Effects of microbubbles and temperature on the germination of the saltmarsh plant, Suaeda maritima (L.) Dumort

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Research Article

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Abstract

Background

Temperature and dissolved gas availability are important parameters for the germination of saltmarsh plants. As a newly developed method to increase the dissolved gas availability, microbubble technology has shown to increase the crop production. However, the effects of microbubbles on the germination of saltmarsh plants have not been studied. The aim of this study is to investigate the effects of the microbubbles on the germination of *Suaeda maritima* (L.) Dumort. in different temperatures (6, 12, 18, 24 and 30ºC).

Results

The highest germination rate was observed at 24 ºC in MBs water. The germination rates at microbubbles were significantly higher than those at control, deionized water at 24 ºC. The germination energy was significantly higher at 24 and 30 ºC than at 6 and 12 ºC in both deionized and microbubbles waters. The mean germination time was significantly shorter at 24 and 30 ºC than at 6 and 12 ºC in both waters. However, no significant effects of microbubbles were observed in terms of germination energy and mean germination time.

Conclusion

This study suggests that microbubbles improves the germination rate of *S. maritima* at the optimal temperature (24ºC), but GE and MGT were not affected by microbubbles but by temperature in *S. maritima*.

Background

Blue carbon is the carbon absorbed by the oceans and coastal ecosystems [7, 30, 35]. Saltmarshes have been considered as a major blue carbon [15, 26, 27]. The saltmarshes in Korea occupy about 2.7% of the total west and south coasts [31]. Therefore, scientists and coastal managers consider the saltmarsh ecosystem as a potential source of blue carbon. The Ministry of Oceans and Fisheries (MOF) of Korea has a goal to absorb and store more than 1 million tons of greenhouse gas (GHG) by 2050 [19]. However, the saltmarsh habitats have been continuously decreased during the past decades due to coastal development [23]. To overcome this issue, MOF is promoting a saltmarsh afforestation project on an area of 660 km^2 of Korea [24]. To assist this effort, it is critical to develop technologies to enhance seed germination and growth in the early stage of the major saltmarsh plants [37].

Many environmental factors influence seed germination. Inter alia salinity and temperature are key factors regulating the germination of saltmarsh plants [5, 18, 36]. The optimum germination temperature is different in different species. For example, the optimal germination temperature for *Phragmites australis* is 10–25°C [13], while *Suaeda maritima* germinates well at 20–25°C [4]. Different populations of the same species also show different optimal germination temperatures due to the acclimation to their original habitats. For example, the Northeast China population of *Phragmites australis* germinated well at the constant temperatures of 29°C. In Tunisia, however, the optimal germination temperature of the same species was found at a constant temperature of 20°C in non-saline water [11]. Thus, it is critical to determine the optimal germination temperature of specific species and even population of saltmarsh plants.

Additionally, the gas availability in media also affects the germination. Recently, in this regard, the micro/nano-bubble techniques have been studied on germination of plants [16, 28]. The bubble sizes between 1 ~ 100 µm are called microbubbles (MBs) [28, 34, 40]. Because of low rising velocity, the residence time of microbubbles can be prolonged in the media [6, 32–34]. Thus, much more gas such as oxygen, carbon dioxide and nitrogen can be dissolved and remained in the water longer, available for plants to absorb. However, the studies on the effects of MBs have mainly focused on the land crops, such as rice and tomatoes [16, 28]. There is a need to exploring the effects of MBs water in other ecologically and economically important plants, like saltmarsh plants.

Suaeda maritima is halophytic shrub widely found in saltmarshes [9, 22]. This plant is edible and is also used for juice and curries, and feed for cattle, goats, and sheep [2, 25]. Furthermore, in 2022, *S. maritima* is selected as one of the species used in saltmarsh afforestation projects in Korea [24].

The aim of this study is to investigate the effects of MBs on the germination of the saltmarsh plant, *Suaeda maritima*, in different temperatures. This is first study examining MBs for the germination of *S. maritima*. The seed germination rates were compared with a single factor of temperature or microbubbles as well as the combined effect of these two factors. This study will provide critical information to understand the early development of *S. maritima*, and therefore, to contribute to the salt marsh plant restoration projects in Korea.

Results

According to the result of two-way ANOVAs, germination rate was significantly influenced by temperature ($p < 0.05$) except for the final day, while, the effects of microbubbles was detected except for the 3rd day ($p < 0.05$). As a result of RM ANOVA analysis, the effects of time ($p < 0.001$), the interaction of temperature and time ($p = 0.002$), and time and microbubbles ($p = 0.032$) were observed. However, significant differences were not detected in the interaction of temperature and microbubbles, as well as the interaction of temperature, time and microbubbles ($p > 0.05$).

The present study showed that 24°C is the optimal temperature for the germination of *Suaeda maritima*, considering germination rate, germination energy and mean germination time. MBs even enhanced the germination rate at this temperature (Fig. 1). The germination rate of *S. maritima* with MBs water was higher at 24°C than all other temperature conditions from the 9th day to the 15th day ($p = 0.012, 0.002$

and *0.046* respectively). From the 15th day, the germination rate of MBs treatment at 18°C became similar to that at 24°C. At control, the germination rates were similar at temperatures from 18 to 30°C. The germination rates of control were saturated at 15–17% at all temperature conditions, while the saturated germination rates of MBs were 13, 18 and 19% at 6, 12 and 30°C respectively. The saturated germination rate at MBs at 24°C was much higher, approximately 33% than those of all other conditions.

At deionized water, the germination energy (GE) of *Suaeda maritima* increased as temperature increased from 8.33% at 6°C to 85% at 30°C (Fig. 2; $p < 0.001$). At MBs, GE was from 0% at 6°C to 100% at 30°C. Significant differences in GE were observed between lower (6 and 12°C) and higher (24 and 30°C) temperatures. However, the MB water effect was not detected ($p > 0.05$).

The mean germination time (MGT) became shorter as temperature increased regardless of the water conditions (Fig. 2; $p < 0.001$). At control, MGT was decreased from 13.3 days at 6°C to 5.4 days at 30°C. At MBs, MGT was decreased from 12.8 days at 6°C to 4.1 days at 30°C. However, the water treatments (MB vs. control) did not significantly influence MGT ($p > 0.05$).

Discussions

The aim of this study was to explore the effect of microbubbles on the germination of *Suaeda maritima* in different temperature conditions. This study suggests that 24°C is the optimal temperature for germination of *Suaeda maritima* in both deionized and MBs waters. A previous study reported that the germination rate of *S. maritima* seeds collected from UK was higher at 20°C compared to lower temperatures (10–18°C) [37]. Another study tested a wider temperature regime (from 5 to 30°C) and found the best germination rate at 25°C, and no germination was observed at 30°C [4]. These results suggest that the optimal temperature of *S. maritima* is 20–25°C.

At 24°C, MBs significantly enhanced the germination rate from the 6th day. This enhancement effect may be due to the high concentration of oxygen in MB water. The seeds of *Spartina alterniflora* were germinated at the atmospheric concentration of oxygen (21%), which is the highest concentration in that study, in comparison to lower oxygen concentrations [38]. Another study also showed that high oxygen concentration enhanced the seed germination of *Brassica kaber* [3]. On the other hand, the enhancement effect can also be related to the reactive oxygen species (ROS) in MB water. ROS can be induced under excessive cold and heat stresses in plant seeds, which may prevent radicle emergence [10]. Therefore, excessive ROS, in general, is harmful for seed's germination by inducing oxidative damage. However, ROS can also increase the germination rate of seeds. For example, MB water enhanced the germination rate by 6–25% compared to the control maybe because of the exogenous ROS in MB water [40]. The germination rates of rice treated MB increased by approximately 14% than treatments with distilled water [16]. Liu suggested that the submicromolar level of ROS can function as signaling molecules, and ROS can be involved in regulation development [20]. ROS may also stimulate the release of seed dormancy through the direct oxidation of a subset of biomolecules [1]. Therefore, the gemination of *Suaeda maritima* seeds may be promoted with proper amount of ROS in MB water. However, the present study did not measure the level of dissolved oxygen and ROS density in media, therefore, there is a need to analyze these factors in the future.

The GE tendency of *Suaeda maritima* increased as temperatures increased, regardless of the presence of microbubbles. In a previous study with cottonwood, GE was drastically reduced the germination rate at low (15°C) and high temperatures (38°C), and the optimal germination temperature of this species was 27–32°C [8]. These results suggest that temperature is a major factor influencing GE and lower or higher temperature may delay the germination of seeds.

The MGT was shorter as the temperature increased, and the microbubble effects were not detected like GE. Seed dormancy induction and release is controlled by phytohormones gibberellins (GA) and dormancy regulators such as delay of germination 1 (DOG1) [17]. DOG1 represses GA and induces expression of cell-wall-remodeling proteins (CWRPs) needed for micropylar endosperm (CAP) weakening. To overcome the expression of CWRP induced by DOG1, a constantly high GA content is necessary. This constantly high GA content is maintained only at colder temperature. Thus, the increased level of DOG1 shifts the germination temperature to colder temperatures, and the reduced level of DOG1 shifts the germination temperature to warmer temperature [12]. The DOG1 expression is down-regulated, and GA level increases in the absence of salt [17]. In a previous study on germination of *Suaeda maritima* at various salinity and temperature conditions, the detrimental effect of salinity was more severe at higher temperatures [29]. In the present study, the *S. maritima* seeds were cultured in non-salt water. Therefore, the absence of salt may be the reason of lower germination rate at low temperature (6°C) [4].

Conclusion

In conclusion, the present study suggests that the optimal temperature for the germination of *Suaeda maritima* seeds is 24°C, and MBs significantly enhanced the germination at this temperature. However, the accumulation of ROS by MBs at both higher and lower temperatures may inhibit and reduce the promotion effect of MBs on seed germination of this plant. Altogether, this is the first study exploring the effect of MBs and temperature on seed germination of the saltmarsh plant, *Suaeda maritima.* The findings from this study will provide critical information for the saltmarsh plant cultivation and afforestation.

Methods

Experimental design

The seeds of *Suaeda maritima* were provided by NeoEnBiz (Bucheon, Korea). The seeds were originally collected from the saltmarsh ecosystem at Shinan (35°01' N, 126°06' E) and maintained at 4°C before the experiment began. The microbubbled deionized water was produced by New Watertech (Goyang, Korea) and stored at room temperature. In all trails, seeds were placed in Petri dishes with filter papers moistened with about 5–7 ml of deionized water or microbubbled deionized water. The seeds and filter papers were moistened every 3 days to avoid desiccation. The Petri dishes were placed at different temperatures, 6, 12, 18, 24 and 30°C. Each Petri dish contained 30 seeds with three replications, total of 90 seeds per treatment. The light intensity was 20 μmol photons m^{-2} s^{-1} and the photoperiod was 12:12 L:D. The germinated seeds were counted every 3 days for 18 days, and the germination rate, germination energy (GE) and mean germination time (MGT) were calculated. These parameters were calculated as follows [14, 21, 39]:

$$\mathrm{Germinationrate}\,(\%) = \frac{\mathrm{numberofgerminatedseeds}}{\mathrm{totalnumberofseeds}} \times 100$$

$$\mathrm{Germinationenergy}\,(\%) = \frac{\mathrm{numberofgerminatedseedsat6thday}}{\mathrm{totalnumberofgerminatedseeds}} \times 100$$

$$\mathrm{MGT} = \Sigma(\mathrm{D} \times \mathrm{n})/\Sigma\mathrm{n}$$

where n is the number of seeds on day D and D is the number of days counted from the beginning of the test.

Statistical analysis

One-way ANOVAs followed by LSD's tests for post hoc analysis ($p < 0.05$) were performed to explore differences in germination rates, germination energy (GE) and mean germination time (MGT) in all temperature conditions. Two-way ANOVAs followed by LSD's tests for post hoc analysis ($p < 0.05$) were performed to explore the influences of temperature and MBs water on germination rate, GE and MGT. Repeated measures ANOVA (RM ANOVA) was used to explore differences in germination rate at different time points. The statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) program version 26.

Abbreviations

MOF Ministry of Oceans and Fisheries

GHG greenhouse gas

MB microbubble

GE germination energy

MGT mean germination time

ROS reactive oxygen species

GA Gibberellins

DOG1 delay of germination 1

CWRPs cell-wall-remodeling proteins

CAP micropylar endosperm

RM ANOVA repeated measures ANOVA

Declarations

Ethics approval and consent to participate

The authors confirm that the cultivars used are available and public. The experimental research and field studies on plants complied with relevant institutional, national, and international guidelines and legislation. The use of all plant materials does not pose any risk to other species in nature. The planting area is the experimental base of our school, and the field collection does not involve endangered or protected species.

Consent for publication

Not applicable.

Availability of data and materials

All data graphs to process data are available upon request to the corresponding author, Jang Kyun Kim (jang.kim@inu.ac.kr).

Competing interests

The authors declare no competing interests.

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Authors’ contributions

NYL, YCP, CY and JK designed the experiment

NYL conducted the experiment

NYL, QX and JSP analyzed the data and wrote the first draft of manuscript.

JK provided fund for the experiment and supervised the experiment

NYL, QX, JSP, YCP, CY and JKK contributed to manuscript revision, read and approved the submitted version.

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Figures

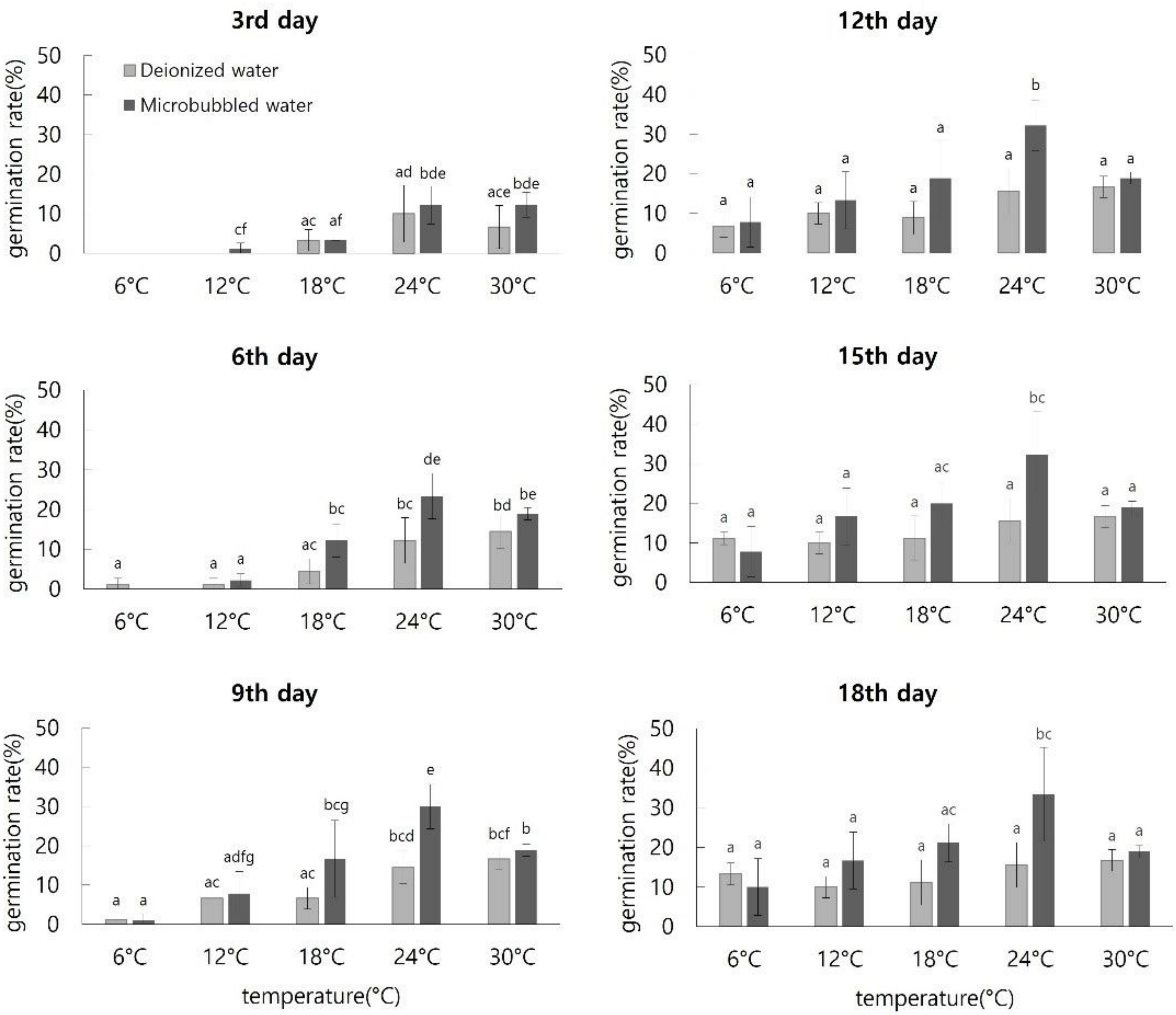

Figure 1

Germination rates of *Suaeda maritima* seeds in deionized water or MBs water at different temperatures at day 3, 6, 9, 12, 15 and 18.

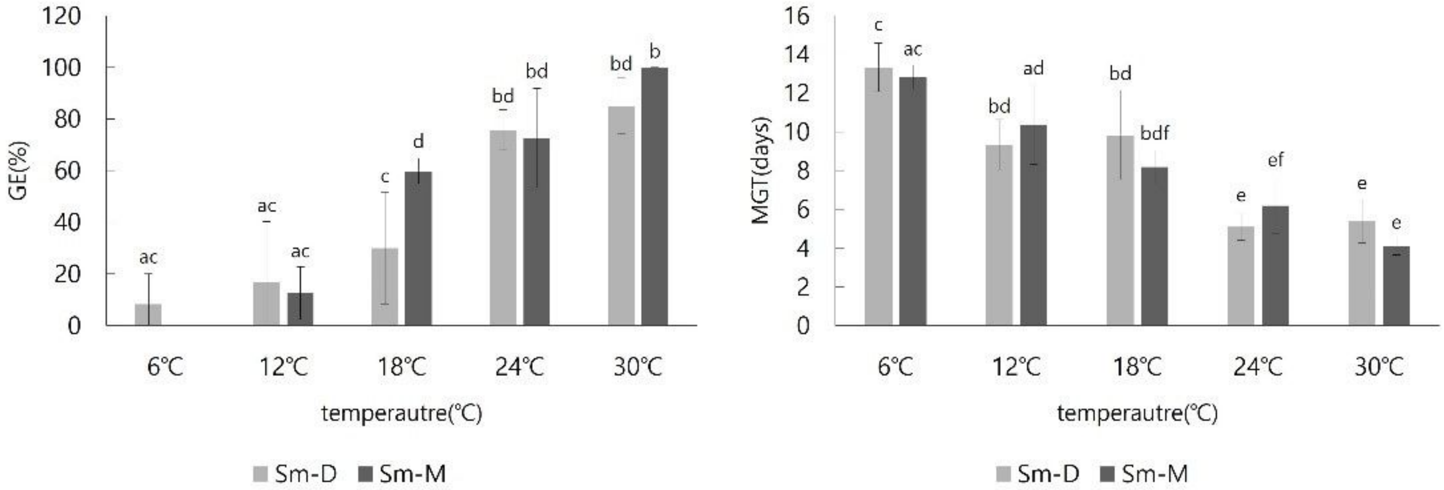

Figure 2

Germination energy (left) and mean germination time (right) of *Suaeda maritima* seeds in and deionized or MBs water at different temperatures.