

The removal of glumes increased the speed of germination process of seeds. The highest seed germination found for treatments with removal of glumes may be associated with overcoming dormancy, which is promoted by the compounds present in structures that were removed, which was proven by results of naked seed germination in water. By studying seeds of tropical grasses, Martins and Silva (1998) reported that treatments which promote physical disruption of the pericarp, eliminating their impermeability, are agents which break dormancy.

The possibility of the mechanisms that regulate the dormancy of some grasses are associated with substances present on the coat of the caryopsis was also observed in *Brachiaria humidicola* (Rendle) Schweick (Costa et al., 2011), *Paspalum notatum* Flüggé and *Poa compressa* L. (Simpson, 2007). Authors such as Vivian et al. (2008) corroborate with this hypothesis, assigning this chemical dormancy in seeds of weeds to the inhibitors present on the external side of the seeds, which can be deactivated by the removal of such structures. In this experiment, the exposure of seeds protected by the glumes to potassium nitrate also promoted germination; however, the speed of this process was lower than in seed where the caryopsis coat were removed, as shown GSI.

The results presented provide relevant information to the *Hymenachne amplexicaulis* management and are strongly related to the ecology of this species. Although this weed also spreads vegetatively, their seeds have high viability and in favorable conditions, quickly germinate. Furthermore, it is likely that the causes of dormancy are related to substances or impediments present in the seed coat, since imbibitions in water and removing of the glumes increases the speed of germination

process. Such information also helps to explain the rapid and aggressive spread of *Hymenachne amplexicaulis* in wetlands.

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