doi: 10.17138/TGFT(10)156-163

Research Paper

The influence of seed structures on dormancy in seeds of *Urochloa* hybrid cultivar 'Mulato II'

La influencia de las estructuras de semillas sobre la dormancia en semillas de Urochloa *híbrido cultivar 'Mulato II'*

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Abstract

This study determined the effects of seed structures on seed dormancy and tested methods to break dormancy in seeds of *Urochloa* hybrid cultivar 'Mulato II'. Seeds stored for 10 months in indoor ambient conditions were studied to determine effects of seed structures on seed germination and their water permeability. Results showed that seed structures presented a barrier to water permeability. Removal of lemmas, puncturing the seed coat, seed structure removal and sulfuric acid immersion all reduced seed dormancy. Water and alcohol extracts from different parts of seeds inhibited seed germination of *Brassica pekinensis* seeds. There were 3 mechanisms responsible for seed dormancy; first, the mechanical barrier of seed structures, which excluded water and reduced gas exchange as well as restricting growth of the embryo; second, an endogenous germination inhibitor mainly found in lemmas; and third, water permeability of the seed coat (including pericarp and testa). The mechanical removal of lemmas and immersion in concentrated sulfuric acid reduced seed dormancy, although mechanical removal of the lemma alone was effective, convenient and safer.

Keywords: Seed extracts, seed germination, vitality index, water permeability.

Resumen

Este estudio determinó los efectos de las estructuras de semillas en su dormancia y probó métodos para romper la dormancia del cultivar híbrido de *Urochloa* 'Mulato II'. Se estudiaron semillas almacenadas durante 10 meses en condiciones de ambiente interior para determinar los efectos de las estructuras de semillas sobre su germinación y permeabilidad al agua. Los resultados mostraron que las estructuras de las semillas presentan una barrera a la permeabilidad al agua. La eliminación de los lemas, la perforación de la cubierta, la eliminación de la estructura de la semilla y la inmersión de ácido sulfúrico redujeron la dormancia de la semilla. Extractos de agua y alcohol de diferentes partes de semillas inhibieron la germinación de semillas de *Brassica pekinensis*. Hubo 3 mecanismos responsables por la dormancia de las semillas: En primer lugar, la barrera mecánica de las estructuras de las semillas, que excluía el agua y reducía el intercambio de gases, además de restringir el crecimiento del embrión; En segundo lugar, un inhibidor endógeno de la germinación que se encuentra principalmente en los lemas; Y tercero, la permeabilidad al agua de la cubierta de la semilla (incluyendo pericarpio y testa). La eliminación mecánica de los lemas y la inmersión en ácido sulfúrico redujo la dormancia de las semillas, aunque la sola eliminación mecánica del lema fue efectiva, conveniente y segura.

Palabras clave: Extractos de semillas, germinación de semillas, índice de vitalidad, permeabilidad al agua.

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Introduction

Grasses of the genus *Urochloa* include annual or perennial forages for cut and carry or grazing systems, as well as for water and soil conservation, in tropical and subtropical regions of the world (Thomas et al. 1987; Li and Guo 1990). The cultivated species of *Urochloa* originated from African savannas and are now widely grown in tropical and subtropical areas in Africa, the Americas, Oceania, and south-east Asia (Kobayashi and Kato-Noguchi 2015; Simeão et al. 2016; Lozano et al. 2017; Castañeda-Pimienta et al. 2017; Moreira et al. 2018). The primary cultivated species include *U. ruziziensis*, *U. brizantha*, *U. decumbens*, *U. humidicola*, and *U. dictyoneura*. The CIAT-bred *Urochloa* hybrid cultivar 'Mulato II' (CIAT36087, *U. ruziziensis* × *U. decumbens* × *U. brizantha*) became widely used after promotion by Semillas Papalotla S.A. seed company (Argel et al. 2005; Argel et al. 2007; Phaikaew et al. 2008). *Urochloa* seed production is mostly in Thailand. Mulato II was introduced to provinces in China (Hainan, Fujian and Yunnan) in 2005 (Li et al. 2009; Zeng et al. 2009; Liu et al. 2013; Deng et al. 2013).

Mulato II shows good spittle bug resistance with high yields of good quality forage, strong tillering ability, a well-developed root system and creeping and erect growth characteristics. Despite excellent forage attributes, adoption is hindered by seed dormancy. Better understanding of dormancy mechanisms and development of dormancy-breaking technologies should promote adoption of Mulato II.

Baskin and Baskin (2004) proposed a seed dormancy classification, which divided seed dormancy into five types: physiological, morphological, morphological physiological, physical and compound dormancy. Studies have been reported on chemical treatment, osmotic regulation and aging treatment for breaking dormancy in *Urochloa* seeds (Whiteman and Mendra 1982; Câmara et al. 2002; Bonome et al. 2006; Batista et al. 2016a). Whiteman and Mendra (1982) reported that the germination rate of *U. decumbens* was 72 % after 20 min of treatment with concentrated sulfuric acid (H_2SO_4) compared to 40 % of intact stored seed. Costa et al. (2011) reported that the germination rate of shelled *U. brizantha* cultivar 'Marandu' seeds was less than 20 %, while the germination rate of denuded seeds reached 60 %. Bonome et al. (2006) showed that the germination uniformity of Marandu seeds immersed in potassium nitrate (KNO_3) solution for 12 h was better than for untreated seeds or seeds immersed in other solutions. Batista et al. (2016b) reported that *U. brizantha* cultivar 'MG-5' seeds aged at 41 °C for 96 h with 0.2 % KNO_3 or 0.2 % calcium nitrate as primers exhibited a seed germination rate of 94 %.

Usberti (2007) reported that dormancy was reduced in seeds of *U. brizantha* by soaking in concentrated sulfuric acid for 15 min and storing at 40 °C while 15 min of concentrated sulfuric acid treatment also reduced the dormancy of *U. decumbens* seeds (Duan et al. 2015). Martins and da Silva (2001) reported that treatment of Marandu seeds with concentrated sulfuric acid for 15 min reduced seed dormancy while Garcia and Cícero (1992) showed that the best method to break the dormancy of Marandu seeds was to soak in sulfuric acid for 15 min followed by soaking in 0.2 % KNO_3 solution. Batista et al. (2016a) showed that after 5 min of treatment with concentrated sulfuric acid and 3 h of hydration with 0.5 mg gibberellic acid/L, seeds of *U. brizantha* showed higher physiological potential and seedling emergence rate. Hare et al. (2014) reported that seeds of Mulato II treated with concentrated sulfuric acid for 10 min had a germination of 80 % and Pereira et al. (2017) reported that when scarified seeds of Mulato II were aged at 42°C and 98 % humidity for 48 hours, their germination ability was enhanced.

The seed structure of Mulato II consists of a lemma and a seed coat (including pericarp and testa) (Figure 1). There are few studies focussing on the relationship between seed structures and seed dormancy. In this study, we sought to measure the effects of seed treatments on the dormancy of Mulato II seeds after storage for 10 months. We aimed to study inhibitory effects of 'Mulato II' seed structure extracts on *Brassica pekinensis* seeds, reveal the influence of the seed structures on seed germination and highlight the most appropriate method to break seed dormancy, using treatments including mechanical scarification and concentrated sulfuric acid.

Materials and Methods

Seed characteristics

Urochloa hybrid cultivar 'Mulato II' seeds were harvested in SiMaoGang, Simao, Pu'er, Yunnan, China (22°30' N, 100°35' E; 1,200–1,250 masl). The seeds were dried, cleaned and stored indoors under ambient conditions for 10 months (from November 2018 to August 2019).

Random seed samples were prepared for the study using a riffle seed divider. Shape and colour of seeds were observed using a magnifying glass. Three replicates of 100 randomly selected seeds were measured for length,

width and thickness using a Vernier calliper. Thousand-seed weight was determined by weighing 10 replicates of 100 randomly selected seeds. The moisture content of seeds was determined using the International Seed Testing Association (ISTA) constant-temperature oven drying method at 130 ºC (Fan et al. 2016; Yan 2017).

Seed treatments/scarification

Seeds were mechanically scarified as:

Punctured seeds with lemmas (A): the seed was held with forceps and the lemmas and seed coat were punctured with a dissecting needle.

Seeds without lemmas (B): the seed was held with forceps and the seed coat and lemma were separated with a dissecting needle and the lemma removed with forceps.

Punctured seeds without lemmas (C): after removing the lemma, the seed coat was punctured with a dissecting needle.

Naked seeds (D): the seed structures were removed by peeling the seed structures (lemma and seed coat) using tweezers.

Control: intact seeds with full seed structures.

For acid scarification, 3 replicates of 100 intact seeds were scarified with 98 % sulfuric acid for 5, 10 or 15 min, or left untreated as control. After repeated rinsing with distilled water, the germination test was conducted.

Determination of seed permeability

The water absorption rate of 100 seeds each of the control, treatments A, B, C and D was assessed by placing them in different beakers, adding 3 ml of distilled water to each and soaking at room temperature (15 °C - 25 °C) for 0, 2, 4, 6, 8, 12, 16, 20, 24, 36, and 48 h. After soaking, surface water was removed by blotting with filter paper and seeds were immediately weighed. The water absorption rate of the seeds was calculated according to the method of Luo et al. (2014).

Water absorption rate of seeds (%) = $(W_2-W_1)/W_1 \times 100$

where:

W_1 is the weight of the seeds before water absorption, and W_2 is the weight after water absorption.

Germination

Seeds from each of the control and treatments A, B, C and D were soaked and disinfected with a 2 % copper sulphate solution for 10 min, washed repeatedly with distilled water and air-dried before germination.

The top of paper germination method was used with 3 replicates of 100 seeds in 90 mm petri dishes (Yan 2017). Seeds were germinated at alternating temperatures of 30/20 °C (16/8 h) with 12/12 h light in an incubator for 14 d with regular watering. Dishes were observed daily to assess germination, which was defined as when the radicle protruded to 1 mm. Germination was counted after 7 and 14 days. After germination, the seedlings were placed in an oven for 15 min at 105 °C and dried at 65 °C for 24 h until a constant weight was reached. The dry weight was noted and the germination percent, germination potential, germination index, and vitality index of the seeds were calculated according to the following formulas (Hu et al. 1992):

Germination potential (GP) = total number germinated within 7 days/number of seeds tested × 100 %

Germination precent (GR) = total number germinated within 14 days/number of seeds tested × 100 %

Germination index (GI) = $\sum Gt / Dt$

Vitality index (VI) = GI × S

where:

Gt is the number germinated in T days

Dt is the corresponding number of germination days

S is the dry weight of a single seedling.

Effect of extracts from different parts of seeds on germination of Brassica pekinensis

Effect of extracts from different parts of the seeds as germination inhibitors were assessed uing the methods of Liu (2015) and Yan (2017). The seed parts were separated into lemmas, seed coat (including pericarp and testa) and caryopsis. Each sample of 1 g was crushed in a mortar and 10 ml of distilled water was added to each in beakers which were sealed with plastic wrap at 4 °C. After leaching for 24 h, the residue was filtered and extracted twice more by rinsing with distilled water. The 3 leachates for each seed part were merged and made up to a volume of 50 ml with distilled water. The concentration of the leachates was 20 mg/ml. A 15 ml sample was diluted with distilled water to a concentration of 10 mg leachate/ml for later use. Another set of 1 g samples of separated lemmas, seed coat and caryopses were crushed, extracted with 20 ml of methanol and leached at 4 °C for 24 h as above. The residue was filtered, extracted twice more with methanol and the 3 leachates for each seed part were merged. The methanol was vaporized in a furnace in a fume hood to obtain solid crystals. The crystals were re-dissolved in 50 ml of distilled water and 15 ml of the solution was taken and diluted with distilled water to 10 mg leachate/ml for later use.

Three replicates of 100 *Brassica pekinensis* seeds per treatment were placed on filter paper moistened with 3 ml of the different leachates and a control using 3 ml of distilled water in 9 cm petri dishes. The dishes were placed in an incubator at alternating temperatures of 30/20 °C (16/8 h) with light cycles of 12/12 h for 4 days. Observations were made every day until the end of the fourth day. If distilled water was added to the petri dish to prevent drying out, the number of germinated *B. pekinensis* seeds was counted after 48 h. The radicle length of *B. pekinensis* was measured using Vernier callipers after 72 h. The inhibition rate was calculated according to the method of Wang (2017):

Inhibition rate (%) = (germination of control group – germination of treatment group)/germination of control group × 100 %.

Data analysis

Excel 2010 and SPSS 21.0 were used for data collation and statistical analysis. SPSS 21.0 was used for one-way variance (ANOVA) analysis and Duncan's test was used to compare the differences among treatments.

Results

Seed characteristics

The seeds of Mulato II were flat, ovoid and light yellow, showing a ventral uplift and abaxial flat surface (Figure 1). The average seed length, width, height, thousand seed weight and water content are shown in Table 1. Seed vitality was 84.38 and initial germination rate was 21 %.

Seed permeability

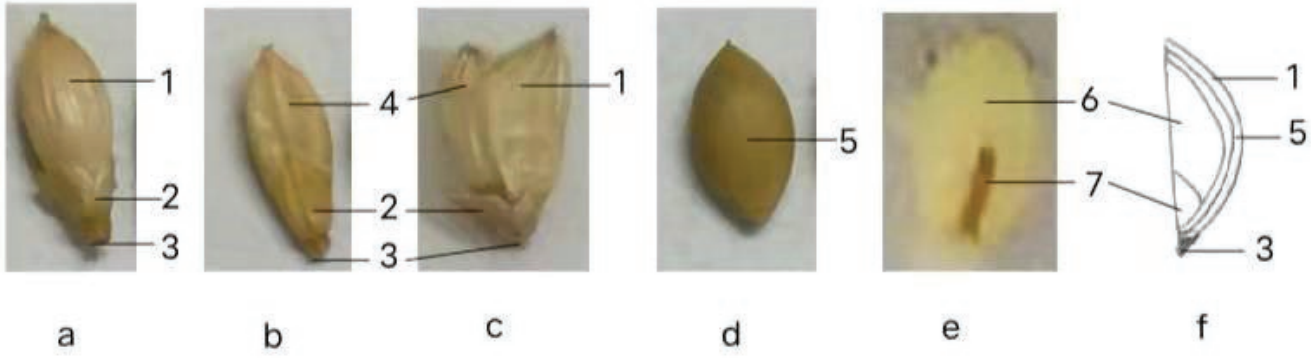
Water absorption rate increased rapidly for 2 h followed by a slow rising trend up to 16 h and reached saturation after 24 h (Figure 2) and was affected by treatment.

Effects of removing seed structures on seed germination

The 4 mechanical seed treatments all significantly improved seed germination compared to the control ($P<0.05$) (Table 2). Removing the seed structures produced the highest germination rate (82.67 %) and seed vitality (84.38 %). The germination potential, germination rate, germination index and vitality index of treatments were significantly different from the control ($P<0.05$).

Effects of concentrated sulfuric acid treatment on seed germination

Seeds soaked in sulfuric acid for 5, 10 and 15 min showed significantly increased seed germination potential, germination rate, germination index and vitality index compared to the control (Table 3). Soaking in concentrated sulfuric acid for up to 15 min eroded the lemma while the seed coat was still well-preserved. Five minutes of soaking was sufficient to increase seed germination rate,



a = Back of spikelet; b = Ventral spikelet; c = Caryopsis appendage; d = Caryopsis; e = Naked seed (without shells); f = Pattern of spikelet slitting along dorsal ventral axis.
1 = Lemma; 2 = Glume; 3 = Rachilla; 4 = Palea; 5 = Seed coat (including pericarp and testa); 6 = Endosperm; 7 = Embryo.

Figure 1. The morphology and appendages of *Urochloa* hybrid cultivar 'Mulato II' seeds.

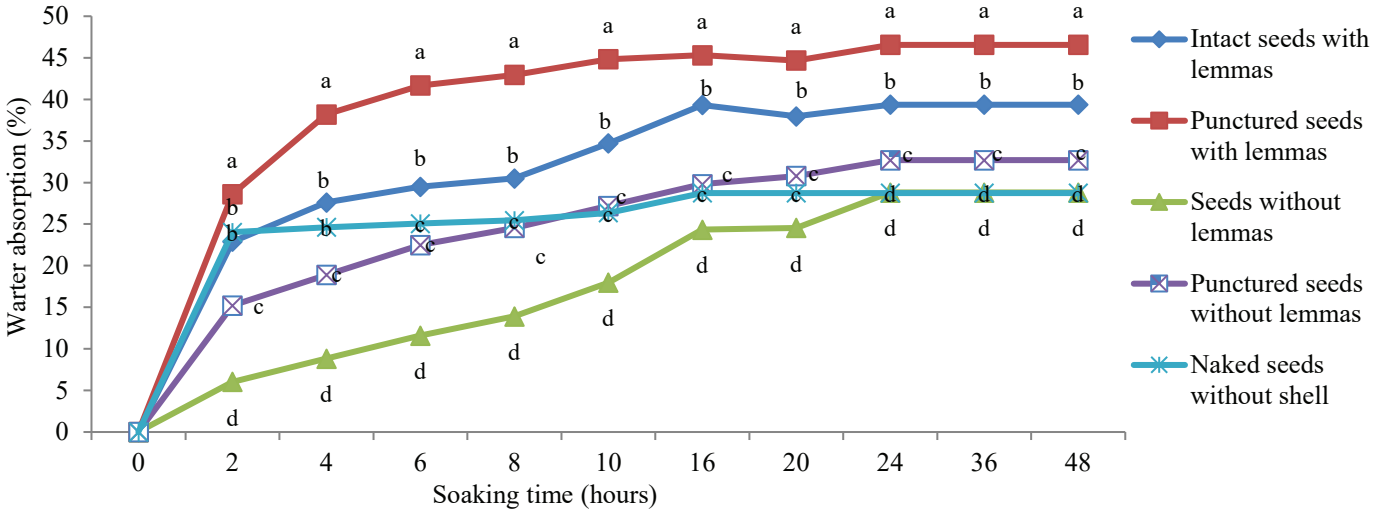
Table 1. Seed characteristics of *Urochloa* hybrid cultivar 'Mulato II'

Length (mm)	Breadth (mm)	Height (mm)	1000 seed weight (g)	Water content (%)	Vitality (%)	Germination percent (%)
5.29 ± 0.21	1.96 ± 0.14	1.21 ± 0.12	6.35 ± 0.02	8.96 ± 0.06	84.38 ± 1.93	21.00 ± 2.67

germination rate and vitality index. The increases in germination rate, germination potential rate, germination index and vitality index of seeds decreased slightly, but not significantly, with the extension of the soaking time with concentrated sulfuric acid. Significant differences were only seen with soaking for 15 min (P<0.05).

Inhibitory effect of extracts from different parts of seeds on germination of Brassica pekinensis *seeds*

Water and alcohol extracts from different parts of the seed all had inhibitory effects on the seed germination of *B. pekinensis* (Table 4). The degree of inhibition decreased with decreasing concentration. The 20 mg leachate/ml water extract from the lemmas had the strongest inhibitory effect on germination of *B. pekinensis* and was significantly different from the control (P<0.05). The caryopsis alcohol extract (10 mg/ml) showed the weakest inhibitory effect and was not significantly different compared with the control, although the root length was significantly different (P<0.05).



Note: Different letters in same soaking time are significantly different (P<0.05).
Figure 2. The change in water absorption by seeds of *Urochloa* hybrid cultivar 'Mulato II'.

Table 2. Effect of mechanical treatment on germination of *Urochloa* hybrid cultivar 'Mulato II' seeds.

Treatment	Germination potential (%)	Germination percent (%)	Germination index	Vitality index
Control	22.67±2.40 e	26.67±1.76 e	6.20 ±1.20 e	0.044 ±0.004 e
A	32.67±2.40 d	32.67±2.40 d	12.44 ±0.97 d	0.060 ±0.003 d
B	64.00±1.15 b	64.00±1.15 b	30.97±1.23 b	0.176 ±0.007 a
C	50.00±2.31 c	50.00±2.31 c	24.03±1.03 c	0.130 ±0.005 b
D	82.67±2.40 a	82.67±2.40 a	40.28± 1.53 a	0.084 ±0.003 c

Note: Values are given as mean ±SE. Values with different letters in the same column are significantly different (P<0.05).

Table 3. Effect of chemical treatment on germination of *Urochloa* hybrid cultivar 'Mulato II' seeds.

Treatment	Germination potential (%)	Germination percent (%)	Germination index	Vitality index
Untreated control	22.67 ± 2.40 c	26.67 ± 1.76 c	6.20 ± 1.20 c	0.044 ± 0.004 c
Soaking in concentrated sulfuric acid for 5 min	78.00 ± 3.16 a	78.00 ± 3.16 a	37.06 ± 0.83 a	0.110 ± 0.002 a
Soaking in concentrated sulfuric acid for 10 min	74.00 ± 2.46 a	74.00 ± 2.46 a	36.16 ± 0.89 a	0.104 ± 0.004 a
Soaking in concentrated sulfuric acid for 15 min	71.33 ± 1.67 b	71.33 ± 1.67 b	34.12 ± 0.62 b	0.092 ± 0.003 b

Note: Values are given as mean ±SE. Values with different letters in the same column are significantly different (P<0.05).

Table 4. Effect of extracts from different parts of *Brachiaria* hybrid cultivar 'Mulato II' on germination of *Brassica pekinensis* seeds.

Extracts from different parts	Concentration (mg/ml)	Germination inhibition (%)	Root length inhibition (%)
Distilled water (control)		0 c	0 f
Water extract of lemmas	20	19.33 ± 6.36 a	68.98 ± 1.73 a
	10	2.00 ± 0 bc	57.13 ± 3.57 ab
Alcohol extract of lemmas	20	7.33 ± 2.40 b	49.18 ± 3.04 bc
	10	2.67 ± 1.33 bc	42.98 ± 0.92 c
Water extract of seed coat	20	2.33 ± 1.33 bc	48.76 ± 4.20 bc
	10	2.00 ± 1.15 bc	45.58 ± 3.51 bc
Alcohol extract of seed coat	20	1.33 ± 1.33 bc	9.77 ± 1.18 e
	10	0.67 ± 0.67 bc	8.69 ± 2.23 e
Water extract of naked seeds	20	2.00 ± 0 bc	45.04 ± 4.61 bc
	10	0.67 ± 0.67 bc	28.59 ± 2.95 d
Alcohol extract of naked seeds	20	1.33 ± 0.67 bc	26.09 ± 0.51 d
	10	0.67 ± 0.0.67 bc	8.75 ± 1.77 e

Note: Values are given as mean ±SE. Values with different letters in the same column are significantly different (P<0.05).

Discussion

This study showed that seed structures caused water absorption barriers. While the seed coat did not hinder water absorption, the presence of lemmas promoted water absorption to the seed overall and helped the seed bind more water. The finding that seed structures were involved in seed dormancy is consistent with research from Whiteman and Mendra (1982), who suggested that the mechanical barrier of the *U. decumbens* seed tegument, which restricts access of oxygen and water, is a cause of dormancy. Câmara et al. (2002) also believed that the seed shell of *U. brizantha* cutlivar 'Marandu' restricted gas exchange and inhibited seed germination, resulting in seed dormancy. Duan et al. (2015) showed that impermeability of the seed coat was the main reason for seed dormancy in *U. decumbens*. Therefore, the permeability of seed structures may be one of the causes of dormancy, but not the main cause.

The results showed that mechanical treatment promoted seed germination, with removal of lemmas giving the highest germination. While puncturing the seed coat and leaving the lemmas intact promoted seed germination, it was not as effective as treatments involving removing lemmas, indicating that lemmas were the main cause of dormancy in this species. Soaking seeds in concentrated sulfuric acid promoted seed germination because acid eroded the lemmas and increased the permeability of the seed coat. Similar results have been found in other studies (Whiteman and Mendra, 1982; Câmara et al. 2002; Duan et al. 2015).

Lemmas, seed coat and caryopses all contain substances that inhibit germination in higher concentrations. Water extract from lemmas was most effective with extract from caryopses the least effective, indicating that the endogenous inhibitor was mainly in the lemmas. This is consistent with results of Duan et al. (2015). Ajala-Luccas et al. (2018) showed that dormancy of *U. humidicola* seed is due to synergistic effects between age, GA/ABA balance and residual structure of the panicoid spikelet covering caryopses. Mechanical removal of lemmas effectively reduced the dormancy of *Urochloa* hybrid cultivar 'Mulato II' seeds and resulted in a higher seed vitality index than use of concentrated sulfuric acid for 5 min. It is more convenient, simpler and safer to remove lemmas mechanically than to immerse the seeds in sulfuric acid. Lemmas can be removed by rubbing seeds on rough surfaces, with coarse sand or beating.

Conclusions

This study showed that removal of lemmas and soaking seeds with concentrated sulfuric acid for 5 min was more effective at breaking seed dormancy than soaking for 10 or 15 minutes. After 5 min, the germination percent, germination index, and vitality index of Mulato II seeds decreased with the increase in soaking time.

There were 3 main reasons for the dormancy of *Urochloa* hybrid cultivar 'Mulato II' seed: mechanical barrier of the seed coat and lemmas, endogenous inhibitor (mainly found in lemmas) and water permeability of the seed coat (including pericarp and testa).

Acknowledgments

This work was supported by the Modern Agricultural Grass Industry Technology System Construction Project in Yunnan Province (2017KJTX0018).

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(Note of the editors: All hyperlinks were verified 5 September 2022).

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(Received for publication 03 January 2021; accepted 30 August 2022; published 30 September 2022)