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Seed Germination of Two Hybrids Obtained via Cross-Pollination between *Miscanthus sinensis* × *Miscanthus sacchariflorus*

Cristina Patanè [1,*], Alessandro Saita [1], Salvatore L. Cosentino [2], Antonella Iurato [2] and Giorgio Testa [2]

[1] CNR-Institute for BioEconomy, Via P. Gaifami 18, 95126 Catania, Italy; alessandroattilio.saita@cnr.it
[2] Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Valdisavoia 5, 95123 Catania, Italy; sl.cosentino@unict.it (S.L.C.); antonella.iurato@unict.it (A.I.); gtesta@unict.it (G.T.)
* Correspondence: cristinamaria.patane@cnr.it; Tel.: +39-095-7338395

Abstract: To date, economically and energy-costly vegetative propagation using rhizomes and tissue culture are the only options for the cultivation of *Miscanthus* spp. Some genotypes of miscanthus produce fertile seeds, offering a valid alternative to vegetative propagation. A preliminary study has been conducted on the seeds of two hybrids of miscanthus obtained via interspecific cross-pollination between *M. sacchariflorus* and *M. sinensis*: 'GRC14' (maternal: *M. sacchariflorus*) and 'GRC10B' (maternal: *M. sinensis*). Seeds were assessed for germination traits in a laboratory (at 25 °C in the dark) just after panicle harvest, and during 1-year storage at room temperature or at 8 °C. In a second experiment, the effects of gibberellic acid (GA_3) solution at different concentrations (0, 50, 100, 300, 500 ppm) on the germination of freshly matured seeds were assessed. Poor germination just after harvest (<30%) indicates the occurrence of a physiological dormancy. Indeed, two months later, germination rose up to 76.7% in 'GRC14' and 50.8%, in 'GRC10B', and peaked at 95.6% in 'GRC14' and at 78% in 'GRC10B', 6 months after harvest. After a total of 12 months, germination was significantly reduced in both hybrids (≈60%). Seeds stored at room temperature lost dormancy earlier than those stored at 8 °C. Overall, germination was significantly improved by GA_3, but the extent of the GA effect was genotype-dependent. In conclusion, a low establishment rate may result from direct seeding when fresh seed is used in the field. In this case, the use of GA_3 is a possible strategy to ameliorate the impact of dormancy on seed germination. In the case of delayed sowings in late winter–early spring, seeds stored at room temperature after harvest may better perform than those stored at 8 °C.

Keywords: miscanthus; seed germination; mean germination time; germination index; seed storage; gibberellic acid

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1. Introduction

The genus *Miscanthus* is comprehensive of a large number of C4 perennial rhizomatous species predominantly native to Asia [1]. Within them, *M. sinensis*, *M. sacchariflorus*, and their hybrids have been recognized as the most promising for bioenergy purposes in Europe, for high water use efficiency, low nutrient requirements, high yields, and high adaptability to diverse environmental conditions [2,3].

Currently, *Miscanthus* × *giganteus* Greef et Deu, due to its high biomass yield potential, is the most common commercially grown miscanthus and the best candidate for bioenergy production in the European Union (EU) [4]. It is a natural sterile triploid hybrid of *M. sinensis* × *M. sacchariflorus*, therefore it does not produce seeds [5]. To date, vegetative propagation using rhizomes and tissue culture are the only options for its cultivation; however, they involve high economic and energy costs [6]. It has been found that some genotypes of *M. sinensis* may yield as much as *M.* × *giganteus* [4], but they also produce fertile seeds, offering a valid alternative to expensive vegetative propagation. Open-field experiments conducted in Europe confirm that there is potential for seed-based propagation

of miscanthus [3]. High multiplication rates (approximately 2000×) and low establishment costs (approximately 75% of those of rhizomes) have been reported in the literature for seed-based hybrids of miscanthus [3]. Moreover, wide morphological diversity occurring in seed-propagated miscanthus did not involve yield losses [5].

The adoption of direct sowing in miscanthus could lower crop establishment costs [7]. To reach commercially viable yield levels, prompt seed germination and plant establishment in fields are crucial. However, low levels of seed germination, associated with the occurrence of a physiological dormancy in fresh seeds [8], may limit the application of direct sowing in miscanthus. Moreover, difficult soil conditions typical of marginal lands, which are proposed for planting of miscanthus to avoid competition with food crops [9], may further adversely affect a successful seed-based crop establishment.

There is an increasing demand for newly developed genotypes of miscanthus adaptable to diverse climatic conditions of Europe [10]. In this regard, the existence of a wide genotypic variation within *Miscanthus* spp. makes it possible to breed and select genotypes suitable to a range of environmental conditions, including marginal lands [11].

The Department of Agriculture, Food and Environment (Di3A) at the University of Catania (Italy), has been carrying out intense research activity on miscanthus since 1988 [12–15]. Recently, the Department has been involved with Aberystwyth University, United Kingdom (UK), in miscanthus seed production, considering the suitability of climatic conditions (in terms of temperature and photoperiod) in Sicily [13]. The breeding activity was planned for developing new seed-based hybrids from *Miscanthus* spp., with desirable agronomic traits (high biomass potential, high water use efficiency, low input requirements, etc.) also suitable to being introduced in cultivation to marginal areas, such as those of a Mediterranean environment [3].

A project has been funded by the Italian Ministry of Agricultural, Food and Forestry Policies (PRIN project), with the overall aim of identifying genotypes of miscanthus suitable for biomass production in semi-arid areas. In the framework of this project, a preliminary study has been conducted to assess the seed germination traits in two new hybrids of *Miscanthus* spp. The final goal of this study is to verify the seasonal timing of germination when potentially mediated by seed dormancy in order to predict the level of success in crop establishment for these two hybrids when seed propagation is adopted in a field.

2. Materials and Methods

2.1. Experiment Location and Plant Material

The study was conducted on the seeds of two hybrids of *Miscanthus* spp. The seeds of the two hybrids were produced in 2020, at the experimental field of the University of Catania (South Italy, 10 m a.s.l., 37°24′35.8″ N Lat, 15°03′31.7″ E Long), via interspecific cross-pollination between *M. sacchariflorus* and *M. sinensis*: 'GRC14' (maternal: *M. sacchariflorus*) and 'GRC10B' (maternal: *M. sinensis*). The two hybrids were selected among genotypes obtained within a wide breeding program for crop biomass production, by crossing several parental *M. sinensis* × *M. sacchariflorus* collected in the South East of Asia in order to select candidate seed-based hybrids adapted to European environments [3,16]. Specifically, in previous field experiments, the two hybrids exhibited traits of adaptation to the hot/dry climate typical of the Mediterranean regions as well as higher water-use efficiency and biomass production than *Miscanthus* × *giganteus* Greef et Deu (unpublished data).

The seeds of the two hybrids were collected from the commercial fields carried out under the supervision of Terravesta Ltd. (Lincoln, UK) at the experimental farm of the Department of Agriculture, Food and Environment (Di3A) of the University of Catania (Italy). The fields were established in 2017 for 'GRC10B', and 2018 for 'GRC14', using rhizomes of *Miscanthus sinensis* and *Miscanthus sacchariflorus* in parallel lines. The fields were not fertilized and were drip-irrigated during summertime, restoring 100% of crop evapotranspiration. Every year, after panicles harvest in November, the plants were cut to allow the re-growth of shoots.

The panicles used for this experiment (approximately 50 per hybrid) were harvested in November 2020, at the full ripening stage, and softly dried at 30 °C in a thermoventilated oven for five days. After drying, some panicles were used for a seed germination test at harvest; others were stored in large paper bags, at room temperature (15–20 °C) and at 8 °C, to assess seed germination traits during storage (up to 12 months).

2.2. Determined Indicators

2.2.1. Spikelet Fertility and 100-Seed Weight

Prior to germination tests at harvest, panicle samples were separated into three portions: apical, central, and basal (Figure 1). After that, spikelets were sampled randomly, separately per each portion of panicle, and 20 individual spikelets (replicated three times) per each part were checked for fertility. This last, expressed as the percentage of total spikelets examined, was measured by splitting all spikelets into two groups: those containing a seed (fertile) and those empty (sterile). Seed weight (mg) was also measured for three replicates of 100 seeds each, separately, per each portion of panicle.

Figure 1. Panicle of miscanthus partitioned into apical, central, and basal sections.

2.2.2. Common Germination Tests without Seed Pretreatment

In seeds just after harvest and in those stored at room temperature (15–20 °C) and at 8 °C (1st experiment), germination tests were performed on 50 seeds, manually threshed, per each portion of panicles, placed in 90-mm plastic Petri dishes containing a single paper filter, and moistened with 5 mL of distilled water. Petri dishes were sealed with Parafilm to prevent water losses by evaporation, then they were incubated in a thermostatically controlled incubator in the dark, in a completely randomized experimental design with four replicates. Seeds were germinated at a constant temperature of 25 °C, considered as optimal or near optimal for seed germination of miscanthus [6]. Germination was scored daily, using a radicle emergence of at least 2 mm as the criterion, until no further seed germination occurred after 72 h. At the end of the test, a final germination percentage (FGP, %), mean germination time (MGT, days), and germination index (GI) were calculated using the following formulas:

$$FGP = (n/Tn) \times 100 \quad (1)$$

$$MGT = \Sigma(ni \times di)/n) \quad (2)$$

$$GI = \Sigma(nd1/d1) + (nd2/d2) + (nd3/d3) + \ldots\ (ndi/di) \quad (3)$$

where *FGP* is the final germination percentage (%) [17]; *n* is total number of seeds germinated; *Tn* is total number of seeds left to germinate; *MGT* is mean germination time (days) [18]; *ni* is the number of seeds germinated on day *i d* is the incubation period in days; *GI* is the germination index; *nd*1, *nd*2, *nd*3, [19] *ndi* indicate the number of seeds

germinated on days 1, 2, 3, . . . , *i*. Higher GI values indicate a higher percentage and rate of germination [20].

Seed germination was assessed just after panicle harvest, and at 2-month intervals, for a total of 12 months.

2.2.3. The Seed Germination Experiment in the Presence of Gibberellic Acid (GA_3) Solution

A second germination experiment was performed contextually in a laboratory to assess germination in seeds of the two hybrids of miscanthus just after harvest and when left to germinate in gibberellic acid (GA_3) solutions. To this end, 50 seeds of each hybrid, replicated 4 times, were randomly chosen among those positioned in the apical, central, and basal parts of the panicles. The germination test was conducted according to the method above described for the 1st experiment. In this 2nd experiment, the seeds were placed in Petri dishes, in a single filter imbibed with 5 mL of one of the following GA_3 solutions: 0 (distilled water), 50, 100, 300, or 500 ppm. GA_3 (Sigma-Aldrich s.r.l., Milano, Italy) was used as the stock 500-ppm solution, and dilutions were prepared from this last solution just before use.

The germination test was conducted at 25 °C (optimal temperature), and the seeds were those of the same seed lots of the 1st experiment.

Germination was scored daily and at the end of the test, FGP, MGT, and GI were calculated, as described above.

2.3. Data Analysis

The time course of cumulative values of seed germination measured at 8 and 12 months after harvest, in seeds stored at room temperature or at 8 °C (1st experiment), and the time course of cumulative values of seed germination measured just after harvest in seeds imbibed in different GA_3 solutions (2nd experiment), was described using a nonlinear iterative regression method (SIGMAPLOT® 11.0 software; Systat Software Inc., San Jose', CA, USA) using the following sigmoidal logistic equation with three parameters:

$$y = a/(1 + (x/x_0)^b) \quad (4)$$

where a is maximal value of y (i.e., maximum germination), x is time (days) starting from seed imbibition, x_0 is time (days) to reach 50% of maximal germination, and b is a fitting parameter of the curve.

Data of the FGP, previously arcsine transformed, and those of the MGT, were statistically analyzed using a two-way ANOVA, separately within each hybrid and portion of panicle, considering 'storage time' and 'storage temperature' (1st experiment), and 'genotype' and 'GA_3 concentration' (2nd experiment) as fixed factors. Data of the FGP, previously arcsine transformed, those of the MGT, and those of the GI, from the germination test conducted on seeds stored 8 and 12 months, were also statistically analyzed using a three-way ANOVA, separately per storage period, considering 'genotype', 'storage temperature', and 'seed position on the panicle' as fixed factors.

For all ANOVAs, the main effects and interaction were evaluated for significance at $p < 0.05$ level according to the Tukey's test.

3. Results

3.1. Spikelet Fertility and 100-Seed Weight

Spikelet fertility was measured on each portion of panicle, in both hybrids of *Miscanthus*, just after panicle harvest. It was rather low, not exceeding 53.3% in 'GRC14' and 43.3% in 'GRC10B' (Table 1).

Table 1. Spikelet fertility (%) and 100-seed weight (mg) in miscanthus 'GRC14' and 'GRC10B' in relation to spikelet position on the panicle. Values (means ± *se*) within columns followed by different letters statistically differ at $p < 0.05$ using Tukey's test.

Spikelet Position	Fertility (%)		100-Seed Weight (mg)	
	GRC14	GRC10B	GRC14	GRC10B
Apical	53.3 ± 0.83 a	38.3 ± 1.67 a	44.5 ± 0.86	63.2 ± 2.11 a
Central	49.2 ± 0.83 b	43.3 ± 2.20 a	44.0 ± 0.89	58.9 ± 1.35 ab
Basal	25.8 ± 1.67 c	32.5 ± 2.89 b	43.3 ± 0.65	56.5 ± 1.43 b
	***	**	ns	*

Significant at $p < 0.05$ (*), 0.01 (**), and 0.001 (***); ns: not significant.

Fertility significantly changed with the spikelet position, progressively and significantly decreasing (down to 25.8%) ($p < 0.001$) from the apical to the basal portion, in 'GRC14', and being the lowest ($p < 0.01$) in the basal portion, while not differing in spikelets of the apical and central parts of panicles, in 'GRC10B'.

Seed weight, as measured at harvest, was 26% greater in 'GRC10B' (100-seed weight 59.5 mg, on average) than in 'GRC14' (43.9 mg). It did not change with seed position along the panicle in 'GRC14' ($p > 0.05$), but it slightly decreased proceeding from the top to the basal portion of the panicle in 'GRC10B' ($p < 0.05$).

3.2. Common Seed Germination Tests (at Harvest and during 1-Year Storage) without Seed Pretreatment

The final germination percentage (FGP) and mean germination time (MGT) were significantly affected by the seed age (months after harvest) in both hybrids of miscanthus (Tables 2 and 3).

Table 2. Main effects of storage time (S) and storage temperature (T) on final germination percentage (FGP) and mean germination time (MGT), in miscanthus 'GRC14', in relation to spikelet position on the panicle. Values (means ± *se*) within columns followed by different letters statistically differ at $p < 0.05$ using Tukey's test.

Source of Variation		Spikelet Position					
		Apical		Central		Basal	
		FGP (%)	MGT (Days)	FGP (%)	MGT (Days)	FGP (%)	MGT (Days)
Storage time (months) (S)	0	18.3 ± 4.6 c	12.3 ± 1.5 a	30.0 ± 1.8 c	6.0 ± 0.4 b	21.7 ± 2.8 d	9.3 ± 1.1 a
	2	68.3 ± 9.3 b	8.7 ± 0.3 b	64.2 ± 8.7 b	9.2 ± 0.7 a	76.7 ± 10.5 b	8.3 ± 0.2 a
	4	60.8 ± 9.8 b	8.3 ± 1.0 b	67.9 ± 11.8 b	8.7 ± 1.0 a	66.7 ± 9.1 c	8.3 ± 1.0 a
	6	95.6 ± 1.6 a	8.4 ± 0.2 b	94.4 ± 1.1 a	7.9 ± 0.3 ab	95.0 ± 1.4 a	8.1 ± 0.2 a
	8	100.0 ± 0.0 a	2.5 ± 0.1 c	100.0 ± 0.0 a	2.2 ± 0.1 c	100.0 ± 0.0 a	2.2 ± 0.1 b
	10	87.2 ± 2.5 a	2.2 ± 0.2 c	93.9 ± 2.0 a	2.0 ± 0.1 c	91.7 ± 2.9 a	2.0 ± 0.1 b
	12	61.7 ± 3.2 b	3.6 ± 0.2 c	58.3 ± 5.4 b	3.3 ± 0.2 c	61.7 ± 3.8 c	3.5 ± 0.3 b
Storage temperature (T)	Room	75.3 ± 6.1 a	6.4 ± 0.9 a	80.4 ± 5.3 a	5.3 ± 0.6 a	81.2 ± 6.1 a	5.8 ± 0.7 a
	8 °C	65.2 ± 6.6 b	6.7 ± 0.9 a	65.0 ± 6.2 b	5.9 ± 0.7 a	65.5 ± 6.0 b	6.2 ± 0.7 a
significance	S	***	***	***	***	***	***
	T	***	ns	***	ns	***	ns
	S × T	***	ns	***	ns	***	ns

Significant at $p < 0.001$ (***); ns: not significant.

Table 3. Main effects of storage time (*S*) and storage temperature (*T*) on final germination percentage (FGP) and mean germination time (MGT), in miscanthus 'GRC10B', in relation to spikelet position on the panicle. Values (means ± *se*) within columns followed by different letters statistically differ at $p < 0.05$ using Tukey's test.

Source of Variation		Spikelet Position					
		Apical		Central		Basal	
		FGP (%)	MGT (Days)	FGP (%)	MGT (Days)	FGP (%)	MGT (Days)
Storage time (months) (S)	0	20.0 ± 3.7 d	5.7 ± 0.2 bc	29.1 ± 6.1 d	6.0 ± 0.0 b	18.6 ± 2.0 d	6.6 ± 0.4 b
	2	39.2 ± 8.5 c	8.8 ± 0.3 a	44.2 ± 7.1 cd	8.7 ± 0.2 a	50.8 ± 10.3 c	9.5 ± 0.2 a
	4	57.1 ± 12.0 b	7.5 ± 1.0 ab	60.0 ± 4.8 bc	6.3 ±0.6 b	53.3 ± 10.8 c	7.3 ± 1.1 b
	6	78.9 ± 1.4 a	8.0 ± 0.5 a	71.7 ± 4.5 ab	7.2 ± 0.5 b	75.6 ± 3.2 ab	6.5 ± 1.0 b
	8	86.1 ± 1.6 a	3.7 ± 0.3 d	88.9 ± 1.9 a	4.1 ± 0.1 c	89.4 ± 1.8 a	4.1 ± 0.2 c
	10	80.0 ± 3.9 a	2.6 ± 0.2 d	68.9 ± 1.6 b	2.2 ± 0.1 d	83.3 ± 0.9 a	3.0 ± 0.2 c
	12	57.2 ± 4.0 b	4.0 ± 0.2 cd	58.3 ± 3.5 bc	3.8 ± 0.1 c	58.9 ± 4.2 bc	4.1 ± 0.2 c
Storage temperature (T)	Room	66.1 ± 5.2 a	5.4 ± 0.5 b	63.4 ± 4.6 a	5.4 ± 0.5 a	61.0 ± 6.0 a	5.9 ± 0.6 a
	8 °C	53.5 ± 6.2 b	6.1 ± 0.6 a	56.9 ± 4.7 b	5.5 ± 0.5 a	61.9 ± 5.7 a	5.8 ± 0.5 a
significance	S	***	***	***	***	***	***
	T	***	*	*	ns	ns	ns
	S × T	***	ns	ns	ns	***	ns

Significant at $p < 0.05$ (*) and 0.001 (***); ns: not significant.

A poor germination was recorded just after harvest (<30%). Two months later, across the two storage temperatures, the FGP significantly improved (storage time—S, $p < 0.001$), rising up to 76.7% in 'GRC14', and to 50.8% in 'GRC10B' (in both hybrids, seeds of the basal part of the panicle). Later on, at 4 months after harvest, no changes in seed germination were observed in both hybrids, but two months later (6 months in total from harvest), a sharp rise in germination occurred, with the mean FGP peaking at 95.6% in 'GRC14', and at 78% in 'GRC10B', in both cases in apical seeds. After that, up to the 10th month, the FGP was kept constant in 'GRC14', or slightly decreased in seeds of the mid portion of panicles in 'GRC10B', but two months later (in total 1 year from harvest), in both hybrids, irrespective of seed position, germination was significantly reduced to approximately 60%. Overall, the germination trend during the 1-year period was similar in the seeds of the three parts of the panicles considered.

Additionally, the thermal conditions of storage (T) significantly affected the seed germination, across the storage months, in all seeds of 'GRC14' ($p < 0.001$), and those in the apical ($p < 0.001$) and median ($p < 0.05$) parts, but not those in the basal part ($p > 0.05$), in 'GRC10B'. In particular, seeds stored at room temperature germinated better overall than those stored at 8 °C. These differences were clearer in the seeds of 'GRC14'.

However, the significant interaction S × T' on the FGP revealed a different evolution of seed germination capacity during storage, depending on the storage temperature. Two months after harvest, FGP significantly improved in seeds stored at both thermal conditions, but those kept at room temperature exhibited a steeper rise in seed germination (up to 100% in 'GRC14' and 70% in 'GRC10B') than those stored at low temperature (up to 53.3% in 'GRC14' and 35% in 'GRC10B') (Figure 2). After that, FGP in 'GRC14' was kept constantly high, exceeding 81.7% during up to 10 months of storage at room temperature, to significantly decline (down to 57–64%) two months later (1 year storage in total). In this hybrid, seeds stored at 8 °C matched in the FGP those kept at room temperature from the 6th month of storage (FGP ≥ 86%) onwards, and as these last, they significantly reduced their ability to germinate at the 12th month of storage.

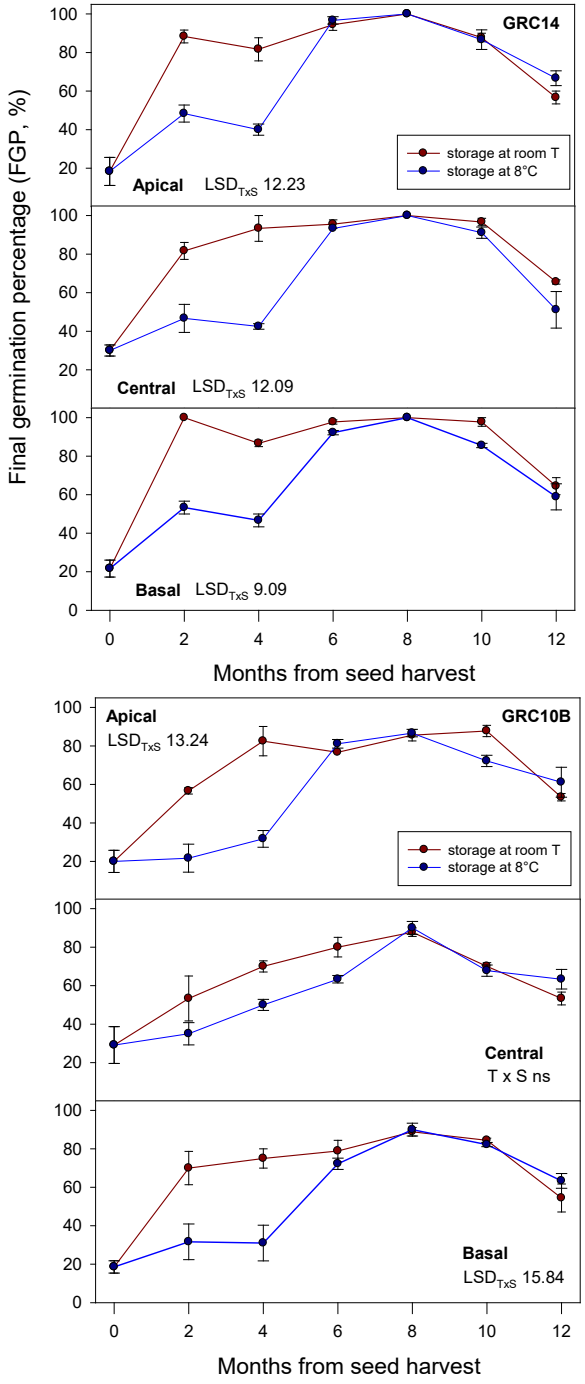

Figure 2. Interaction effect of 'storage time' × 'storage temperature' on final seed germination percentage (FGP, %) in miscanthus 'GNT 43A' (on the left) and 'GRC10B' (on the right) in relation to spikelet position on the panicle. Data are means ± *se* ($n = 4$). Value for LSD ($p < 0.05$) is reported in the case of significant interaction. ns: not significant.

A similar trend was observed in seeds of 'GRC10B', whose FGP, however, never achieved 100%, with the best results being between the 4th and the 6th month of storage at room temperature (up to approx. 80%), and later, at the 8th month of storage at 8 °C. Seeds at 8 °C, as those at room temperature, had a significant decline in FGP at the 12th month.

In relation to spikelet position, overall, just after harvest, seeds positioned in the middle part of the panicle exhibited a higher seed germination (30% in 'GRC14', 29.1% in GRC10B') than those of the apical and basal parts. Moreover, the basal seeds of 'GRC14' stored at room temperature, differently than those of the apical and central parts, full

germinated (100%) already at two months after harvest. In this hybrid, seeds on the top of the panicle, during 10 months of storage at the same temperature, experienced a significant decline in germination capacity (FGP < 88%), which did not occur in seeds in the central and basal parts (FGP ≥ 97%). In 'GRC10B', the differences in seed germination related to storage temperature were overall less evident in seeds of the central part of the panicle.

Overall, seeds of 'GRC14' germinated better than those of 'GRC10B'.

Mean germination time (MGT), which indicates the germination speed, was affected by the time of storage but not (when excluded by a few cases) by the temperature of storage.

In 'GRC14', germination proceeded quite slow just after harvest, and apical, central, and basal seeds, took, respectively 12.3, 6.0, and 9.3 days, to germinate (S, $p < 0.001$) (Figure 3). Afterwards, germination speed did not vary, or slightly increased (in seeds of the central part of panicle) until the 6th month of storage. Two months later, the germination time drastically dropped, and seeds germinated in approximately 2 days. Later on, the germination time was maintained constant until 1 year of storage.

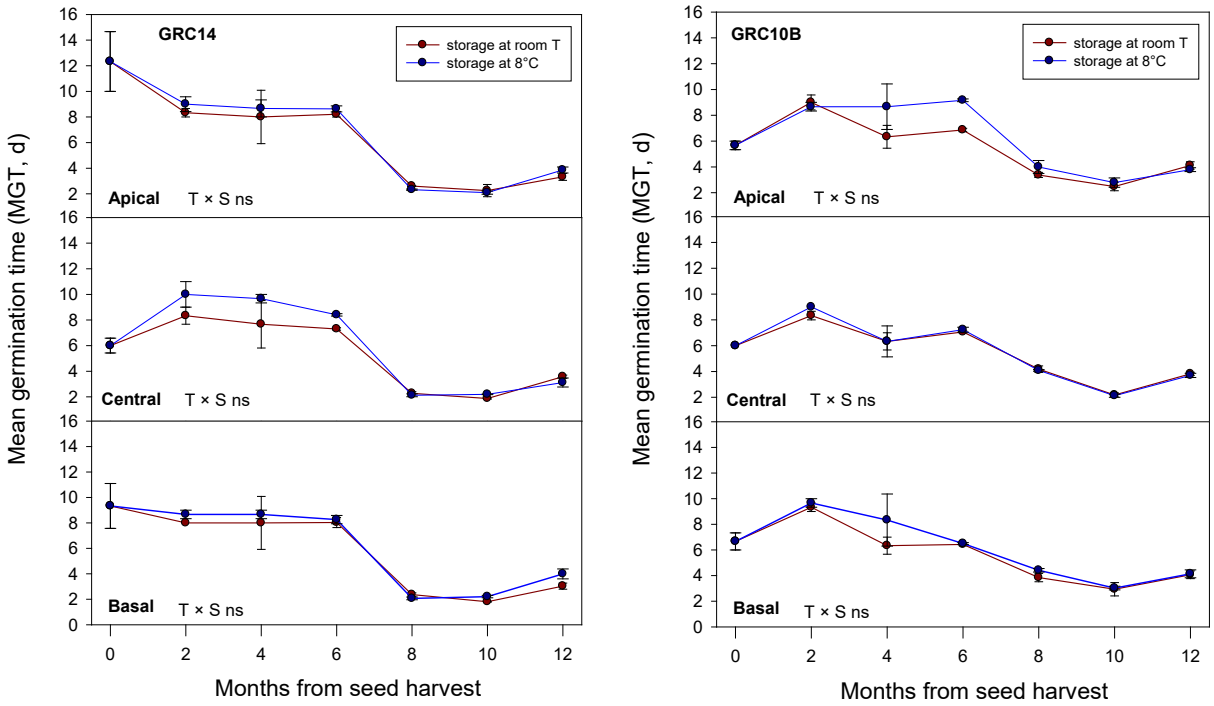

Figure 3. Interaction effect of 'storage time' × 'storage temperature' on mean germination time (MGT, days) in miscanthus 'GRC14' (on the left) and GRC10B' (on the right) in relation to spikelet position on the panicle. Data are means ± *se* ($n = 4$). ns: not significant.

In 'GRC10B', seeds just after harvest germinated in approximately 6 days, irrespective of their position on the panicle, but two months later, their MGT significantly increased (S, $p < 0.001$) according to the number of seeds germinated. Afterwards, the germination speed remained unchanged (apical and basal seeds, stored at 8 °C) or progressively improved, down to a minimum MGT (2.2–3.0 days) recorded at 10 months of storage. Two months later, the germination time did not change (apical and basal seeds) or was slightly extended (central seeds).

Overall, the lowering of the temperature of storage to 8 °C did not alter the germination speed as compared to that of seeds stored at room temperature. No interaction 'S × T' was highlighted during the ANOVA.

3.3. Cumulative Germination Course at 8- and 12-Month Storage Stages

The course of cumulative seed germination for the two hybrids at 8 and 12 months of storage at room temperature or at 8 °C is illustrated in Figure 4. This course is well

described ($R^2 \geq 0.98$) by a sigmoidal logistic equation whose trend indicates an initial phase of low germination (lag phase), followed by a steep rise in germination, up to a maximum (*a* parameter of the curve) (Table 4). The lag phase was negligible in seeds of 'GRC14' after 8 months of storage, and, consequently, steeper was the rise in germination up to the maximum possible for these seeds. Overall, germination in 'GRC10B' started later and proceeded slower than in 'GRC14', especially in seeds at 8 months of storage.

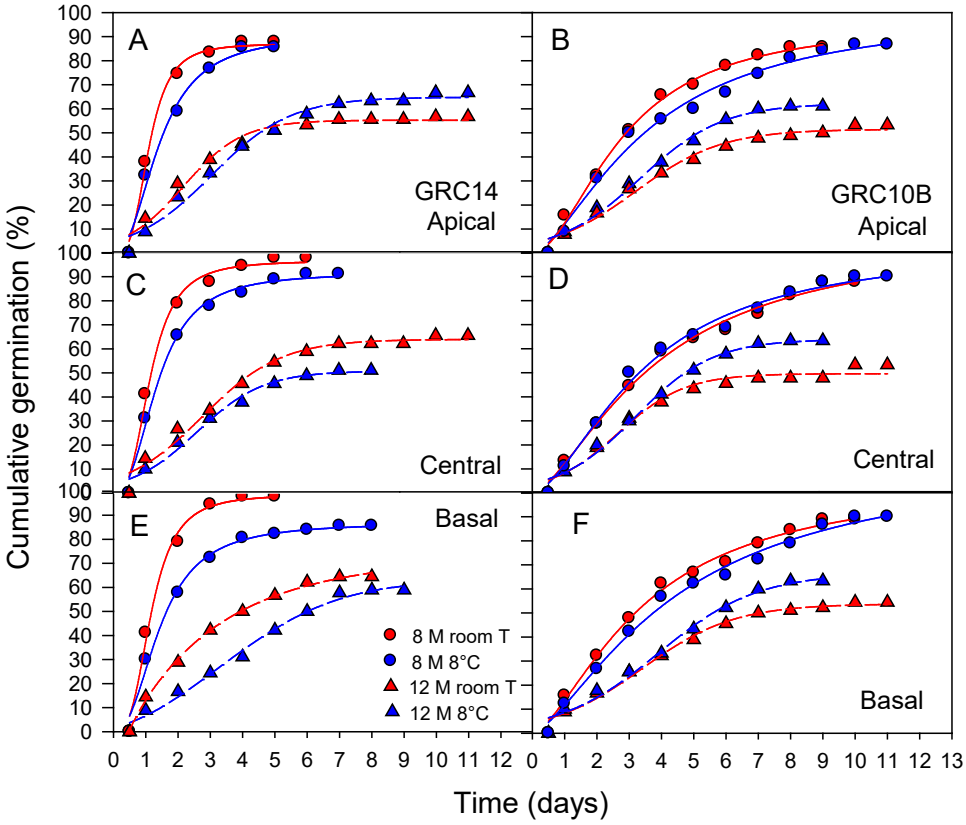

Figure 4. Time course of cumulative seed germination in miscanthus 'GRC14' (**A**,**C**,**E**,) and 'GRC10B' (**B**,**D**,**F**) at 8 (solid lines) and 12 (short-dash lines) months of storage (8 M, 12 M) at room temperature (red lines) or at 8 °C (blue lines) in relation to seed's position on the panicle (**A**,**B**: apical; **C**,**D**: central, **E**,**F**: basal). Symbols represent the observed germination percentage with time.

Table 4. Values of parameters of the sigmoidal logistic function interpolating data of seed germination of miscanthus 'GRC14' and 'GRC10B' at 8 (8 M) and 12 months (12 M) of storage at room temperature or at 8 °C, in relation to seed position on the panicle.

Storage Time	Storage Temperature	Seed Position	GRC14			GRC10B		
			a	R^2	$\sum x^2$	*a*	R^2	$\sum x^2$
8 M	Room	Apical	87.1	0.99	42.9	98.2	0.99	36.3
		Central	96.6	0.99	88.1	107.6	0.99	64.8
		Basal	98.6	0.99	74.8	105.7	0.99	59.3
	8 °C	Apical	90.8	0.98	91.5	101.2	0.99	112.5
		Central	91.6	0.99	68.1	102.9	0.99	79.1
		Basal	86.9	0.99	67.9	114.6	0.99	77.1
12 M	Room	Apical	74.1	0.99	20.2	78.9	0.99	34.2
		Central	60.8	0.99	17.0	77.5	0.99	30.4
		Basal	89.5	0.99	53.8	106.6	0.99	48.9
	8 °C	Apical	59.5	0.99	25.5	61.3	0.99	10.7
		Central	73.5	0.99	58.9	55.6	0.99	25.2
		Basal	74.3	0.99	27.4	65.6	0.99	23.4

The observation of the values of the *a* parameter, which indicates the maximum value on the curve, reveals that a 12-month storage involved an overall depressive effect on seed germination (lower *a* values), which also started later and proceeded slower than in seeds stored for 8 months.

The final germination percentage (FGP) measured at 8 months was affected by genotype (G, $p < 0.001$), being full in 'GRC14' (FGP 100%) but not in 'GRC10B' (FGP 88.2%) (Table 5, Figure 5). Such difference was not observed in seeds that were 1-year-old, whose FGP did not change with the genotype ($p > 0.05$). Neither storage temperature nor seed position on the panicle significantly affected the FGP, both in seeds at 8 and 12 months. No significant interaction among the experimental factors was highlighted during the ANOVA on the FGP.

Table 5. Main effects of genotype (G), storage temperature (T), and seed position on the panicle (P) on final germination percentage (FGP), mean germination time (MGT) and germination index (GI), in seeds of miscanthus at 8 and 12 months of storage. Values (means ± *es*) within columns followed by different letters statistically differ at $p < 0.05$ using Tukey's test.

Source of Variation		Storage Time					
		8 Months			12 Months		
		FGP (%)	MGT (Days)	GI	FGP (%)	MGT (Days)	GI
Genotype (G)	GRC14	100.0 ± 0 a	2.29 ± 0.1 b	16.1 ± 0.4 a	60.6 ± 2.3 a	3.48 ± 0.1 b	8.0 ± 0.3 a
	GRC10B	88.2 ± 1.0 b	3.98 ± 0.1 a	10.4 ± 0.2 b	58.2 ± 2.1 a	3.94 ± 0.1 a	6.7 ± 0.2 b
Storage temperature (T)	Room	93.7 ± 1.7 a	3.11 ± 0.2 a	13.1 ± 0.6 a	58.0 ± 1.9 a	3.66 ± 0.1 a	7.6 ± 0.4 a
	8 °C	94.4 ± 1.5 a	3.17 ± 0.3 a	13.4 ± 0.9 a	60.7 ± 2.5 a	3.77 ± 0.1 a	7.1 ± 0.2 a
Seed position (P)	Apical	93.1 ± 2.2 a	3.08 ± 0.2 a	12.5 ± 0.7 b	59.4 ± 2.5 a	3.77 ± 0.1 a	7.4 ± 0.4 a
	Central	94.4 ± 1.9 a	3.17 ± 0.3 a	13.4 ± 1.0 ab	58.3 ± 3.1 a	3.55 ± 0.1 a	7.4 ± 0.3 a
	Basal	94.7 ± 1.8 a	3.17 ± 0.3 a	13.7 ± 1.1 a	60.3 ± 2.7 a	3.81 ± 0.2 a	7.3 ± 0.4 a
Significance	G	***	***	***	ns	**	***
	T	ns	ns	ns	ns	ns	ns
	P	ns	ns	*	ns	ns	ns
	G × T	ns	*	**	ns	ns	***
	G × P	ns	*	*	ns	ns	ns
	T × P	ns	ns	ns	ns	ns	ns
	G × T × P	ns	ns	ns	ns	ns	ns

Significant at $p < 0.05$ (*), 0.01 (**) and 0.001 (***); ns: not significant.

The germination speed was affected by genotype, being higher (significantly lower MGT) in 'GRC14', both in seeds stored 8 and 12 months (respectively 2.29 and 3.48 days, in 'GRC14', and 3.98 and 3.94 days in 'GRC10B'). As for the FGP, no effect on the MGT was exerted by storage temperature and seed position (T, P, $p > 0.05$). However, significant 'G × T' interaction ($p < 0.05$) in seeds at 8 months of storage revealed that the germination speed was affected by genotype, depending on storage temperature, the MGT being not affected in 'GRC14', and being significantly higher in seeds stored at 8 °C, in 'GRC10B'. Similarly, significant 'G × P' interaction indicated that germination speed, across storage temperatures, was affected by genotype, depending on seed position on the panicle, the MGT being unaffected in 'GRC14', and slightly ($p < 0.05$) but significantly lower in the seeds positioned in the apical part of the panicle in 'GRC10B'.

No interaction on both the FGP and MGT at 8 and 12 months storage was observed among the three experimental factors (G × T × P, $p > 0.05$).

The germination index (GI), which was calculated from the number of seeds germinated each day and the relative day of germination, was reduced as seed storage was prolonged from 8 to 12 months. In both cases, the GI significantly changed with genotype (G, $p < 0.001$), being greater in 'GRC14' (16.07 and 7.96, against 10.36 and 6.73 in 'GRC10B', calculated, respectively at 8 and 12 months). The GI was not affected by the temperature of storage (T, $p > 0.05$), and it varied with seed position, although solely in seeds stored for

8 months, slightly increasing as we move from apical to basal seeds. Significant interaction 'G × T' indicated that the effect of temperature on the GI was genotype-dependent. In particular, the GI in 'GRC14' was higher in seeds stored at 8 °C, at 8 months, and in those stored at room temperature, at 12 months. Contrastingly, in the seeds of 'GRC10B', the GI did not change with storage temperature (Figure 6).

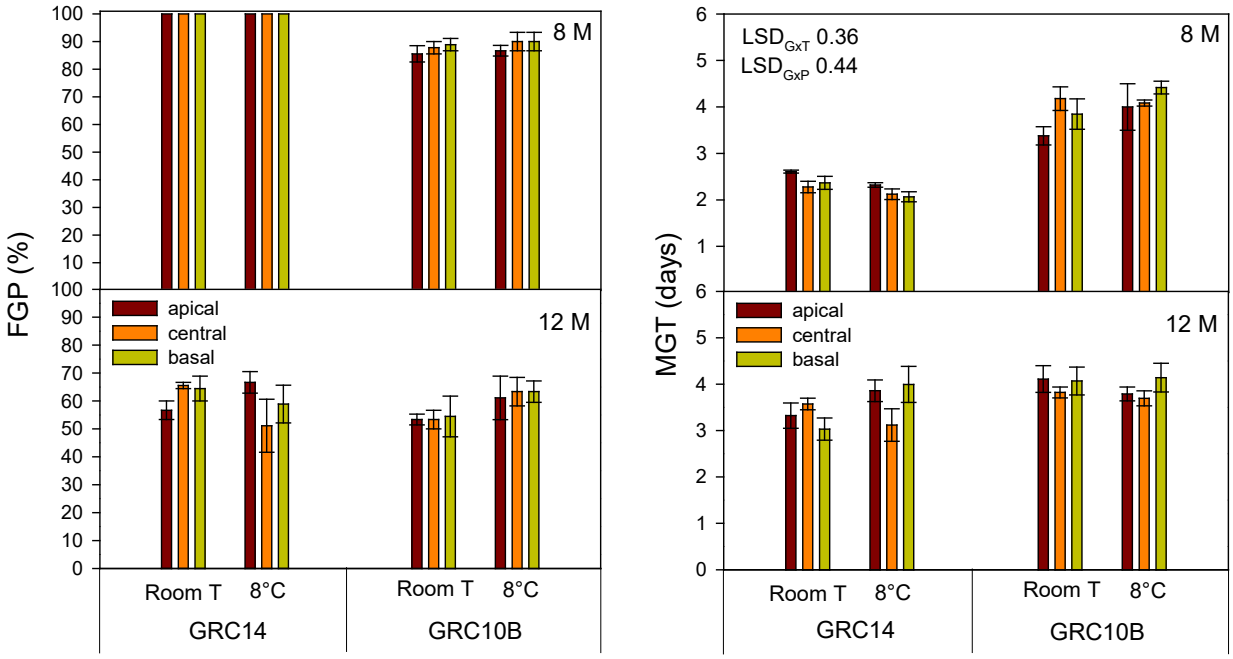

Figure 5. Interaction effect of 'genotype ('GRC14', 'GRC10B') × storage temperature (room temperature, 8 °C) × seed position (apical, central, basal)' on final seed germination percentage (FGP, on the left) and mean germination time (MGT, on the right) in seeds of miscanthus at 8 (8 M) and 12 months (12 M) of storage. Data are means ± *se* (n = 4). Value for LSD ($p < 0.05$) is reported in the case of significant interaction.

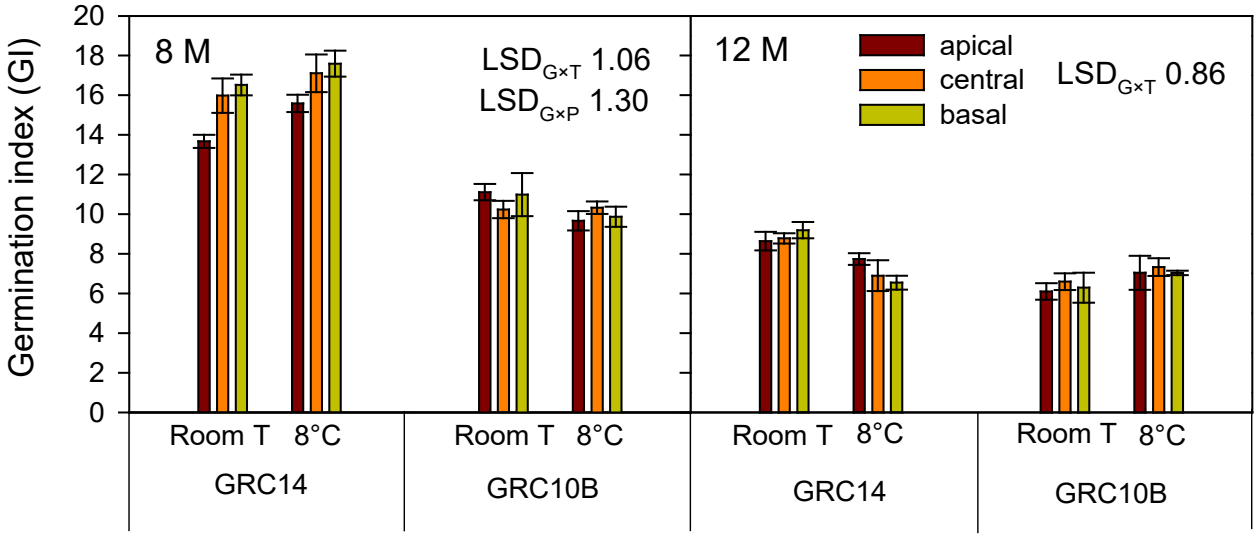

Figure 6. Interaction effect of 'genotype ('GRC14', 'GRC10B') × storage temperature (room temperature, 8 °C) × seed position (apical, central, basal)' on the germination index (GI) in seeds of miscanthus at 8 (on the left) and 12 months (on the right) of storage. Data are means ± *se* (n = 4). Value for LSD ($p < 0.05$) is reported in the case of significant interaction.

3.4. Experiment #2: Effects of GA_3 on Seed Germination at Harvest

The course of cumulative seed germination for the two hybrids of miscanthus during imbibition in distilled water and at different concentrations of GA_3 is illustrated in Figure 7.

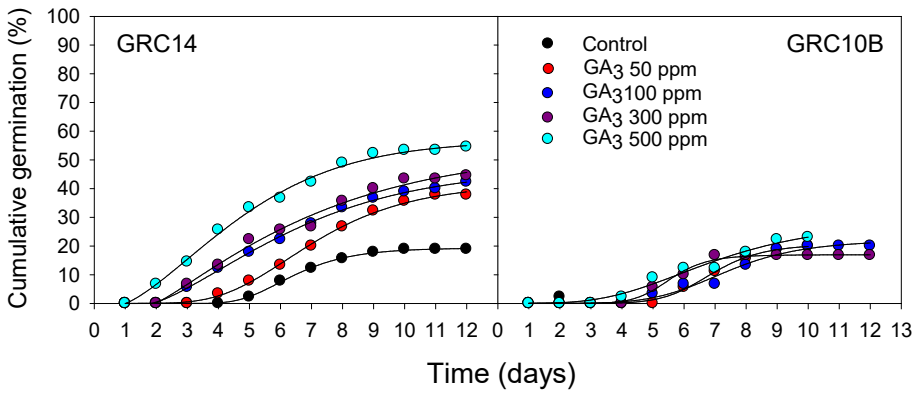

Figure 7. Time course (solid curves) of cumulative seed germination at different gibberellic acid (GA_3) concentrations of the imbibition solution in miscanthus 'GRC14' (on the left) and 'GRC10B' (on the right). Symbols represent the observed percentage with time.

As previously discussed, the course is well described ($R^2 \geq 0.98$) by the sigmoidal logistic equation, with an initial phase of low germination, followed by a rise in germination up to a maximum (*a* parameter) (Table 6). In 'GRC14', the logistic function slightly overestimated (from +11 to +18%) the maximum germination percentage (*a* parameter) in the GA_3 solution. In 'GRC10B', the overestimation was evident at 500 ppm only (+26%).

Table 6. Values of the parameters of the sigmoidal logistic function interpolating data of seed germination of miscanthus 'GRC14' and 'GRC10B' under different gibberellic acid (GA_3) concentrations of the imbibition solution.

GA_3 Concentration (ppm)	GRC14			GRC10B		
	a	R^2	$\sum x^2$	*a*	R^2	$\sum x^2$
0	19.3	0.99	1.25	-	-	-
50	42.1	0.99	3.48	20.7	0.99	2.51
100	48.4	0.98	8.33	22.3	0.98	17.95
300	52.8	0.99	39.18	17.0	0.99	5.83
500	61.0	0.99	19.75	28.0	0.98	17.57

From the analysis of the interpolation curves of cumulative germination values, it is evident how the length of the initial phase of low germination, which was maximized in distilled water (control), was progressively reduced by the rise in GA_3 concentrations. In other words, the increase in GA_3 concentration promoted the start of seed germination that, anyway, approached final values (*a* parameters) which were similar at all concentrations.

When the main factors are taken into account, a significant effect of both was observed during the ANOVA. Across concentrations, 'GRC14' germinated better than 'GRC10B' (36.6% and 16.7%, respectively (Table 7). Across genotypes, germination was significantly promoted by GA_3, with no differences among concentrations.

Table 7. Main effects of 'genotype' (G) and 'GA_3 concentration' (C) on the final germination percentage (FGP), mean germination time (MGT), and germination index (GI) in miscanthus. Values (means ± se) within columns followed by different letters statistically differ at $p < 0.05$ using Tukey's test.

Source of Variation		FGP (%)	MGT (Days)	GI
Genotype (G)	GRC14	39.6 ± 3.5 b	6.5 ± 0.3 a	2.25 ± 0.3 a
	GRC10B	16.7 ± 2.4 a	6.1 ± 0.9 a	0.80 ± 0.1 b
GA_3 concentration (ppm) (C)	0 (distilled water)	10.6 ± 4.1 b	4.5 ± 1.1 d	0.58 ± 0.2 c
	50	28.9 ± 5.5 a	7.5 ± 0.3 a	1.25 ± 0.3 bc
	100	31.7 ± 5.8 a	7.2 ± 0.4 ab	1.59 ± 0.4 b
	300	31.2 ± 6.1 a	6.4 ± 0.4 bc	1.70 ± 0.4 ab
	500	38.3 ± 7.4 a	5.9 ± 0.4 c	2.50 ± 0.6 a
Significance	G	***	ns	***
	C	***	***	***
	G × C	ns	***	**

Significant at $p < 0.01$ (**) and 0.001 (***); ns: not significant.

No interaction for 'G × C' was highlighted during the ANOVA. However, the germination responses of the two hybrids to the hormone differed in their extent. Indeed, in both hybrids, imbibition in the GA_3 solution led to a significant improvement of germination over the control, and the promoting effect of gibberellic acid did not differ with the concentration (Figure 8). However, in 'GRC14', the FGP peaked at 54.4% (500 ppm GA_3), whilst in 'GRC10B', the beneficial effect of the hormone upon seed germination was less evident, and an overall low FGP was recorded for this hybrid (max 22% at 500 ppm GA_3).

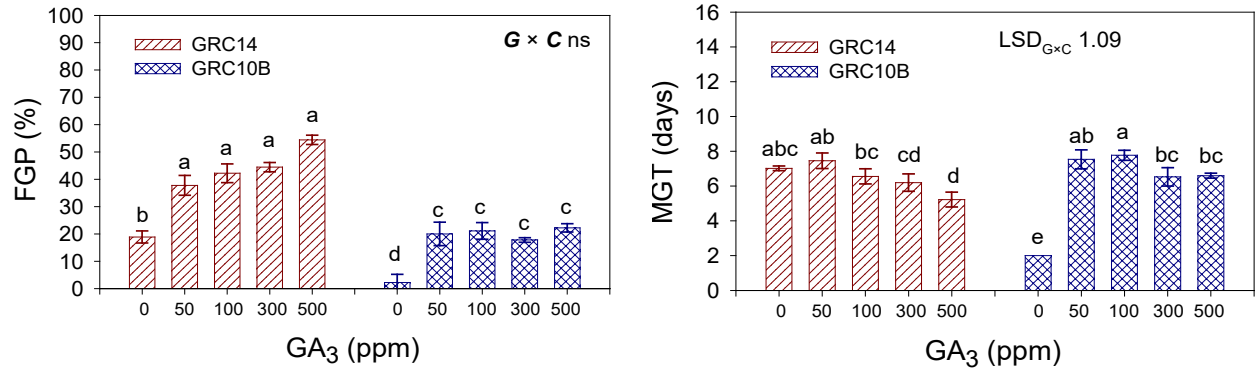

Figure 8. Interaction effect of 'genotype × GA_3 concentration' on final seed germination percentage (FGP, %) (on the left) and mean germination time (MGT, days) (on the right) in miscanthus 'GRC14' and 'GRC10B'. Data are means ± *se* ($n = 4$). Different letters indicate significance at $p < 0.05$ according to Tukey' test. Value for LSD ($p < 0.05$) is reported in the case of significant interaction.

The two hybrids did not differ in germination speed (G, $p > 0.05$). Instead, germination speed was strongly affected by GA concentrations (C, $p < 0.001$). As a result, the MGT was maximized at 300 ppm GA_3 (9.0 days) and minimized at 0 ppm GA_3 (distilled water). However, low MGT in distilled water is associated with the very low seed germination that occurred under these experimental conditions.

A significant interaction for 'G × C' on the MGT revealed how GA concentration differed in its effect, depending on genotype. Indeed, in 'GRC14' the time of germination was significantly reduced only at the highest concentration of the hormone (500 ppm), whilst in 'GRC10B', the MGT was minimized in the control, basically because under these experimental conditions, the germination was very poor (2.2%).

The germination index (GI) was affected by 'genotype' and 'GA_3 concentration', being significantly higher in 'GRC14' (G, $p < 0.001$) across the GA concentrations, and at the highest GA_3 concentration (500 ppm, $p < 0.001$) across hybrids. A significant 'G × C'

($p < 0.01$) interaction on this trait was highlighted during the ANOVA. In particular, 'GRC14' responded positively to increasing GA concentration, progressively raising its GI from 0.83 (in distilled water) to 3.9 (at 500 ppm GA_3) (Figure 9). Differently, the GI in 'GRC10B' did not change with GA_3 concentration.

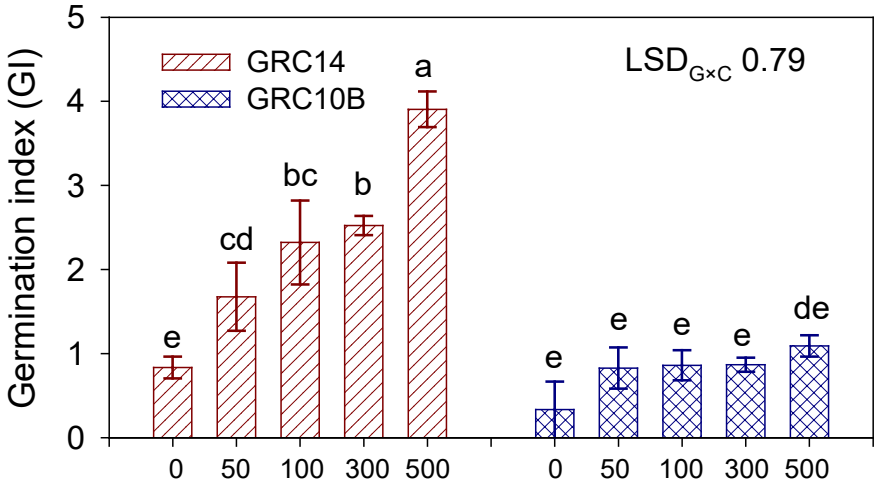

Figure 9. Interaction effect of 'genotype × GA_3 concentration' on the germination index (GI) in miscanthus 'GRC14' and 'GRC10B'. Data are means ± *se* ($n = 4$). Different letters indicate significance at $p < 0.05$ according to Tukey' test. Value for LSD ($p < 0.05$) is reported.

4. Discussion

In this study, the seed germination traits were studied in two new hybrids of *Miscanthus* spp., just after harvest and during a 1-year storage.

Spikelet fertility did not exceed 54% in both hybrids, and progressively decreased when moving from the apical to the basal part of the panicle. Overall, fertility was lower in 'GRC10B'. A percent seed set (our spikelet fertility) widely ranging from 84 to 0.7% was reported by Rounsaville et al. [1] in a pool of 23 genotypes, among diploids and triploids of *M. sinensis*, which indicated how this trait may greatly vary with genotype.

Seed weight was greater in the hybrid from maternal *M. sinensis* ('GRC10B', 100 seeds = approx. 60 mg). The mean seed weight measured in this hybrid was much smaller than that reported in the literature for several accessions of *M. sinensis* (100 seeds = 86 to 100 mg) [6]. In these accessions, however, seed germination did not change with seed size, overall approaching 100% after 6 months of storage at 4 °C. Similarly, a higher seed weight measured in 'GRC10B' than in 'GRC14' in our experiment did not involve better germination, both in terms of the final percentage and speed. Furthermore, in 'GRC10B', lower weight in seeds of the basal part of the panicle did not lead to poorer germination performances than those of the apical and central seeds. These results disagree with those of Dwianty et al. [21], who found that a delayed germination was associated with the low seed weight in *M. sinensis*.

When seeds of both hybrids of miscanthus were germinated in distilled water, poor germination occurred just after harvest (November), which suggests the potential occurrence of a physiological dormancy. Seed dormancy is an ecological strategy that controls the timing of germination in seeds, preventing their germination until climate conditions become favorable to seedling growth and establishment [22]. Indeed, prompt germination of freshly matured seeds may result in substantial losses in yield in agricultural productions [23].

Dormant seeds often require a period of cold or warm stratification after ripening to release their dormancy. Seeds in many plant species are unable to germinate at ripening [24,25]. However, in some cases, these seeds are not dormant but only require specific conditions (light, alternating temperatures) to germinate. In our study, full or near-full seed germination under dark conditions and at constant temperature, after a certain period

of seed storage (after-ripening), corroborates the hypothesis that a dormancy took place in the seeds of the two hybrids at maturity, which prevented a prompt germination just after panicle harvest. After-ripening is a physiological process by which dry seeds naturally lose dormancy with time [8]. It is reasonable that seed dormancy occurring in miscanthus could be regulated by the climatic course in its sub-tropical area of origin. Indeed, in eastern Asia, winter is the major dry season, thus in nature, in this area, a prompt seed germination in November could lead to the seedling experiencing unfavorable weather conditions (in terms of water availability) to its growth.

Hsu et al. [26] in their studies on the seed germination of miscanthus observed that seeds require no specific pretreatment to break dormancy, but if they are not chilled, germination starts to decline after 6 months when stored at room temperature. In our experiment, we found that seeds stored at room temperature retain a high germination capacity for a long time, and that their germination starts to decline only after approximately one year of storage. Seeds stored at a low temperature (8 °C) started to germinate well, later (approximately 6 months after harvest) than those kept at room temperature during storage, i.e., they seem to release dormancy more slowly. However, as these last, they lost part of their germination capacity at 12 months of storage. A genetic difference was also observed in dormancy release, with seeds of 'GRC14' being more likely to germinate approximately a couple of months earlier than those of 'GRC10B'.

The high levels of seed germination measured in this experiment (at 8 months of storage) also indicate that the weather conditions experienced by the plants during flowering/seed formation were favorable for producing mature seeds. Contrastingly, no seed germination was found by Jørgensen et al. [10] in some hybrids of miscanthus cultivated in Denmark, a probable adverse effect of the climatic conditions during late flowering in September on the formation of mature fertile seeds.

Base temperatures ranging from 9.6 to 11.6 °C were estimated for seed germination in *Miscanthus sinensis* [6,27]. In our experiment, high levels of germination were achieved after 6 months (May) in seeds stored at 8 °C, or earlier, in seeds stored at room temperature. Much higher temperatures (>15 °C) than those needed for the germination of miscanthus generally occur in Sicily between April–May (i.e., when seeds are likely to germinate) up to an altitude of 800 m [28]. Therefore, these thermal conditions would not limit seed germination in the two hybrids of miscanthus examined, either for seeds stored at room temperature or for those stored at 8 °C after harvest. Indeed, at that time, both groups of seeds had totally released their dormancy.

Various dormancy-breaking techniques are used in laboratories to promote germination [8,29]. Among them, gibberellic acid (GA_3) is effective in stimulating seed germination in dormant seeds [30]. In our experiment, gibberellic acid (GA_3) exerted the same stimulatory effect on the germination of freshly matured seeds, at all concentrations, although values of FGP higher than 50% were achieved only at the highest concentration (500 ppm) and in 'GRC14'. From these results we may speculate that a breaking of dormancy occurred in the seeds of miscanthus via the treatment with gibberellic acid. GA concentrations used in the present study were those reported in the literature in seed germination experiments conducted on different plants [30].

The effects of GA_3 at much lower concentrations (up to 150 ppm) on seed germination of *Miscanthus sinensis* were examined by Awty-Carroll et al. [31]. The authors reported that the GA treatment had the biggest effect on the germination percentage at the highest concentration, when seeds were germinated under high levels of water stress combined with low-light conditions. In this regard, the hormone would help seeds of miscanthus to germinate in marginal lands of low soil water availability. In turn, the same authors reported that the germination rate was promoted by GA_3 at its highest level (150 ppm), when seeds were germinated at low water stress combined with low-light conditions. Overall, the effect of GA was more relevant on germination speed than on the final percentage, supplying the promoting effects of light when this last is low. According to the authors, light is an optional booster for seed germination in miscanthus, but it does not stop it.

Indeed, in our experiment, germination occurred in full even under no light conditions. Similarly, Christian et al. [8], working on seed germination of *M. sinensis*, observed that the seeds of the varieties used in their experiment did not require light to germinate.

Very high GA_3 concentrations (up to 900 ppm) were also found to stimulate germination in some plants [25,32]. Therefore, we may speculate that higher GA concentrations than those used in our experiment would further promote germination in 'GRC14' and boost germination in 'GRC10B' as well.

The germination index (GI) is another index frequently considered in seed germination studies [19,33], which indicates the speed of germination (the higher the GI, the faster the seed germination). In the present experiment, a higher GI in the seeds indicates the greater speediness of this hybrid in germination. This result was also confirmed in the GA experiment. However, a remarkable decrease in the GI value in 'GRC14' (50%) with respect to 'GNT43B' (35%), extending the seed storage from 8 to 12 months, indicates the faster loss of the germination capacity in this hybrid.

5. Conclusions

The use of direct seeding in miscanthus propagation, at least in the two hybrids considered, is applicable in those areas where the climatic requirements are satisfied. However, this study provides evidence that a short-lasting physiological dormancy occurs in seeds of the two hybrids of miscanthus, and a low establishment rate may result from direct seeding, when fresh seed is used. In this case, the use of GA is a possible strategy to ameliorate the impact of dormancy on seed germination. In case of delayed sowings in late winter–early spring, prompt germination is expected, and seeds stored at room temperature (15–20 °C) after harvest may perform better than those stored at 8 °C.

However, the differing environmental conditions that seeds experience in fields determine complex interactions that may greatly affect the success of a seed-based crop. Therefore, further studies are required to establish the agronomic technique for miscanthus in the hope that it may lead to an adequate plant establishment when direct seed sowing is used.

Author Contributions: Conceptualization, C.P. and A.I.; methodology, C.P. and A.S.; software, C.P.; validation, A.I.; formal analysis, C.P. and A.S.; investigation, C.P. and A.S.; resources, S.L.C. and G.T.; data curation, C.P. and A.I.; writing—original draft preparation, C.P.; writing—review and editing, S.L.C. and A.I.; visualization, C.P. and G.T.; supervision, S.L.C.; project administration, S.L.C. and G.T.; funding acquisition, S.L.C. and G.T. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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