

Germination analysis of tassel hyacinth [*Muscari comosum* (L.) Mill.] seeds: first results

D. Castronuovo^{1,a}, L. Cardone¹, M.M. Pandolfo¹ and V. Candido²

¹School of Agriculture, Forest, Food and Environmental Sciences, University of Basilicata, Viale dell'Ateneo Lucano 20, 85100 Potenza, Italy; ²Department of European and Mediterranean Cultures, University of Basilicata, Via Lanera 10, 75100 Matera, Italy.

Abstract

Tassel hyacinth [*Muscari comosum* (L.) Mill.], a species belonging to the *Liliaceae* family, is a widespread spontaneous plant typical of the Mediterranean area, which bulbs are used for food, especially in Southern Italy where they represent a traditional meal. Tassel hyacinth can be propagated both by bulbs and by seeds but, since bulb growth is very slow, gametic propagation modality seems to be increased to speed up the production of bulbs. Unfortunately, *Muscari comosum* seeds are not easy to germinate and often pre-germinative treatments are needed. In this research, tassel hyacinth seeds were subjected to different pre-germinative treatments to estimate differences in times, speeds, and percentages of germination. Namely, soaking and osmo-priming pre-germinative treatments, both conducted at two different temperatures that is 4 and 20°C, were investigated. Seeds were immersed in distilled water for the soaking treatment whereas in the osmo-priming, to vary the osmotic pressure of the solution, polyethylene glycol (PEG) and potassium nitrate (KNO₃) were used. Moreover, to determinate the effect of the pre-germinative treatments, germination was conducted at three different temperatures: 5, 10 and 20°C. Results pointed out that, regarding the osmo-priming treatments, the use of PEG has given the best results, in terms of T50 (seeds 50% germination time), MGT (Mean Germination Time), compared to soaking and KNO₃. In addition, also the percentage of germination was enhanced by the application of the PEG exceeding 98%. Regarding the T50 of the seeds, the best results were obtained with the soaking treatments. Germination was also influenced by the temperature, resulting that seed treated at 4°C and germinating at 10°C (that is as known the best germinating temperature for tassel hyacinth) have reached the best results; on the other hand, the germination for the seeds kept at 20°C was quite completely inhibited.

Keywords: priming, osmo-priming, germination, temperature

INTRODUCTION

Tassel hyacinth [*Muscari comosum* (L.) Mill.] is a perennial bulbous plant belonging to the *Liliaceae* family. The species grows from sea level up to 2,000 m a.s.l., and its distribution area extends from Northern Africa to South-Western and Central Europe including Iran and Arabia (Candido et al., 2017). This species was known since ancient times; in fact, all the people who lived in the Mediterranean basin such as Egyptians, Greeks and Romans used it for both medicinal and food purposes (Casoria et al., 1999). In Italy tassel hyacinth is known as “lampascione”, and especially in the Southern Italian regions, such as Puglia, Basilicata, Campania, and Sicily, it is used in various traditional recipes (Candido et al., 2012).

M. comosum adapts to different types of soil, but prefers light, and well-aerated ones with a good percentage of organic matter (Matteo, 2010). The reproductive organs are both the bulbils that are mainly formed at the base of the bulb and the seeds. Each flower stem is capable of producing up to 300 seeds. Therefore, the propagation of the plant can take place both by agamic and gametic way.

Before planting the bulbs, the soil must be worked with deep plowing capable of

^aE-mail: donato.castronuovo@unibas.it



oxygenating even the deeper layers of soil (Matteo, 2010). The bulbs are planted in autumn when they are in vegetative rest and are placed at a depth of 10 cm, and spaced by 30 and 10 cm, respectively between the rows and in the rows, to obtain a density of 33-34 plants m⁻². As far as sowing is concerned, this is done before autumn, since the seed needs an optimal soil temperature of 10°C to germinate, a condition reached in late autumn-early winter in Southern Italy (Candido et al., 2017).

The remarkable nutritional, therapeutic and organoleptic properties make the “lampascione” a species to be protected and rediscovered. The great rusticity makes the plant easy to cultivate and its rediscovery could lead to the birth of new markets and the revaluation of marginal or underdeveloped areas; in fact, the enhancement of the *M. comosum* is part of the operational and planning strategies to be adopted for the redevelopment and enhancement of the internal areas of the Mediterranean countries.

In Italy bulbs for the food market are commonly imported mainly from Morocco, Tunisia, and Egypt. In these countries, to supply the fresh market and processing industry, bulbs are still collected from the wild. To reach a suitable tassel hyacinth cultivation in Southern Italy there is a need to develop an appropriate propagation method, since bulb growth is very slow as it normally takes 3-4 years for bulbs to reach a marketable size (Maiellaro and Macchia, 1996).

This study deals with some results of a research conducted on the germination capacity of *M. comosum* seeds as propagation material to be used as a valid alternative to bulbs cultivation. Therefore, to evaluate some differences in time, speed, and percentage of germination three pre-germinative methods, namely soaking, cold priming and osmo-priming, were applied to tassel hyacinth seeds.

MATERIALS AND METHODS

The research activity was carried out from September 2019 to January 2020 at the Vegetable crops and Floriculture laboratory of the University of Basilicata (Potenza, Italy) using the seeds of *M. comosum* collected from spontaneous plants in the countryside of Metaponto in Southern Italy (40°24'N, 16°48'E; 10 m a.s.l.) in June 2018 and then stored in glass jars in the dark.

In particular, the seeds were subjected to two pre-germinative treatments, soaking and osmo-priming. The soaking was carried out only with distilled water and was taken as control treatment while two different compounds namely potassium nitrate (KNO₃) and polyethylene glycol 8000 (PEG 8000) were used for osmo-priming. The seeds were treated for 3 days at two different temperatures, i.e. in a cold room at 4°C and at room temperature (20°C).

Before starting the experiment, 1,000 seeds were selected for each experimental treatment and weighed using a precision balance with 4 decimal places (TE214S, Sartorius, Germany), the values of which are shown in Table 1.

For the soaking treatments two glass jars, previously sterilized in an autoclave, were filled with 20 mL of distilled water each before placing the seeds inside them. The potassium nitrate osmo-priming treatment was made by using a 3% solution of KNO₃ (Π=14.3 atm), and filling two glass jars of 20 mL of solution each prior to inserting seeds in them. The PEG 8000 osmo-priming treatment was carried out preparing a 32% solution of polyethylene glycol (PEG 8000; Π=22.2 atm) (Stanley and Strey, 2003) to fill two glass jars of 20 mL solution each before putting the seeds in them. The water was previously heated in an oven at a temperature of 40°C for 30 min, to have a better dissolution of the used compounds. Moreover, before placing the jars in the cold room and at room temperature, a ventilation system by using air pumps was placed inside each jar to supply fresh air to avoid asphyxiation.

After the treatment, seeds were filtered in a previously sterilized sieve and then washed in tap water to eliminate any impurities. After this, seeds were dried for 24 h at room temperature (20°C) to restore the initial seeds moisture content (9.5%). On 24/09/2019 each treatment was divided into nine Petri dishes of 100 seeds each to be germinated at three different temperatures.

Table 1. Weight of 1,000 seeds before the pre-germinative treatments.

Treatments	Temperature of the treatment (°C)	Weight of 1,000 seeds (g)
Soaking	20	7.3659±0.321
	4	7.2011±0.411
KNO ₃	20	7.0336±0.501
	4	7.1735±0.347
PEG 8000	20	7.1559±0.380
	4	7.1646±0.521

Thereafter, the pre-treated seeds were germinated in 3 different environments: 1) climatic cell at 10°C (*M. comosum* optimal germination temperature); 2) cold room at 4°C; 3) room temperature at 20°C. Portable probes (Cox Tracer, Sandved International ApS, Denmark) with data acquisition every 30 min were used to monitor the temperatures in the pre-germination and seed germination environments. Therefore, as reported in Table 2, the experiment involved a comparison of 18 experimental theses (3 types of pre-germination × 2 pre-germination environments × 3 germination environments). In addition, 3 replicates per theses were carried out for a total of 54 plots (Petri dishes).

Table 2. Treatments.

Treatments ^a	Pre-germinative treatments	Pre-germinative treatments temperature (°C)	Seeds germination temperature (°C)
Soaking AA	Soaking	20	20
Soaking AO	Soaking	20	10
Soaking AF	Soaking	20	4
Soaking FA	Soaking	4	20
Soaking FO	Soaking	4	10
Soaking FF	Soaking	4	4
KNO ₃ osmo-priming AA	KNO ₃ osmo-priming	20	20
KNO ₃ osmo-priming AO	KNO ₃ osmo-priming	20	10
KNO ₃ osmo-priming AF	KNO ₃ osmo-priming	20	4
KNO ₃ osmo-priming FA	KNO ₃ osmo-priming	4	20
KNO ₃ osmo-priming FO	KNO ₃ osmo-priming	4	10
KNO ₃ osmo-priming FF	KNO ₃ osmo-priming	4	4
PEG 8000 osmo-priming AA	PEG 8000 osmo-priming	20	20
PEG 8000 osmo-priming AO	PEG 8000 osmo-priming	20	10
PEG 8000 osmo-priming AF	PEG 8000 osmo-priming	20	4
PEG 8000 osmo-priming FA	PEG 8000 osmo-priming	4	20
PEG 8000 osmo-priming FO	PEG 8000 osmo-priming	4	10
PEG 8000 osmo-priming FF	PEG 8000 osmo-priming	4	4

^a“AA” indicates a pre-treatment temperature and a germination temperature both of 20°C; “AO” indicates a pre-treatment temperature of 20°C and a germination temperature of 10°C; “AF” indicates a pre-treatment temperature of 20°C and a germination temperature of 4°C; “FA” indicates a pre-treatment temperature of 4°C and a germination temperature of 20°C; “FO” indicates a pre-treatment temperature of 4°C and a germination temperature of 10°C; “FF” indicates a pre-treatment temperature and a germination temperature both of 4°C.

For seeds germination, 8.5 cm diameter Petri dishes were used inside which two discs of filter paper were placed. The seeds were moistened with 5 mL of distilled water and the plates closed with parafilm. Three Petri dishes for each thesis were placed in a cold room at 4°C, another three were placed in a cold room at 10°C and the last three dishes were positioned at room temperature of 20°C. The first week the plates were checked every two days and water was added in case of desiccation of the bibulous paper. During the experiment seeds were considered germinated when the hypocotyl reached a length equal or greater than

1 mm. At each take-over the germinated seeds were selected and removed from the Petri dishes.

To evaluate the effect on seeds of the different applied treatments, T50 (median germination time), MGT (mean germination time), GI (germination index), Z (synchrony of germination), and percentage of germination were calculated.

In particular, the T50 was calculated using the Coolbear et al. (1984) formula modified by Farooq et al. (2005) as follows:

$$T50 = T_i + \frac{\left(\frac{N}{2} - N_i\right) * (T_i - T_j)}{N_i - N_j} \quad (1)$$

where N is the final number of germinated seeds, N_i and N_j are the numbers of germinated seeds at time T_i and T_j , with $N_i < \frac{N}{2} < N_j$.

The mean germination time represents the value of the germination energy and the germinability of the seeds. It was calculated using the Ellis and Roberts (1981) formula:

$$MGT = \frac{\sum (n_i * d_i)}{N} \quad (2)$$

where n_i is the number of seeds germinated on each day, d_i 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 germination index (GI) was calculated using the formula of the Association of Official Seed Analysts (AOSA, 1983):

$$GI = \frac{G_1}{D_1} + \frac{G_2}{D_2} + \dots + \frac{G_n}{D_n} \quad (3)$$

where G_1, G_2, G_n represent the number of seeds germinated each day, and D_1, D_2, D_n 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:

$$Z = \frac{\sum C_{n_i,2}}{C \sum C_{n_i,2}} \quad (4)$$

where n_i is the number of seeds germinated at time i , C_{n_i} is the combination of seeds germinated at time i , for two seeds germinated at the same time. Z is a value that varies between 0 and 1, with $Z=1$ if all seeds germinate at the same time, $Z=0$ if at least two seeds germinate on the same day (Ranal and Santana, 2006).

The germination percentage was calculated using the formula below:

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

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

Obtained data were subjected to the analysis of variance (ANOVA) by separating the statistically different means with the Least Significant Difference (LSD) test with $P \leq 0.05$. The open-source software R was used for data processing.

RESULTS AND DISCUSSION

Effects of the pre-germinative treatments

Observing the T50 values, the treatment that reported the best result was achieved by the PEG8000 and the worst one (higher T50) by using the KNO_3 (Table 3). Synchrony of germination (Z), mean time (MGT) and germination index (GI) did not show significant differences between treatments (Table 3). The germination percentages (GP) were higher

with the osmo-priming PEG 8000, equal to 98.5%, while the seeds pre-treated with soaking and KNO₃ osmo-priming germinated with an average percentage of 96% (Table 3).

Table 3. Effects of pre-germinative treatments on seeds' germination parameters.

Treatments	Seeds germination parameters ^a				
	T50	Z	MGT	GP	GI
Soaking	38.9 ab	0.128 a	39.9 a	96.1 b	14.5 a
KNO ₃ osmo-priming	39.7 a	0.123 a	39.9 a	96.9 ab	14.4 a
PEG 8000 osmo-priming	38.1 b	0.119 a	39.9 a	98.5 a	14.7 a
Significance ^b	*	n.s.	n.s.	*	n.s.
Pre-germinative treatments temperature	T50	Z	MGT	GP	GI
Cold room at 4°C	38.2 a	0.120 a	39.0 b	96.8 a	14.2 a
Room temperature at 20°C	39.6 a	0.126 a	40.3 a	97.5 a	14.9 a
Significance ^b	n.s.	n.s.	*	n.s.	n.s.
Seeds germination temperature	T50	Z	MGT	GP	GI
Cold room at 4°C	41.1 a	0.119 a	41.0 a	97.2 b	13.6 a
Cold room at 10°C	35.4 b	0.107 a	36.9 b	99.3 a	14.2 a
Room temperature at 20°C ^c	—	—	—	—	—
Significance ^b	*	n.s.	*	*	n.s.

^aValues in the columns not having any letters in common are significantly different at 0.05P.

^bSignificance at 0.05P, n.s. = no significant differences.

^cNo seeds germination occurred at room temperature of 20°C.

Effects of the pre-germinative treatments temperatures

The temperatures of the treatments did not affect the germination parameters (Table 3). Only the MGT showed a value one day longer by using the pre-germination treatments at room temperature (Table 3).

Effects of the germination temperatures

At the optimal temperature (10°C) 35.4 days were needed to germinate 50% of the seeds while at 4°C it took almost 6 days more (Table 3). The germination synchrony showed no difference between the two temperatures (Table 3). The MGT of the seeds placed to germinate at 10°C was equal to 36.9 days; on the other hand, at 4°C the TMG was of 41.0 days (Table 3). The percentage of germination was always above 95% with a slightly better result at 10°C respect to the lower temperature (Table 3). There was no seeds germination at room temperature of 20°C (Table 3).

Interactive effects of pre-germinative treatments and pre-germinative temperatures on seeds germination parameters

The temperature conditions which the seeds undergone before the germination had significant effects on the T50 of all three treatments (Table 4). The synchrony of germination (Z) resulted lower by using PEG 8000 pre-germination treatment at 4°C (Table 4). The best MGT value was reached by the PEG 8000 at 4°C compared to the other treatments (Table 4). Also, for the germination index (GI), the PEG 8000 osmo-priming treatment, both at 4°C and at room temperature, showed the best results (Table 4). Finally, the germination percentages in all tests exceeded 95% with a peak of 98.8% for the PEG 8000 osmo-priming treatment at 4°C (Table 4). On the contrary, the lowest percentage was found in Soaking and Osmopriming KNO₃ treatments at 4°C.

Interactive effects of pre-germinative treatments and germinative temperatures on seeds germination parameters

As shown in Table 5, for each pre-germinative treatment the T50 presents significant

differences between the two seed germination temperatures. In fact, for each treatment germinated at 10°C, the T50 tends to reduce by more than 4 days compared to the equivalent placed to germinate at 4°C. Among the three treatments placed at optimal temperature of 10°C, the PEG 8000 osmopriming reported the best result, with a T50 of 35.6 days (Table 5). A very similar trend was also observed for the MGT. The synchrony of germination has recorded similar values for all the treatments studied, except for the PEG 8000 osmopriming at 4°C which reported a lower value than the ones obtained for the seeds germinated at 4°C and subjected to the other two pre-treatments (Table 5). The percentage of germination recorded significantly higher values when the osmotic treatments made by PEG 8000 and KNO₃ were applied at 4°C. On the contrary, the lowest percentages were recorded with seeds pre-treated with soaking and KNO₃ were placed to germinate at 10°C (Table 5). The GI was lower with the KNO₃ and PEG 8000 osmopriming treatments and with the lowest germination temperature (Table 5).

Table 4. Effects of pre-germinative treatments and pre-germinative temperatures on seeds germination.

Seeds germination parameters ^a					
Pre-germinative treatments × pre-germinative temperature	T50	Z	MGT	GP	GI
Soaking at 20°C	39.5 ab	0.128 a	40.5 a	96.7 ab	14.6 ab
Soaking at 4°C	38.3 b	0.127 a	39.2 ab	95.5 b	14.5 ab
KNO ₃ osmo-priming at 20°C	41.1 a	0.122 a	40.8 a	97.8 ab	14.4 ab
KNO ₃ osmo-priming at 4°C	38.3 b	0.124 a	39.1 ab	96.0 b	14.5 ab
PEG 8000 osmo-priming at 20°C	38.3 b	0.128 a	39.4 ab	98.2 ab	15.5 a
PEG 8000 osmo-priming at 4°C	37.9 b	0.110 b	38.8 b	98.8 a	13.8 b
Significance ^b	*	*	*	*	*

^aValues in the columns not having any letters in common are significantly different at 0.05P.

^bSignificance at 0.05P.

Table 5. Effects of pre-germinative treatments and germination temperature on seeds germination.

Seeds germination parameters ^a					
Pre-germinative treatments × germination temperature	T50	Z	MGT	GP	GI
Soaking × 10°C	36.1 b	0.125 a	37.6 b	95.2 b	14.8 a
Soaking × 4°C	41.7 a	0.130 a	42.2 a	97.0 ab	14.2 a
KNO ₃ osmopriming × 10°C	36.5 b	0.123 ab	35.9 b	95.3 b	15.1 a
KNO ₃ osmopriming × 4°C	42.9 a	0.124 a	43.9 a	98.5 a	13.8 b
PEG 8000 osmopriming × 10°C	35.6 b	0.122 ab	36.5 b	97.7 ab	15.6 a
PEG 8000 osmopriming × 4°C	40.6 a	0.116 b	41.6 a	99.3 a	13.7 b
Significance ^b	*	*	*	*	*

^aValues in the columns not having any letters in common are significantly different at 0.05P.

^bSignificance at 0.05P.

Interactive effects of pre-germinative treatments, temperatures, and germination environments on seeds germination parameters

Seeds placed to germinate at a temperature of 4°C reported significantly higher values of T50 compared to the ones germinated at 10°C. The best result was reached by the “Soaking FO” treatment (Table 6). The lowest MGT was achieved by seeds germinated at 10°C, having values between 34.8 and 38.2 days. The lowest value was reached by the “PEG 8000 osmopriming FO” treatment (Table 6). The highest Z value was recorded with the “Soaking FF”

treatment, while lower ones were obtained with the “Soaking FO”, “Soaking AO”, “PEG 8000 osmo-priming AF”, “PEG 8000 osmo-priming AO”, and “KNO₃ osmo-priming FF” treatments. In average, germination rate almost exceeded 95% just seeds of the “KNO₃ osmo-priming FF” treatment reached the lowest value equal to 94.3% (Table 6). The germination index values were higher for the theses “Soaking AF”, “Soaking FF”, “KNO₃ osmo-priming AO”, and “KNO₃ osmo-priming FO”. On the other hand, lower values were recorded by “KNO₃ osmo-priming AF” and “KNO₃ osmo-priming FF” (Table 6).

Table 6. Effects of pre-germinative treatments, temperatures, and germination environments on seeds germination.

Treatments ^b	Seeds germination parameters ^a				
	T50	Z	MGT	GP	GI
Soaking AF	41.4 a	0.127 ab	40.8 ab	99.3 a	15.3 a
Soaking AO	36.2 c	0.096 c	37.9 de	100.0 a	12.9 ab
Soaking FF	42.9 a	0.143 a	42.8 a	97.0 b	15.5 a
Soaking FO	34.8 c	0.091 c	36.1 f	99.7 a	12.6 ab
KNO ₃ osmo-priming AF	42.2 a	0.110 bc	41.4 ab	95.0 bc	11.7 c
KNO ₃ osmo-priming AO	35.2 c	0.118 ab	36.6 f	98.0 ab	15.8 a
KNO ₃ osmo-priming FF	42.8 a	0.108 c	42.9 a	94.3 c	11.2 c
KNO ₃ osmo-priming FO	35.3 c	0.116 ab	36.6 f	100.0 a	16.1 a
PEG 8000 osmo-priming AF	38.4 b	0.109 c	38.6 cd	98.3 ab	13.4 bc
PEG 8000 osmo-priming AO	36.0 c	0.104 c	38.2 d	99.3 a	13.8 bc
PEG 8000 osmo-priming FF	38.8 b	0.118 bc	39.6 bc	99.0 a	14.2 ab
PEG 8000 osmo-priming FO	35.0 c	0.115 bc	34.8 g	98.7 ab	14.2 ab
Significance ^c	*	*	*	*	*

^aValues in the columns not having any letters in common are significantly different at 0.05P.

^b“AF” indicates a pre-treatment temperature of 20°C and a germination temperature of 4°C; “AO” indicates a pre-treatment temperature of 20°C and a germination temperature of 10°C; “FF” indicates a pre-treatment temperature and a germination temperature both of 4°C; “FO” indicates a pre-treatment temperature of 4°C and a germination temperature of 10°C.

^cSignificance at 0.05P.

In this study, to evaluate the germination capabilities of tassel hyacinth seeds three different temperatures, 20, 10, and 4°C, were applied during the germination process. Moreover, all tested seeds undergone three pre-germinative treatments (soaking, KNO₃ and PEG 8000 osmo-priming) at two different temperatures (20 and 4°C). According to Doussi and Thanos (2002), who reported that *M. comosum* seeds can germinate in a temperature range between 5 and 15°C with an optimal value of 10°C, the seeds pre-treated at 4°C and placed to germinate at 10°C have produced the best results for all the considered germination parameters, compared to seeds treated at 4 and 20°C and germinated both at room temperature (20°C) and at sub-optimal temperature (4°C). Therefore, this study has confirmed that *M. comosum* benefits from lower pre-germinative temperature compared to that applied at room temperature.

As regards the seeds placed to germinate at room temperature, it has been noted that values above 15°C completely inhibit germination. The germination at 4°C, on the other hand, still allowed the seeds to germinate, although with an elongation of the T50 from 2 to 7 days compared to germination which took place at 10°C.

As for the pre-termination treatments, the use of PEG 8000 as an osmotic agent has given the best results, in terms of T50 and MGT, compared to soaking and KNO₃ osmo-priming. In addition, the percentage of germination was also enhanced by the application of the PEG 8000 exceeding 98% in both tests. As regards the germination time of 50% of the seeds, the best results were obtained with the “Soaking FO” thesis which reported the lowest values. These results can be explained by a probable positive effect of pre-treatments on the first stage of germination that made pre-treated seeds readier to germinate (Dastanpoor et al.,

2013; Ghiyasi et al., 2008; Varier et al., 2010).

CONCLUSIONS

According to the results carried out in this study, it can be said that the gametic propagation of the “lampascione” can be simply achieved thanks to the good germination capacity of the seeds and even thanks to their easy availability by the spontaneous plants of the Mediterranean area. Moreover, pre-germinative treatments can be usefully applied to improve the germination capability of seeds and so quicken the earliness of germination and the emergence of the seedlings, and therefore to shorten the phase of bulbs enlargement. The latter can be considered one of the most important steps of the *M. comosum* cultivation cycle, since bulbs reach the right dimensions and characteristics to be marketed only after 3-4 years from sowing.

ACKNOWLEDGEMENTS

Author Contributions: Conceptualization D.C. and V.C.; Methodology D.C., V.C., and M.M.P.; Statistics analysis D.C. and L.C.; Validation D.C., V.C., L.C., and M.M.P.

Literature cited

AOSA (1983). Seed Vigor Testing Handbook. Contribution No. 32 to the Handbook on Seed Testing (Association of Official Seed Analysis).

Candido, V., Castronuovo, D., Matteo, D., Fascetti, S., and Miccolis, V. (2012). Seed-propagated Tassel hyacinth: effects of sites and sowing dates. Paper presented at: European Botanic Gardens in a Changing World: Insights into EUROGARD VI.

Candido, V., Castronuovo, D., Fascetti, S., Rosati, L., and Potenza, G. (2017). Seed-propagated *Muscari comosum* (L.) Mill.: effects of sowing date and growing conditions. *Plant Biosyst.* 151 (3), 484–492 <https://doi.org/10.1080/11263504.2016.1194337>.

Casoria, P., Menale, B., Muoio, R., and Orto, B. (1999). *Muscari Comosum*, Liliaceae, in the food habits of South Italy. *Econ. Bot.* 53 (1), 113–115.

Coolbear, P., Francis, A., and Grierson, D. (1984). The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. *J. Exp. Bot.* 35 (11), 1609–1617 <https://doi.org/10.1093/jxb/35.11.1609>.

Dastanpoor, N., Fahimi, H., Shariati, M., Davazdahemami, S., and Hashemi, S.M.M. (2013). Effects of hydropriming on seed germination and seedling growth in sage (*Salvia officinalis* L.). *Afr. J. Biotechnol.* 12 (11), 1223–1228.

Doussi, M.A., and Thanos, C.A. (2002). Ecophysiology of seed germination in Mediterranean geophytes. 1. *Muscari* spp. *Seed Sci. Res.* 12 (3), 193–201 <https://doi.org/10.1079/SSR2002111>.

Ellis, R.A., and Roberts, E.H. (1981). The quantification of aging and survival in orthodox seeds. *Seed Sci. Technol.* 9, 373–409.

Farooq, M., Basra, S.M.A., Ahmad, N., and Hafeez, K. (2005). Thermal hardening: a new seed vigour enhancement tool in rice. *J. Integr. Plant Biol.* 47 (2), 187–193 <https://doi.org/10.1111/j.1744-7909.2005.00031.x>.

Ghiyasi, M., Seyahjani, A.A., Tajbakhsh, M., Amirnia, R., and Salehzadeh, H. (2008). Effect of osmopriming with polyethylene glycol (8000) on germination and seedling growth of wheat (*Triticum aestivum* L.) seeds under salt stress. *Res. J. Biol. Sci.* 3, 1249–1251.

Maiellaro, F., and Macchia, F. (1996). Germinazione ed ontogenesi della plantula di *Leopoldia comosa* (L.) Parl. *G. Bot. Ital.* 130 (1), 497 <https://doi.org/10.1080/11263509609439714>.

Matteo, D. (2010). The propagation of *Muscari comosum*. MSC degree in Agricultural Sciences Academic Year 2008–2009 (University of Basilicata).

Ranal, M.A., and Santana, D.G. (2006). How and why to measure the germination process? *Rev. Bras. Bot.* 29 (1), 1–11 <https://doi.org/10.1590/S0100-84042006000100002>.

Stanley, C.B., and Strey, H.H. (2003). Measuring osmotic pressure of poly(ethylene glycol) solutions by sedimentation equilibrium ultracentrifugation. *Macromolecules* 36 (18), 6888–6893 <https://doi.org/10.1021/ma034079e>.

Varier, A., Vari, A.K., and Dadlani, M. (2010). The subcellular basis of seed priming. *Curr. Sci.* 99 (4), 450–456.