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Original Research Article

Effects of Disinfection Conditions and Culture Media on *in vitro* Germination of Sea Daffodil (*Pancratium maritimum*)

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ABSTRACT

In this study, we investigated the disinfection methods and germination conditions for seeds of sea daffodil (*Pancratium maritimum*). Seeds were exposed to 20% and 40% NaOCl (Sodium hypochlorite) solutions for 15, 20, and 25 minutes, as disinfection treatments. The rate of 20% NaOCl was the most appropriate concentration for percentage of seed germination (58%) and plant formation (34%). Effects of three culture media on seed germination, plant formation, and growth were investigated: A. Agar (4 gl^{-1}) with sucrose (30 gl^{-1}); B. MS (Murashige - Skoog) with agar (4 gl^{-1}) and sucrose (30 gl^{-1}); C. MS with agar (4 gl^{-1}), sucrose (30 gl^{-1}), and plant growth regulators (1 mgl^{-1} BA, 0.1 mgl^{-1} 2,4-D). Evaluated plant growth parameters were total plant weight; number, width, and length of leaf; thickness and length of root; and diameter, length, and weight of bulb. All culture media were found to be successful in terms of in vitro germination of the seeds. The most suitable medium for plant formation and all the plant growth parameters were found to be Medium B. This is an innovative report in that it has been thoroughly investigated in terms of disinfection practices and seed germination media with different evaluation parameters.

Keywords: Amaryllidaceae, Disinfection, Growth parameters, Plant formation, Seed germination

INTRODUCTION

Sea daffodil (*Pancratium maritimum* L.), which is adapted to sandy coastline regions, is one of the most attractive species among bulbous plants (Nikopoulos et al. 2008). It belongs to Amaryllidaceae family and has 2n = 22 chromosomes. *Pancratium maritimum* is naturally seen on the coast of Atlantic Ocean, Mediterranean, Black, and Caspian Seas (Zahreddine et al. 2004, Sanaa and Fadhel 2010, Sanaa et al. 2012). *P. maritimum* has been used in European and American gardens as an ornamental plant for centuries (Kilinc and Yuksel 1995).

The height of the adult *P. maritimum* plant is generally 30 - 60 cm on a sand surface (Grassi et al. 2005). It has attractive, fragrant white flowers, which are seen in the summer season (from July to October). Each peduncle includes 1 - 6 fruits described as pods or capsules and consisting of loculusites. Each pod has different number of loculusites, called carpels, and approximately 15 - 20 seeds (Demir et al. 2010, Korkmaz and Celikel 2013). According to our visual observations, the pod shapes change from spherical to cylindrical, and the seeds, irregularly shaped when they are alone, come together like pieces of a puzzle by arranging perfectly in the each carpel. The seeds, which occur from late September to early December, are sharp-edged, black, numerous, and fairly lightweight (about 50 mg each) due to a large presence of auriferous parenchyma. Distribution of seeds occurs through bird, wind, and sea streams, due to the ease with which the seeds float on the water (Grassi et al. 2005, Sanaa and Fadhel 2010).

The *P. maritimum* plant has decorative value and medicinal properties owing to its alkaloid substances (Berkov et al. 2004, Nikopoulos and Alexopoulos 2008, Bogdanova et al. 2009, Sanaa and Fadhel 2010, Berkov et al. 2010). Some of the alkaloids have pharmacological properties, such as anti-tumor (pancratistatin and ungiminorine), anti-viral (lycorine), anti-cholinesterase (galanthamine), and analgesic activities (lycorine and galanthamine). Furthermore, bulb and leaf extracts of *P. maritimum* have acaricidal, insecticidal, and antifungal activities (Berkov et al. 2004).

As over-collection, urbanization, tourism development, and other human activities have increased, *P. maritimum* has lost its natural habitats to a serious degree. For this reason, it is considered a threatened and protected species in Turkey, Bulgaria, Greece, France, Spain, Italy, and Lebanon (Zahreddine et al. 2004, Gümüş and Ellialtıoğlu 2006, Nikopoulos and Alexopoulos 2008, Panayotova et al. 2008, Georgiev et al. 2010, Korkmaz and Çelikel 2013).

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Seed germination and seedling growth are affected by various environmental conditions (Koksal et al. 2015). Seed germination and seedling emergence are critical life stages with generally high mortality for most plants. Various biotic and abiotic stress factors, such as sand instability, drought, salinity, extreme high temperatures in summer or low temperatures in winter, and herbivore predation can restrict seed germination and seedling emergence in coastal sand dunes. As a coastal plant, seedling survival of *P. maritimum* is also affected by deeply burial in sand, desiccation of the surface layer of sand, frost, and sand movement and seawater inundation during storms (Maun 1994, Greipsson and Davy 1996, Bach 1998, Chen and Maun 1999, Balestri and Cinelli 2004).

Knowledge of seeds' germination and emergence abilities is one of the most important prerequisites for maintaining existing populations and restoring threatened species (Balestri and Cinelli 2004). Recent studies show that the *P. maritimum* L. population's management and restoration were hindered by low germination capacity. Indeed, approximately 3% of seeds could form seedlings. Difficulties inherent in seed germination and seedling growth in in situ conditions revealed the necessity for developing protocols for in vitro propagation (Balestri and Cinelli 2004, Georgiev et al. 2010).

It is well known that micropropagation is an up-to-date technique for the preservation of plant genetic material, the development of plant breeding programs, and the fast production of large quantities of propagation material. Generally, seed propagation has been adopted for the production of *Pancratium maritimum* L. (Gümüş 2015). As it is known, seed germination in in vitro conditions is more rapid and controlled than germination in situ. Owing to high success rates, seed germination in tissue culture is attractive for some species (Pierik 1997, Cantos 1998, Dragasski et al. 2003).

Despite these conveniences, desired success in production can be limited, mainly due to contamination in tissue culture studies, which require rigor. Although using seeds as a starting material in in vitro studies decreases contamination risk, knowledge of optimal disinfection procedures for each plant species is critical (Nikopoulos et al. 2008). In vitro conditions with an optimal growth environment and unsuccessful disinfection cause the development of bacteria and fungi (Yıldız and Er 2002). The purpose of disinfection is to eliminate all microorganisms that hinder the in vitro process. However, disinfection should not restrict the explant's viability and regeneration ability, which are known to be affected by the disinfectant concentration and duration (Yıldız and Er 2002, de Jesus et al. 2016). In order to achieve surface disinfection in in vitro studies, various disinfectants such as ethanol, hydrogen peroxide, bromine water, mercuric chloride, silver nitrate, and antibiotics are utilized, the most commonly used among them being sodium hypochlorite (NaOCl). Almost all kinds of bacteria, fungi, and viruses can be inhibited by sodium hypochlorite (Yıldız and Er 2002).

Successful plant tissue culture depends on the choice of nutrient medium and the medium's content, which is a determining factor for growth, explant conditions, and other culture conditions. Most plant species can be grown on completely defined media. Moreover, in some stages of the tissue culture process, such as seed germination, regeneration, and micropropagation, it is a requirement to determine a suitable culture media. The most widely used culture media are Murashige - Skoog (MS) or its modifications. Distinguishing properties of the MS culture medium are its high content of nitrate, potassium, and ammonium compared to other culture media (Gamborg et al. 1976).

As *P. maritimum* is a threatened species and has low seed germination ability and seedling capacity, this increases the importance of its cultivation under controlled conditions. So, determining an in vitro germination process for *P. maritimum* is necessary to provide easier, faster, and more economical production. In this study, we focused on finding the most suitable disinfection treatments and in vitro germination media for sea daffodil (*Pancratium maritimum* L.). This study is also used evaluation criteria to reveal the effects of disinfection treatment and germination media.

MATERIALS AND METHODS

Plant material

This study was conducted in a tissue culture laboratory at the Department of Horticulture, Agriculture Faculty, Cukurova University in Adana, Turkey. In this study, mature seeds of sea daffodil (*P. maritimum*) were used as a

plant material. Seeds were collected from the sand coasts of the Hatay (36° 5' 35" - 35° 56' 21") and Mersin (36° 23' 11" - 34° 4' 30") Provinces in the Mediterranean, in October 2015.

Seed disinfection

After collection, the aeriferous parenchyma part was removed from seed's surface by hand. Pre-treatments were applied to all seeds for surface disinfection. Pre-treatments to seeds consisted of washing under tap water, soaking in 70% alcohol for one minute, and washing in sterile distilled water in a flow bench. After pre-treatments, two different sodium hypoclorite concentrations (20 and 40% NaOCl) were applied for three different durations (15, 20, 25 minutes) for surface sterilization of the seeds. Disinfected seeds were transferred into petri dishes containing a semi-solid Murashie-Skoog nutrient media (MS with 4 gl^{-1} agar) under aseptic conditions.

Cultures were observed for three weeks for determining contamination. Furthermore the effects of disinfection treatments were evaluated in terms of germinated seed number and percentage of seed germination and plant formation, in order to detect the best treatment. The germinated seed number per week for twelve weeks was determined by counting. Percentage of seed germination and plant formation were estimated at the end of the twelve-week culture period.

***In vitro* seed germination**

Pretreated seeds, as explained in the seed disinfection section, were disinfected by the most suitable method (20% NaOCl for 15 minutes) according to results of seed disinfection experiments. For germination, seeds were transferred into three different nutrient media: A. Agar (4 gl^{-1}) with sucrose (30 gl^{-1}); B. MS with agar (4 gl^{-1}) and sucrose (30 gl^{-1}); C. MS with agar (4 gl^{-1}), sucrose (30 gl^{-1}), and plant growth regulators (1 mgl^{-1} BA, 0.1 mgl^{-1} 2,4-D).

Percentages of germination and plant formation (%) were determined by observing two-day intervals for one month. In order to determine the effects of nutrient media on plant growth, total plant weight, number, width, and length of leaf, thickness and length of root, and diameter, length, and weight of bulb were investigated at the end of a three-month culture period. All measurements were uniformly recorded on specific points of plants. Root thickness, leaf width, and bulb diameter were measured by digital caliper. Length of root, leaf, and bulb were determined using a ruler. Fresh weight of bulb and total plant were weighed using a digital top-loading weighing balance. Leaf number was determined by counting.

Statistical analysis

The seed disinfection experiment was conducted using a completely randomized experimental design with two factors (NaOCl concentration and duration). Treatments had five replications (Petri dishes) with five explants each. The seed germination experiment was carried out using a completely randomized experimental design with a single factor: every treatment had five replications with five explants each. All quantitative data expressed as percentages were subjected to arcsine transformation. Data were subjected to ANOVA and the means were separated using the LSD multiple range test at $p \leq 0.05$. All statistical analyses were performed using the JMP8 software package.

RESULTS

Seed disinfection

According to the results obtained via observation, contamination percentages were fairly low (data not shown). All tested disinfection methods were found to be successful. However, some differences in germination and plant formation were found. Per weekly observation for twelve weeks, the number of germinated seeds increased for up to 7 - 10 weeks in the 20% NaOCl concentration at three durations (15, 20, and 25 minutes), and then remained constant after 10 weeks (Figure 1). On the other hand, the number of germinated seeds in the 40% NaOCl concentration increased for up to 5 - 8 weeks and then remained constant.

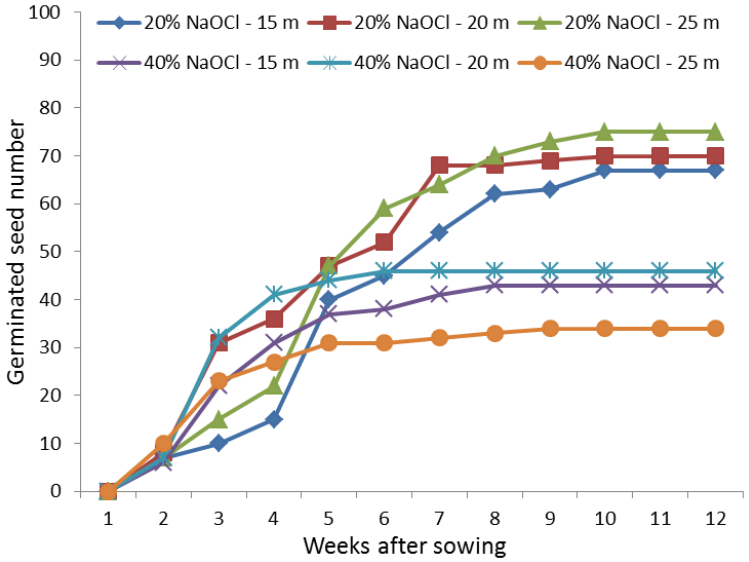

Figure 1. The number of germinated seed during culture.

Germination percentage results related to disinfection treatments are presented in Table 1. Percentage of germination in the 20% NaOCl concentration (58%) was higher than in the 40% concentration (34%). Duration of treatment and interaction effects (concentration x duration) were not statistically significant (Table 1).

Table 1. The effect of disinfection treatments on the *in vitro* germination and plant formation percentage (%) of *Pancratium maritimum* seeds.

	Germination percentage			Plan formation percentage		
Duration	NaOCl concentration (%)			NaOCl concentration (%)		
(min)	20	40	Mean	20	40	Mean
15	57±14 (49)	36±5 (37)	46 (43)	33±8 (35)	10±9 (17)	22 (26)
20	56±10 (49)	38±4 (38)	47 (43)	33±12 (35)	8±4 (16)	21 (26)
25	62±13 (52)	27±9 (31)	45 (42)	47±11 (43)	10±7 (17)	28 (30)
Mean	58[a] (50)	34[b] (35)		38[a] (38)	9[b] (16)	

Values in parenthesis were transformed. [NS]Non-significant, *p < 0.05

$LSD_{duration}$: [NS], LSD_{con}: 7.812[*], $LSD_{con\ x\ duraion}$: [NS], $LSD_{duration}$: [NS], LSD_{con}: 8.125[*], $LSD_{con\ x\ duraion}$: [NS]

Plant formation percentage results are shown in Table 1. Whereas percentage of plant formation in seeds treated with the 20% NaOCl concentration (38%) was found relatively high, the percentage for the 40% NaOCl concentration (9%) was significantly lower. Duration of treatment and interaction effects (concentration x duration) were not statistically significant. The effects of disinfection treatments on the in vitro germination and plant formation are also presented in Figure 2.

Figure 2. *Pancratium maritimum* plantlets from seeds which disinfected by NaOCl.

In vitro germination of the seeds

Regarding in vitro germination percentage of the seeds, three different nutrient media were found to be successful, with no statistically significant differences (Figure 3a). Plant formation percentage was also defined (Figure 3b). The highest plant formation percentage (52%) was found in Medium B, MS without plant growth regulators (MS, 4 gl^{-1} agar, 30 gl^{-1} sucrose). Plant formation percentage in Medium A (4 gl^{-1} agar, 30 gl^{-1} sucrose) and Medium C (MS, 4 gl^{-1} agar, 30 gl^{-1} sucrose, 1 mgl^{-1} BA, 0.1 mgl^{-1} 2,4-D) was 25% and 18%, respectively. The effects of germination media are also presented in Figure 4.

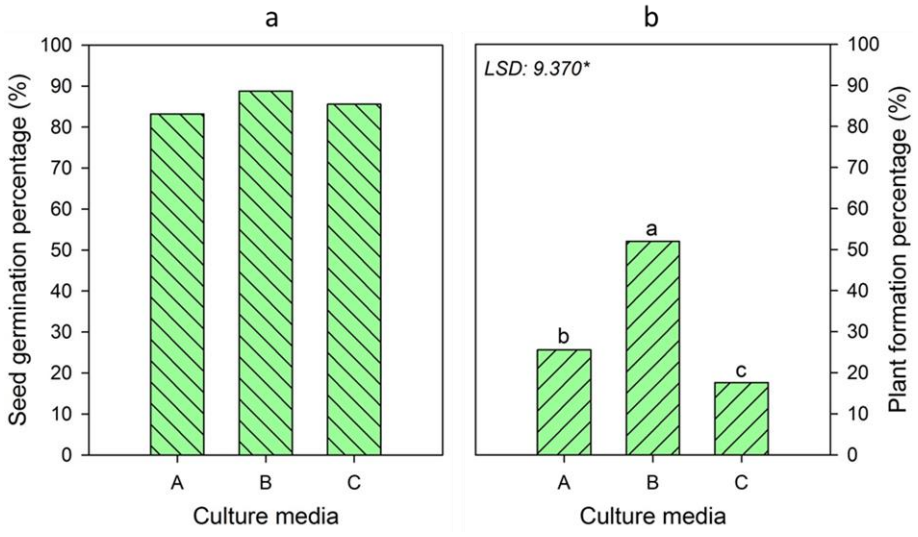

Figure 3. The effects of *in vitro* germination media. a). Seed germination percentage b). Plant formation percentage (A: 4 gl^{-1} agar, 30 gl^{-1} sucrose - B: MS, 4 gl^{-1} agar, 30 gl^{-1} sucrose - C**:** MS, 4 gl^{-1} agar, 30 gl^{-1} sucrose, 1 mgl^{-1} BA, 0.1 mgl^{-1} 2,4-D - ^{NS}Nonsignificant - *$p < 0.05$)

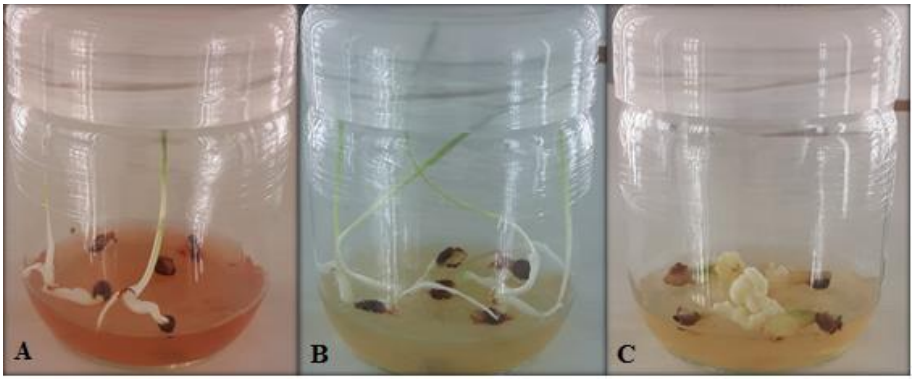

Figure 4. *Pancratium maritimum* plantlets after *in vitro* seed germination (A: 4 gl^{-1} agar, 30 gl^{-1} sucrose - B: MS, 4 gl^{-1} agar, 30 gl^{-1} sucrose - C**:** MS, 4 gl^{-1} agar, 30 gl^{-1} sucrose, 1 mgl^{-1} BA, 0.1 mgl^{-1} 2,4-D)

After seed germination, growth of plantlets was maintained in the same media. Table 2 presents the evaluations of plant growth parameters including total plant weight; leaf number, width, and length; thickness and length of root; and bulb diameter, length, and weight. Significant differences in plant growth due to the effects of media were determined. The best plant growth was found in Medium B, followed by Medium C and Medium A. The general overview of plants in Figure 5 supports this result. Total plant weight was highest in Mediums B (1.27 g) and C (1.17 g). Leaf number was greater in Mediums B (3.56 unit) and C (2.56 unit) than in Medium A (Table 2). Leaf width was also greater in Mediums B (1.58 mm) and C (1.82 mm). These differences in leaf width are presented in Figure 6. Mediums A (12.48 cm) and B (17.17 cm) produced longer roots than Medium C. There were no significant differences in leaf length, root thickness, bulb diameter, bulb length, and bulb weight among the three culture media. Bulb formation was observed in all mediums, but some differences among bulblets were detected according to visual observations (data not shown). Bulbs were surrounded by callus in Medium C. On the other hand, bulbs secreted some phenolics in Medium A. Although no significant differences in bulb parameter (diameter, length, and weight) were determined among media used in the study, Medium B was determined to be the most promising medium for bulb formation (Figure 7).

Table 2. Plant growth parameters of plantlets after *in vitro* seed germination.

Parameters	*Germination media*			
	A	B	C	*LSD*
Total plant weight (*g*)	0.67±0.22^{b}	1.27±0.01^{a}	1.17±0.17^{a}	*0.322***
Leaf number (*unit*)	1.22±0.19$^{b.}$	3.56±0.84^{a}	2.56±0.51^{a}	*1.153***
Leaf width (*mm*)	0.97±0.11^{b}	1.58±0.14^{a}	1.82±0.13^{a}	*2.463****
Leaf length (*cm*)	11.44±0.91	20.95±4.08	15.01±5.81	---^{NS}
Root thickness (*mm*)	0.82±0.09	1.20±0.14	1.11±0.48	---^{NS}
Root length (*cm*)	12.48±1.92^{a}	17.17±4.87^{a}	1.62±0.58^{b}	*6.079***
Bulb diameter (*mm*)	4.69±0.36	4.82±0.72	4.33±0.72	---^{NS}
Bulb length (*cm*)	2.88±0.29	2.66±0.21	2.20±0.62	---^{NS}
Bulb weight (*g*)	0.23±0.04	0.28±0.07	0.31±0.05	---^{NS}

^{NS}: Nonsignificant, $^{**}p < 0.05$, $^{***}p < 0.001$

A: 4 gl^{-1} agar, 30 gl^{-1} sucrose

B: MS, 4 gl^{-1} agar, 30 gl^{-1} sucrose

C: MS, 4 gl^{-1} agar, 30 gl^{-1} sucrose, 1 mgl^{-1} BA, 0.1 mgl^{-1} 2,4-D

Figure 5. Plant formation of *Pancratium maritimum* (**A**: 4 gl^{-1} agar, 30 gl^{-1} sucrose - **B**: MS, 4 gl^{-1} agar, 30 gl^{-1} sucrose - **C:** MS, 4 gl^{-1} agar, 30 gl^{-1} sucrose, 1 mgl^{-1} BA, 0.1 mgl^{-1} 2,4-D)

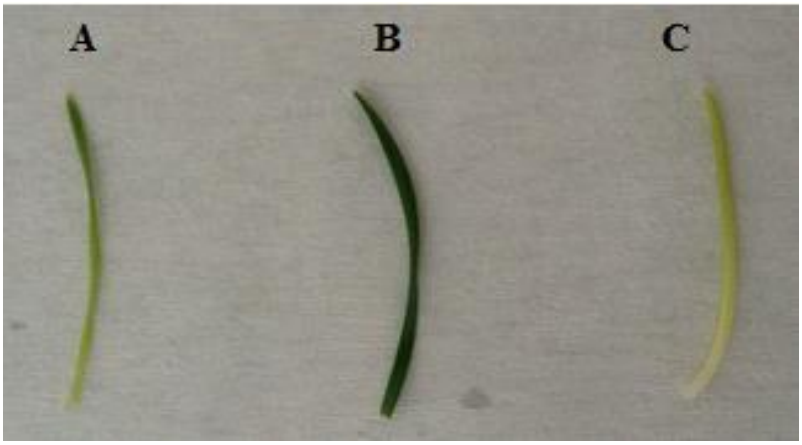

Figure 6. An aspect of *Pancratium maritimum* leaves (**A**: 4 gl^{-1} agar, 30 gl^{-1} sucrose - **B**: MS, 4 gl^{-1} agar, 30 gl^{-1} sucrose - **C:** MS, 4 gl^{-1} agar, 30 gl^{-1} sucrose, 1 mgl^{-1} BA, 0.1 mgl^{-1} 2,4-D)

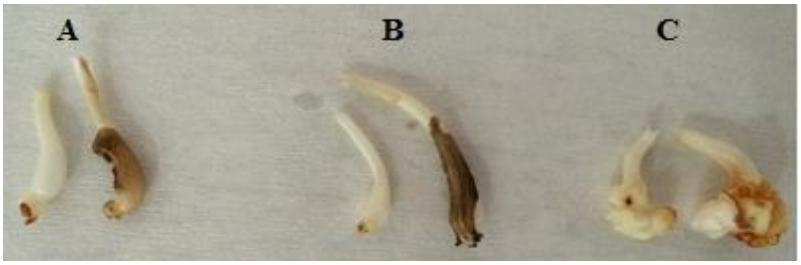

Figure 7. An aspect of *Pancratium maritimum* bulbs (**A**: 4 gl^{-1} agar, 30 gl^{-1} sucrose - **B**: MS, 4 gl^{-1} agar, 30 gl^{-1} sucrose - **C:** MS, 4 gl^{-1} agar, 30 gl^{-1} sucrose, 1 mgl^{-1} BA, 0.1 mgl^{-1} 2,4-D)

DISCUSSION

Disinfection of plant material is the primary requirement for in vitro studies. Knowledge of disinfectant concentration and sterilization period has great importance for plant growth in in vitro culture (Yıldız and Er 2002). It is well known that disinfectants that have direct contact with the explant have various effects on growth processes in in vitro cultures (Yıldız et al. 1997, Yıldız and Er 2002). Sodium hypoclorite (NaOCl), which has been generally used for disinfection of plant materials in in vitro studies, has a beneficial effect on the seed disinfection of various plants (Lee et al. 2005, Picolotto et al. 2007, Zeng et al. 2013, Yıldız and Ekiz 2014, Trivedi and Tiwari, 2016, de Jesus et al. 2016). In our study, concentrations and durations were not statistically significant for disinfection achievement; namely, all treated NaOCl concentrations (20 and 40%) and durations (15, 20, and 25 minutes) were highly successful. Unlike our results, Tort (1997) revealed that the length of application period (2, 5, and 10 minutes) and NaOCl concentration (3, 6, 9, and 12%) of solutions were effective for anise seed (*Pimpinella anisum* L.) and cotton (*Gossypium hirsutum* L.) plants.

The successful results of all disinfection methods can be attributed to the use of seed as a starting material in this study. Nikopoulos and Alexopoulos (2008) achieved a rate of 98 - 100% success in disinfection using seeds from closed pods as starting material. Similarly, Georgiev et al. (2010) successfully disinfected young pods using 20% commercial detergent. Picolotto et al. (2007) emphasized that 5% sodium hypochlorite solution was effective for disinfection of jabuticaba seeds. In addition, Gümüş and Ellialtıoğlu (2006) determined a 40% infection rate using NaOCl in different concentrates for the disinfection of the twin scales of *P. maritimum*.

Although not all disinfection treatments showed statistically significant disinfection achievement in this study, all applications were found to be effective in terms of seed germination and plant formation. According to disinfection treatment results, NaOCl concentrations were significant for both germination and plant formation, whereas duration period was not important. While seed germination could not continue after 5 - 8 weeks at 40% NaOCl concentration, 20% NaOCl treatments allowed germination until the tenth week. Percentage of germination and plant formation was limited by 40% NaOCl compared to 20% NaOCl. Similar to our results, the negative effects of increasing disinfectant concentrations on shoot regeneration have been reported previously. Yıldız and Er (2002) determined that seed germination, seedling growth, and shoot regeneration were negatively affected by increasing the concentration (40, 60, and 80%) and temperature (0, 10, 20, and 30°C) of NaOCl solutions.

It is important to determine the most appropriate medium to provide easier, faster, and more economical germination when seed is used as a starting material in tissue culture studies. Up to now, various germination media have been tried in in vitro germination studies for the sea daffodil (*Pancratium maritimum* L.) and a high germination rate has usually been reported. Likewise, a high in vitro germination percentage (more than 80%) was found for all culture media in this study. Panayotova et al. (2008) determined that mature *Pancratium maritimum* seeds were rapidly germinated in a water-agar (8 gl^{-1} agar) medium. Another study supporting this result was conducted by Nikopoulos and Alexopoulos (2008). They emphasized that *Pancratium maritimum* seeds were germinated in semi-solid MS culture media (4 gl^{-1} agar, 30 gl^{-1} sucrose) that contained different concentrations of GA_3 (0, 1, 2, 5, 10 mgl^{-1}) and a 75 - 90% germination rate was achieved in all media at the end of the incubation period (70 days). Apart from *Pancratium maritimum*, the effects of different culture media on the germination of other plants have been revealed. Undomde et al. (2014) found the greatest germination rate for *Dendrobium nobile* hybrids in mature seeds (4 months old) in media containing lower sucrose doses among all concentrations (0, 10, 20, and 40 gl^{-1} sucrose). Bektaş et al. (2013) revealed the highest germination rate (44.2%) for *Orchis coriophora* L in an Orchimax medium including activated charcoal plus 1 mgl^{-1} indole -3-acetic acid.

In addition to previous studies, plant formation percentage from germinated seeds was also determined in our study. Medium B was found to be the most suitable for plant formation (52%). These results indicated that the plantlets could maintain their growth in the same media after germination, meaning it is not necessary to transfer them from a germination medium to a plant growth medium. In this respect, it is possible to save in costs, labor, and time. Similarly, Nikopoulos et al. (2008) emphasized that *P. maritimum* microplants that formed 4 - 5 leaves in a culture medium were preserved for at least 14 months at those conditions without the need for recultivations.

Plant growth parameters were also examined to evaluate the quality of the plants in the study. The effects of media on plant growth parameters were more evident in Mediums B and C. Although Mediums B and C showed similar results on shoot growth, with regard to root and bulb formation, higher values were obtained from Medium B. Since there is not any literature evaluated detailed plant growth parameters in in vitro germination studies, it is not possible to comparatively discuss the results of the plant growth parameters examined in our study. However, plant growth parameters detected in our study were considered important for determining plant quality and deciding optimum plant growth level for the acclimatization stage.

CONCLUSIONS

Our study attempted to determine the most appropriate method for the disinfection of *Pancratium maritimum* seeds. Determining the rate of contamination is not a sufficient parameter for revealing the success of disinfection in in vitro studies. Additionally, it is necessary to determine some plant growth indicators, such as germination percentage, regeneration rate, and plant formation percentage. Indeed, we found contamination percentage negligible, with extremely low levels for all disinfection treatments in this study, but seed germination and plant formation percentage were affected by disinfection concentrations. Due to the absence of any differences relating to the duration of the application, for the purposes of saving time, a 15 - minute application at the 20% NaOCl level was considered adequate. In the later stage of this study, achievement of germination was considered via percentage of seed germination, plant formation, and plant growth parameters for the sea daffodil. When all germination achievement parameters were considered, the most suitable culture medium for sea daffodil was determined to be Medium B (MS with 4 gl^{-1} agar and 30 gl^{-1} sucrose). Determining plant growth parameters for in vitro culture studies could be useful for deciding the optimum growth level for the transfer stage of plants. As far as we know, this study is the most detailed yet regarding the effects of disinfection treatments on plant formation and evaluating the effects of in vitro germination media on plant growth parameters for *P. maritimum*.

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REFERENCES

Bach CE (1998). Seedling survivorship of the Beach Morning Glory, *Ipomoea pes-caprae* (Convolvulaceae). Aust J Bot 46: 123-133.

Balestri E, and Cinelli F (2004). Germination and early - seedling establishment capacity of *Pancratium maritimum* L. (Amaryllidaceae) on coastal dunes in the North-Western Mediterranean. J Coastal Res 20: 761-770.

Bektaş E, Cüce M, and Sökmen A (2013). *In vitro* germination, protocorm formation, and plantlet development of *Orchis coriophora* (Orchidaceae), a naturally growing orchid species in Turkey. Turk J Bot 37: 336-342.

Berkov S, Evstatieva L, and Popov S (2004). Alkaloids in Bulgarian *Pancratium maritimum* L. Z. Naturforsch, C 59: 65-69.

Berkov S, Pavlov A, Georgiev V, Weber J, Bley T, Viladomat F, Bastida J, and Codina C (2010). Changes in apolar metabolites during *in vitro* organogenesis of *Pancratium maritimum*. Plant Physiol Biochem 48: 827-835.

Bogdanova Y, Pandova B, Yanev S, and Stanilova M (2009). Byosynthesis of Lycorine by *In vitro* Cultures of P*ancratium maritimum* L. (Amaryllidaceae). Biotechnol & Biotec Eq 23: 919-922.

Cantos M (1998). Embriyo rescue and development of *Juniperus oxycedrus* and macrocarpa. Seed Sci Technol 26: 193-198.

Chen H, and Maun MA (1999). Effects of sand burial depth on seed germination and seedling emergence of *Cirsium pitcheri*. Plant Ecol 140: 53-60.

de Jesus VAM, Araújo EF, Neves AA, Santos FL, dos Santos Dias LA, and da Silva RF (2016). Ratio of seeds and sodium hypochlorite solution on the germination process of papaya seeds. J Seed Sci 38: 057-061.

Demir Z, Müderrisoğlu H, Aksoy N, Özkan AS, Uzun S, and Özkara H (2010). Effects of second–housing and recreational use on *Pancratium maritimum* L. population in Western Black Sea Region of Turkey. J Food Agric & Environ 8: 890-894.

Dragasski M, Economo AS, and Vlahos JC (2003). Bulblet formation *in vitro* and plantlet survival extra vitrum in *Pancratium maritimum* L. Acta Hort 616: 347-352.

Gümüş C (2015). Kum zambağı (*Pancratium maritimum* L.) bitkisinde yapılan araştırmalar üzerinde bir inceleme. Derim 32: 89-105.

Gümüş C, and Ellialtıoğlu Ş (2006). Kum zambağı (*Pancratium maritimum*)'nın doku kültürü ile çoğaltılma olanağı üzerine bir çalışma. Özzambak ME, Zeybekoğlu E, editörler. III. Ulusal Süs Bitkileri Kongresi Bildiri Kitabı, 8- 10 Kasım 2006; İzmir, Türkiye. s: 435-439.

Gamborg OL, Murashige T, Thorpe TA, and Vasil IK (1976). Plant tissue culture media. In Vitro 12: 473-478.

Georgiev V, Ivanov I, and Pavlov A (2010). Obtaining and selection of *Pancratium maritimum* L. *in vitro* cultures with acetylcholinesterase inhibitory action. Biotechnol & Biotec Eq 24: 149-154.

Grassi F, Cazzaniga E, Minuto L, Peccenini S, Barberis G, and Basso B (2005). Evaluation of biodiversity and conservation strategies in *Pancratium maritimum* L. Northern Tyrrhenian Sea. Biodivers and Conserv 14: 2159-2169.

Greipsson S, and Davy AJ (1996). Sand accretion and salinity as constraints on the establishment of *Leymus arenarius* for land reclamation. Ann Bot 78: 611-618.

Kilinc M, and Yuksel S (1995). *Pancratium maritimum* L. (Amaryllidaceae) üzerinde morfolojik, anatomik ve ekolojik bir araştırma, Doga Turk J Bot 19: 309-320.

Korkmaz E, and Çelikel FG (2013). Türkiye kıyılarında doğal yayılış gösteren kum zambağının korunması ve kültüre alınması üzerine yapılan araştırmalar. Erken K, Pezikoğlu F, editörler. V. Süs Bitkileri Kongresi Bildiriler Kitabı, 6-9 Mayıs 2013, Yalova, Türkiye, Cilt-II, s: 855-859.

Koksal N, Agar A, and Yasemin S (2015). The effects of top coat substrates on seedling growth of marigold. J Appl Biol Sci 9: 66-72.

Lee YI, Lee N, Yeung EC, and Chung MC (2005). Embryo development of *Cypripedium formasanum* in relation to seed germination *in vitro*. J Amer Soc Hort Sci 130: 747-753.

Maun MA (1994). Adaptations enhancing survival and establishment of seedlings on coastal dune systems. Vegetatio 111: 59-70.

Nikopoulos D, and Alexopoulos AA (2008). *In vitro* propogation of an endangered medicinal plant: *Pancratium maritimum* L. J Food Agric & Environ 6: 393-398.

Nikopoulos D, Nikopoulos D, and Alexopoulos AA (2008). Methods for the preservation of genetic material of *Pancratium maritimum* (Amaryllidaceae). J Food Agric & Environ 6: 538-546.

Panayotova LG, Ivanova TA, Bogdanova YY, Gussev CV, Stanılova MI, Bosseva YZ, and Stoeva TD (2008). *In vitro* cultivation of plant species from sandy dunes along the Bulgarian Black Sea Coast. Phytologia Balcanica. 14: 119-123.

Picolotto L, Schuch MW, Souza JA, Silva LC, Ferri J, and Fachinello JC (2007). Efeito do hipoclorito de sódio, fotoperíodo e temperatura no estabelecimento in vitro de jabuticabeira. Sci Agraria 8: 19-23.

Pierik RLM (1997). In vitro Culture of Higher Plants, 1st ed. Netherlands Springer Publishers s: 348.

Sanaa A, Boulila A, Bejaoui A, Boussaid M, and Fadhel NB (2012). Variation of the chemical composition of floral volatiles in the endangered Tunisian *Pancratium maritimum* L. populations (Amaryllidaceae). Ind Crop Prod 40: 312-317.

Sanaa A, and Fadhel NB (2010). Genetic Diversity in Mainland and Island Populations of Endangered *Pancratium maritimum* L. (Amaryllidaceae) in Tunisia. Sci Hortic 125: 740-747.

Trivedi M, and Tiwari RK (2016). Method of enhancing seed germination in *Chlorophytum* sp. Int Res J Eng Tech 3: 21-25.

Tort N (1997). Anason (*Pimpinella anisum* L.) ve pamuk (*Gossypium hirsutum* L.) tohumlarının *in vitro* ortamda sterilizasyonu üzerinde bir araştırma. Anadolu, J. of AARI 7: 41-50.

Undomde W, Wen PJ, Lee CY, Chin SW, and Chen FC (2014). Effect of sucrose concentration and seed maturity on *in vitro* germination of *Dendrobium nobile* hybrids. Plant Growth Regul 72: 249–255.

Yıldız M, Avcı M, and Özgen M (1997) Studies on sterilization and medium preparation techniques in sugarbeet (*Beta vulgaris* L.) regeneration. Turkish–German Agricultural Research Symposium V, 29 September–4 October, Antalya, Turkey. pp: 125-130.

Yıldız M, and Er C (2002). The effect of sodium hypochlorite solutions on *in vitro* seedling growth and shoot regeneration of flax (*Linum usitatissimum*). Naturwissenschaften 89: 259-261.

Yıldız M, and Ekiz H (2014). The effect of sodium hypochlorite solutions on *in vitro* seedling growth and regeneration capacity of sainfoin (*Onobrychis viciifolia* Scop.) hypocotyl explants. Can J Plant Sci 94: 1161-1164.

Zahreddine H, Clubbe C, Baalbaki R, Ghalayini A, and Talhouk SN (2004). Status of native species in threatened Mediterranean habitats: The case of *Pancratium maritimum* L. (sea daffodil) in Lebanon. Biol Conserv 120: 11-18.

Zeng S, Zhang Y, da Silva JAT, Wu K, Zhang J, and Duan J (2013). Seed biology and *in vitro* seed germination of *Cypripedium*. Crit Rev Biotechnol, Early Online: 1–14.