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Effects of Pre-Germinative Treatments and Temperatures on Tassel Hyacinth [*Muscari comosum* (L.) Mill.] Seeds

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Abstract: *Muscari comosum* (L.) Mill. is a spontaneous plant that grows in the whole Mediterranean area, including the Basilicata and Puglia regions (southern Italy), where it has received inclusion in the Italian National List for Traditional Agri-Food Product (TAP). The food and medicinal uses of bulb are ancient due to its antioxidant properties and high variety of nutrients, such as starch, sugars, and minerals. *Muscari* seed is characterized by morpho-physiological dormancy, and in order to achieve uniform germination, some pre-germinative treatments are needed. In this research, the effects of hydro-priming and osmo-priming, i.e., PEG 8000 and KNO_3, as well as three germination temperatures (4, 10, and 20 °C), have been evaluated. In general, the average results pointed out that the pre-treatments increased the germination index (GI) by 5% and the germination percentage (GP) by 3% compared to the no-primed control. The germination temperature of 10 °C significantly reduced the median germination time (T50) by 5.4 days and the mean germination time (MGT) by 5 days compared to temperature at 4 °C. In particular, the best results were obtained by "hydro-priming treatment × 10 °C" interaction, in terms of T50 (34.9 days) and MGT (36.3 days). This combination decreased the T50 by 10.5 days and the MGT by 9.6 days compared to the "control × 4 °C" interaction. Pearson's correlation matrix results highlighted a significant positive link between T50 and MGT (r = 0.993). In conclusion, these techniques enhanced the germination potential so that the use of pre-treated seeds could be included in a cultivation protocol to improve the germination phase and satisfy the growing demand for Italian bulbs.

Keywords: seed dormancy; hydro-priming; osmo-priming; polyethylene glycol; potassium nitrate; biodiversity; lampascione; wild edible species

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1. Introduction

Muscari comosum (L.) Mill. [*Tassel hyacinth*; syn. *Leopoldia comosa* (L.) Parl.] is a widespread herbaceous geophyte belonging to the *Liliaceae* family and distributed over south-western and central Europe, the Mediterranean area, and eastwards to Iran and Arabia [1].

The food and medicinal use of the bulb is ancient, and its excellent properties were known by the Egyptians, the Greeks, and all the other inhabitants of the Mediterranean area and Asia Minor [2,3]. In particular, in Turkey [4] and in Italian regions as Sardinia [5], Sicily [6], Basilicata [3,7], Puglia [8], Campania, Molise, and Calabria [9], the bulb is used in traditional gastronomy due to its characteristic strong bitter taste [2,10] and interesting biological activities such as anti-inflammatory, anti-cancer, anti-hyperglycemic, diuretic, aphrodisiac, and antioxidant effects [9–11]. Recently, some authors have reported the anti-obesity effects of raw bulbs due to their high total polyphenol and flavonoid content [9].

From a botanical point of view, *M. comosum* is characterized by globose-ovate bulbs wrapped in reddish-pinkish tunics, particular racemes with violet-blue sterile flowers above brownish-green fertile flowers, obovoid capsules, blackish and globose seeds, and

linear leaves [1,2,12]. Propagation can occur from seeds, bulbs, and bulblets, i.e., small bulbs that form laterally from the mother bulb during the growing season [12]. In Italy, it is commonly named "lampascione", and according to dialect, takes other local names such as "lambagione", "lampagione", "muscaro", "cipuddine", and "pampasciuli" [8]. It received inclusion in the Italian National List for Traditional Agri-Food Products (TAP) of the Italian Ministry of Agricultural, Food, and Forestry Policies for the Puglia and Basilicata regions, and is generally employed in preserves in oil and in various local recipes [13]. The processing methods of these products are practiced on the territory according traditional and antic rules, characteristics which attract the majority of consumers [14,15]. In the case of "lampascione", its harvesting occurs in uncultivated fields before the flowering phase, using a tool similar to a spade with a very narrow blade capable of going deep into the soil. At the beginning of the vegetation, a cut is made to leave a small green spike [1]. Unfortunately, today, the availability of Italian bulbs has been reduced due to the spread of agriculture and agronomic operations such as deep ploughing, and due to the uncontrolled harvesting [16]. Therefore, in order to satisfy the growing demand for bulbs and remedy the limited disposability of imported bulbs from northern Africa (particularly Tunisia and Morocco), seed sowing at high density in shallow containers was recommended for the first two years. Two-year-old bulbs, with a mean diameter of 1.5 cm, can be hand-transplanted and will reach the commercial dimensions (2.5–5 cm) in two years [17].

The epigeal germination of *M. comosum* occurs between November and December in southern Europe, and the range of temperature is 5–15 °C [1,12]. The germination is an important process which influences the yield and quality of crop vegetables [12,18]. Doussi and Thanos (2002) [12] investigated the ecophysiological aspects of seed germination of *M. comosum* and found that white light, simulating daylight, decreases the germination rate, and the optimal temperature for germination is 10 °C. Seeds of *M. comosum* are characterized by a morpho-physiological dormancy due to a hard coating with low water permeability. The long period of dormancy (7–8 months) and low germination rate represent a problem in *M. comosum* cultivation. Many studies on the positive effects of the pre-germinative treatments, such as decreasing the mean germination time of seeds, increasing germination uniformity, and increasing the tolerance of seeds to stress environmental (such as salt stress and thermal stress), have been investigated in different species [16,18,19].

In the case of *Muscari* spp., Labbaf et al. (2023) [20] studied how sulfuric acid and stratification treatments influence the germination of seeds. These authors indicated that only the combination of scarification treatment with sulfuric acid at 15 min and a 45-day stratification period allowed for the germination of *Muscari armeniacum*, and sulfuric acid treatment at 5 min with a 45-day stratification period seeds resulted in a rapid and uniform germination of *Muscari neglectum*. Kirmizi (2023) [21] studied the effects of moist chilling (3 to 12 months), the application of gibberellic acid (GA_3) (250 to 1000 mgL^{-1}) and four temperature regimes (20, 20–10, 25–15, and 15–10 °C) on the seed dormancy and germination of *Muscari bourgaei* Baker, an ornamental and endemic plant of Turkey. This author reported that the highest germination (90%) was obtained after 9 months of moist chilling treatment and with 1000 mg L^{-1} GA_3 application at 15–10 °C. This type of study provides important information for ex situ conservation protocols of rare plants and allows for the safeguarding of biodiversity as an attraction for winter sports and tourism.

As of today, the literature lacks scientific evidence on the effects of pre-germinative treatment, such as priming and its interaction with temperature germination, of *M. comosum* seeds. Priming is a technique which involves hydration and drying of the seeds with water (hydro-priming), with moistened solid carriers such as vermiculite or calcium silicate (matric-priming) or with osmo-priming agents [19]. The osmo-priming is the most common technique involving the hydration of seeds in aerated solutions with low osmolarity, such as potassium nitrate (KNO_3) and polyethylene glycol (PEG). This latter is the most used, and it does not damage the seed embryos [19,22].

The aim of the present study is to evaluate the effects of three pre-germinative treatments, hydro-priming and osmo-priming with KNO_3 or PEG 8000, three germination temperatures (20, 10, and 4 °C), and their interactions.

2. Materials and Methods

2.1. Experimental Description and Research Design

On *Muscari comosum* (L.) Mill. seeds, collected in June 2018 from spontaneous plants in the countryside of Metaponto in southern Italy (40°24′ N, 16°48′ E; 30 m a.s.l.), some germination tests were carried out from September to November 2019 at the Vegetable Crops and Floriculture laboratory of the University of Basilicata (Potenza, Italy). In particular, pre-germinative treatments, i.e., hydro-priming and osmo-priming, were applied. The hydro-priming was carried out only with distilled water, whereas potassium nitrate (KNO_3) (Fisher Scientific Italia s.r.l., Segrate, Milano, Italy) and polyethylene glycol 8000 (PEG 8000) (Fisher Scientific Italia s.r.l., Segrate, Milano, Italy) were used for the osmo-priming. Moreover, seeds not subjected to any treatment were considered as control.

On 23 September 2019, for each pre-germinative treatment plus the control, 1000 seeds were selected and weighed using a precision balance (TE214S, Sartorius, Goettingen, Germany). Afterwards, they were disinfected using a 10% solution of hydrogen peroxide for 10 min, and distilled water was used to remove the disinfectant solution [23]. Finally, seeds were subjected to different pre-germination treatments, which were carried out for 24 h at a temperature of 4 ± 0.5 °C and in dark conditions [24].

For each pre-germinative treatment, two glass jars, which had previously been sterilized in an autoclave, were filled with 20 mL of distilled water for the hydro-priming, 3% KNO_3 solution ($\Psi = -1.35$ MPa) for the potassium nitrate osmo-priming, and 32% PEG8000 solution ($\Psi = -1.51$ MPa) for the polyethylene glycol osmo-priming. Then, 500 seeds were placed inside each jar. The distilled water used for the KNO_3 and PEG 8000 osmo-priming treatments had previously been heated in an oven at a temperature of 40 °C in order to achieve a better dissolution of the used compounds. After the pre-germinative treatments, seeds were filtered in a previously sterilized sieve and then washed in distilled water to eliminate any impurities. Then, seeds were dried for 24 h at room temperature (20 °C).

On 25 September 2019, three replicates of 100 seeds from each pre-germinative treatment were placed on two discs of filter paper (90 mm diameter, Whatman filter No.1, GE Healthcare Life Sciences, Buckinghamshire, UK); afterwards, the discs were placed into acrylic plastic Petri dishes 8.5 cm in diameter. Seeds were moistened with 5 mL of distilled water, and the dishes were closed with parafilm. Then, Petri dishes were placed into three germination chambers (GDH-H 500, Falc Instruments s.r.l., Treviglio, Bergamo, Italy) set at 20 °C, 10 °C (*M. comosum* optimal germination temperature), and 4 °C, respectively, in the dark. The germinated seeds were checked daily starting from the 5th day of the germination period, which lasted 53 days, until at least the 90% of the seeds were germinated.

Therefore, a randomized block design with 12 treatments (3 types of pre-germination treatments × 3 germination temperatures plus a control, i.e., no pre-germination treatment × 3 germination temperatures), with three replicates consisting each one by a single Petri dish, was carried out for a total of 36 dishes.

As reported in Table 1, the experimental treatments are indicated as follows: S-A (hydro-priming pre-germinative treatment and germination temperature of 20 °C), S-O (hydro-priming pre-germinative treatment and germination temperature of 10 °C), S-F (hydro-priming pre-germinative treatment and germination temperature of 4 °C), K-A (KNO_3 osmo-priming pre-germinative treatment and germination temperature of 20 °C), K-O (KNO_3 osmo-priming pre-germinative treatment and germination temperature of 10 °C), K-F (KNO_3 osmo-priming pre-germinative treatment and germination temperature of 4 °C), P-A (PEG 8000 osmo-priming pre-germinative treatment and germination temperature of 20 °C), P-O (PEG 8000 osmo-priming pre-germinative treatment and germination temperature of 10 °C), P-F (PEG 8000 osmo-priming pre-germinative treatment and germination temperature of 4 °C), C-AA (no pre-germinative treatment and germination

temperature of 20 °C), C-O (no pre-germinative treatment and germination temperature of 10 °C), and P-F (no pre-germinative treatment and germination temperature of 4 °C).

Table 1. Trial experimental theses.

Experimental Theses	Pre-Germinative Treatments	Seeds Germination Temperature (°C)
S-A	Hydro-priming	20
S-O	Hydro-priming	10
S-F	Hydro-priming	4
K-A	KNO_3 osmo-priming	20
K-O	KNO_3 osmo-priming	10
K-F	KNO_3 osmo-priming	4
P-A	PEG 8000 osmo-priming	20
P-O	PEG 8000 osmo-priming	10
P-F	PEG 8000 osmo-priming	4
C-A	Control	20
C-O	Control	10
C-F	Control	4

2.2. Germination Indexes

In order to evaluate the effects of the different applied treatments on the seeds, the median germination time (T50), mean germination time (MGT), germination index (GI), synchrony of germination (Z), and percentage of germination (GP) were calculated.

In particular, the T50, which indicates the time required to achieve 50% of the final maximum germination [25], was calculated using the Coolbear et al. (1984) [26] formula, modified by Farooq et al. (2005) [27], as follows:

$$T50 = Ti + \frac{\left(\frac{N}{2} - Ni\right) * (Ti - Tj)}{Ni - Nj} \quad (1)$$

where N is the final number of germinated seeds and Ni and Nj are the numbers of germinated seeds at times Ti and Tj, with $Ni < \frac{N}{2} < Nj$.

The MGT, representing the average length of time until the maximum germination of the seeds [28], was calculated using the Ellis and Roberts formula (1981) [29]:

$$MGT = \frac{\Sigma(ni * di)}{N} \quad (2)$$

where ni is the number of seeds germinated on each day, di is the number of days from the beginning of the test, and N is the total number of seeds germinated at the termination of the experiment.

The GI, defined as a weighted sum of the daily numbers of germinated seeds and representing an index that describes the germination percentage/speed relationship [30], was calculated using the formula of the Association of Official Seed Analysts [31]:

$$GI = \frac{G1}{D1} + \frac{G2}{D2} + \ldots + \frac{Gn}{Dn} \quad (3)$$

where G1, G2, and Gn represent the number of seeds germinated each day, and D1, D2, and Dn indicate the day on which the seeds germinated, counting the days from sowing until the last counting day.

The synchrony of germination was calculated using the following formula:

$$Z = \frac{\Sigma\, C_{n_{i,2}}}{N} \quad (4)$$

where $Cn_{i,2} = \frac{n_i(n_i-1)}{2}$, $N = \frac{\Sigma n_i(\Sigma n_i-1)}{2}$, and n_i is the number of seeds germinated at time i. Z is a value that varies between 0 and 1, with Z = 1 if all seeds germinate at the same time and Z = 0 if at least two seeds germinate on the same day [26,28].

The germination percentage, representing the seed viability index, was calculated using the formula below:

$$GP = \frac{n}{N} \times 100 \quad (5)$$

where n is the number of germinated seeds, and N is the number of total seeds sown.

2.3. Statistical Analysis

The Shapiro–Wilk ($p \leq 0.05$) and Bartlett ($p \leq 0.05$) tests were applied to test the normality and homogeneity of variances, respectively. Then, the data were subjected to an analysis of variance (two-way ANOVA) according to the randomized block experimental design, with the typology of the pre-germinative treatment and the germination temperatures as sources of variation. Mean values were separated with the Student–Newman–Keuls (SNK) test at a significance level of $p \leq 0.05$.

Moreover, a correlation matrix, implemented using the Pearson correlation coefficient method, was utilized to evaluate the correlations between the pre-germinative treatments and the seeds' germination temperatures using the investigated germination parameters.

All statistical procedures were computed using the software RStudio: Integrated Development for R, version 2023.09.1 Build 494 [32].

3. Results

3.1. Effects of the Pre-Germinative Treatments on Seeds Germination

As shown in Table 2, the comparison of the studied pre-germinative treatments on the analysed seeds' germination parameters reported significant differences for all the investigated indices. In particular, the pre-germinative treatments yielded similar data, whilst the control always achieved the worst results (Table 2).

Table 2. Effects of the pre-germinative treatments on seeds' germination parameters.

Treatments [1]	T50 (Days)	MGT (Days)	GI (-)	Z (-)	GP (%)
Hydro-priming	38.57 b	39.33 b	14.27 a	0.116 a	99.25 a
KNO_3 osmo-priming	37.31 b	38.11 b	14.05 a	0.122 a	99.16 a
PEG 8000 osmo-priming	36.76 b	37.57 b	13.99 a	0.123 a	99.50 a
Control	44.85 a	44.36 a	9.26 b	0.106 b	95.92 b
Significance [2]	*	*	*	*	*

[1] Mean values followed by different letters are significantly different at $p \leq 0.05$, according to SNK test. [2] *, significance at $p \leq 0.05$.

3.2. Effects of the Germination Temperature on Seed Germination

Since no seed germination occurred at 20 °C, Table 3 reports data only for the germination temperatures of 10 and 4 °C (Table 3).

Table 3. Effects of the germination temperature on seeds' germination parameters.

Germination Temperature [1]	T50 (Days)	MGT (Days)	GI (-)	Z (-)	GP (%)
4 °C	42.33 a	42.85 a	12.83	0.130 a	98.25
10 °C	36.92 b	37.84 b	12.96	0.109 b	97.83
Significance [2]	*	*	n.s.	*	n.s.

[1] Mean values followed by different letters are significantly different at $p \leq 0.05$, according to SNK test. [2] n.s., no significant difference; *, significance at $p \leq 0.05$.

The germination temperature had effects with significant differences on T50, MGT, and Z; on the contrary, no significant differences were found for GI and GP (Table 3). In all

the parameters with differences, the germination temperature of 4 °C had higher values for the seeds' germination parameters (Table 3).

3.3. Interactive Effects of Pre-Germination Treatments and Seeds' Germination Temperatures

The interactive effects of the pre-germinative treatments and the seeds' germination temperature are reported in Figure 1. Statistically, significant differences were observed for T50 (Figure 1a), MGT (Figure 1b), and GI (Figure 1c), whereas Z (Figure 1d) and GP (Figure 1e) showed no significant differences. Concerning to T50, the best results were recorded by S-O, K-O, and P-O, with the shortest median time of germination, while the treatment C-F had the longest T50 time (Figure 1a). The MGT followed the T50 trend, with the S-O, K-O, and P-O treatments achieving the shortest length to maximum seed germination and the C-F resulting the worst one (Figure 1b). Lastly, the germination index (GI) reported higher values for S-O, K-O, and P-O, indicating that those treatments had high seed germination percentages and speeds (Figure 1c).

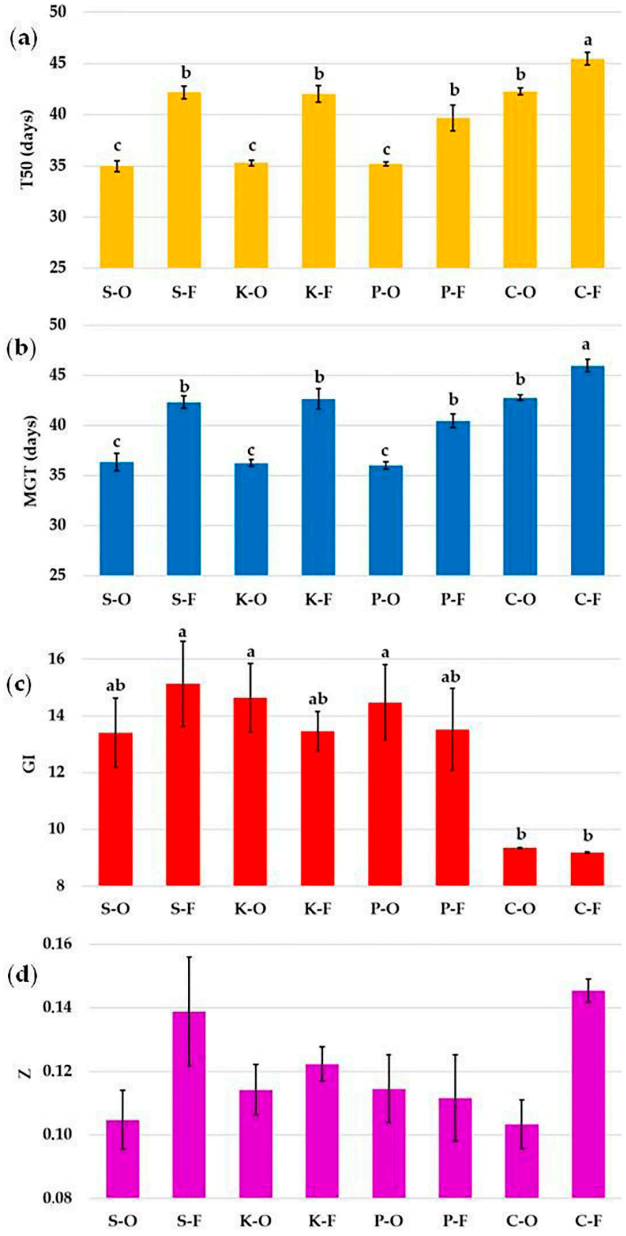

Figure 1. *Cont.*

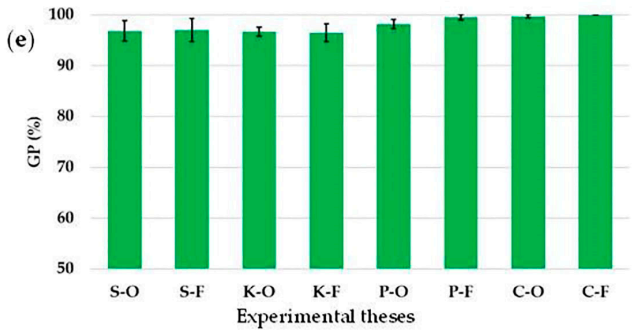

Figure 1. Interactive effects of pre-germination treatments and seeds' germination temperatures on (**a**) T50, (**b**) MGT, (**c**) GI, (**d**) Z, and (**e**) GP. Mean values followed by different letters are significantly different at $p \leq 0.05$, according to SNK test.

3.4. Correlation Matrix

As shown in Table 4, the correlation matrix, built using the Pearson correlation coefficient method, highlighted the correlations of the analyzed seeds' germination parameters. A high correlation was found between T50 and MGT, with a Pearson coefficient equal to 0.993 (Table 4).

Moreover, for a visual reading of the correlation matrix (Table 4), a correlogram was built, with positive correlations displayed in blue and negative correlations in red, and with the color intensity and the size of the circles proportional to the correlation coefficients (Figure 2).

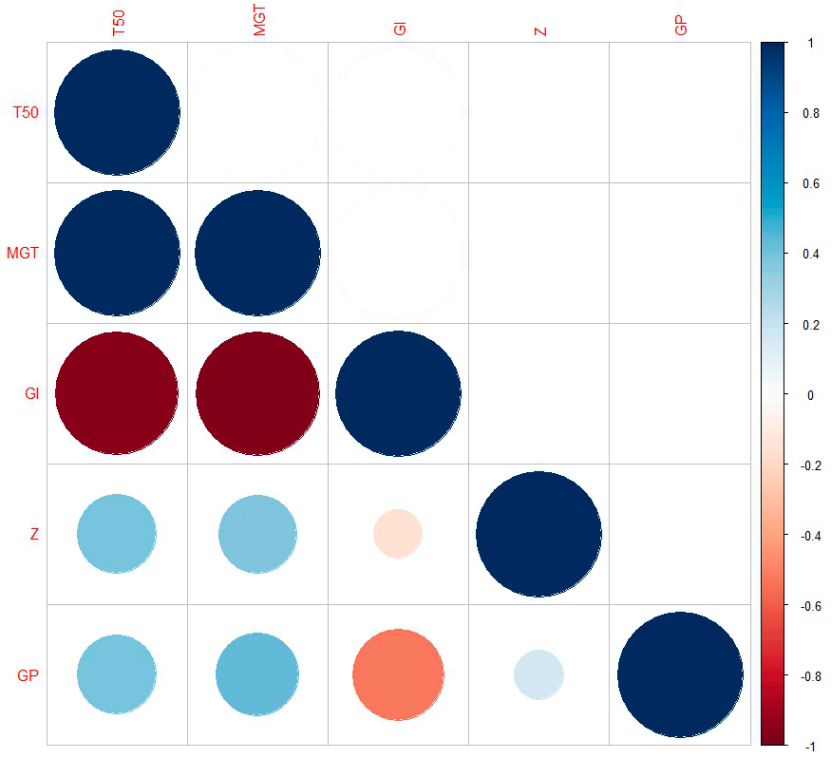

Figure 2. Correlogram of the studied germination parameters. Color intensity and the size of the circles are proportional to the correlation coefficients.

Table 4. Correlation matrix of the studied germination parameters.

	T50	MGT	GI	Z	GP
T50	1.000				
MGT	0.993	1.000			
GI	−0.385	−0.400	1.000		
Z	0.552	0.544	0.326	1.000	
GP	0.403	0.446	−0.002	0.463	1.000

4. Discussion

The process of seed germination is mainly influenced by environmental factors such as temperature and light [33], and can be distinguished into three principal phases: an initial phase (phase I), which involves water imbibitions by the seeds; phase II, which concerns the reactivation of metabolism (hydrolytic enzyme synthesis, respiration, translocation of nutrients and subcellular structures, and cell elongation); and phase III, characterized by the protrusion of radicles. The most critical phase is II, where the pre-germinative treatments act at a biochemical level [34,35].

In general, the species with the most difficult germination, such as the "lampascione", are subjected to pre-germination treatments, such as scarification, hydro-priming, osmo-priming, matrix-priming, and bio-priming, to obtain rapid and uniform germination [34–36].

In this study, the hydro-priming and osmo-priming treatments, as well as the effect of germination temperature on *M. comosum* seeds, were evaluated. The results reveal that the pre-germinative treatments significantly influenced all of the studied parameters, and the treated seeds presented more interesting values than the untreated ones (Table 2). In particular, these techniques increased the germination index, synchrony of germination, and germination percentage compared to the control (Table 2). On the other hand, these pre-treatments decreased the median germination time and mean germination time (Table 2).

The positive obtained effects of pre-germinative treatments are similar to those of Labbaf et al. (2023) [20], who, by investigating the effects of sulfuric acid and stratification priming on seed germination, found a decrease in germination time to 15 days using a 5 min sulfuric acid treatment plus a 45-day stratification treatment.

The favorable effects of hydro-priming and osmo-priming on *M. comosum* can be attributed to the improvement of the metabolic processes of the seeds and the physiological modifications, such as the efficiency of water use, the accumulation of osmolytes (proline, glycinbetaine, and polyamines), the biosynthesis of some hormones (gibberellins, abscisic acid, and ethylene), and the translocation of reserved food materials [18,24,34]. In addition, the effect of priming can be attributed to the early replication of DNA, an increase in RNA and protein synthesis, greater availability of ATP, and faster growth of the embryos [34].

The beneficial effects of priming have been observed in several field crops, such as tomato [26], wheat [34], maize [37], rice [22,38], sorghum [39], melon [24], alfalfa [35], carrot [40], and faba bean [41], but no studies on *M. comosum* have been reported in the literature. When studying the effect of seed priming on *Liliaceae* species, Selvarani and Umarani (2011) [40] reported an increase in the vigor of the seedlings, greater formation, and mobilization of reserves for the seedling growth of onion plants.

Interaction effect between pre-germinative treatments and temperature was found significant for all parameters, except for Z and GP (Figure 1). In particular, the best results were obtained using the "hydro-priming treatment × 10 °C" interaction in terms of T50 (34.9 days) and MGT (36.3 days). This combination decreased the T50 by 10.5 days and the MGT by 9.6 days compared to the "control × 4 °C" interaction (Figure 1).

The most important factor in the breaking of dormancy and germination is the temperature [21]. No seed germination was found at a temperature of 20 °C. This result is in agreement with Casoria et al. (1999) [42], who reported that germination does not occur above 15 °C. A germination temperature of 10° significantly reduced the median germination time, mean germination time, and synchrony of germination. In particular, the

temperature at 10 °C reduced the T50 and MGT by about 5 days compared to temperature of 4 °C (Table 3).

To provide a more thorough understanding of the plant's response to different environmental conditions, intermediary ranges of temperatures, such as between 4 and 9 °C and between 10 and 15 °C, will be evaluated in future research. Being that the seed germination responses to temperature are species-specific [43], future research could be carried out to study the effect of pre-treatments on different *Muscari* spp. widely present in Basilicata region, such as *Muscari atlanticum* Boiss. et Reuter and *Muscari botryoides* (L.) Mill., whose boiled or fried bulbs are used in traditional gastronomy [44].

The mean germination time, median germination time, and germination index were the best indices for the assessment of the germination performance compared to the synchrony of germination and final germination percentage, as reported in Figure 2.

Seed germination is a crucial process that influences crop yield and quality [45]. Further studies of the effects of seed pre-treatment on the growth, development, yield, and quality of "lampascione" bulbs will be conducted. It will be interesting to determine how these pre-treatments can help heighten to the profitability of "lampascione" cultivation by increasing the fresh weight and diameter of bulbs. On the other hand, germination pre-treatments could enhance seed quality through the accumulation of antioxidants (total ascorbate, dehydroascobate and catalase activity) responsible for the improvement of cell membrane integrity [46,47].

The study of molecular mechanisms underlying the seed dormancy and germination of *M. comosum* could be important for improvement of crop yield and quality. Further studies are necessary in order to identify these mechanisms of the regulation of the metabolic and signaling aspects of different plant hormones, mainly abscisic acid and gibberellins [45,48].

5. Conclusions

In recent years, and mainly during the COVID-19 pandemic, the consumption of local and traditional agri-food products has increased due to their high food safety and nutritional aspects. "Lampascione" bulbs are characterized by a long cultivation cycle, which could be considered sustainable if seeds are sown in containers for the first two years. This study suggests that the performance of *M. comosum* seed germination can be improved by employing seed priming techniques, such as hydro-priming and osmo-priming with PEG and KNO_3. These techniques—mostly hydro-priming—are cheap, eco-friendly, and easy to implement. Moreover, further studies are also needed because seed priming treatments can improve the plant defense system through increased activity of antioxidant enzymes (superoxide dismutase, catalase, and glutathione reductase), allowing the plant to overcome abiotic stresses (high temperature and drought) and adapt better to climate change. Furthermore, the aforementioned seed treatments may be useful for reducing the vegetative cycle of *M. comosum* in order to obtain marketable bulbs earlier. In conclusion, this study provides practical information for farmers regarding improving the seed germination and the subsequent field performance of "lampascione".

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