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EFFECTS OF PLANT GROWTH REGULATORS ON EELGRASS (*ZOSTERA MARINA* L.) GERMINATION

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Abstract

Eelgrass (*Zostera marina* L.) plays a vital role in coastal ecosystems, but its restoration is often hindered by low seed germination rates. This study was aimed to evaluate the effects of plant growth regulators (PGRs) on enhancing eelgrass seed germination under controlled conditions. Seeds were treated with different concentrations of GA_3, 2,4-D and NAA. Results demonstrated that PGRs application significantly increased germination rates compared to control. These findings indicated that PGRs could be an effective tool for increasing the success of eelgrass restoration initiatives.

Seagrass, is a monocotyledonous plant found in marine environments. Seagrass beds are essential for the spawning of species such as cuttlefish, bigfin reef squid and Temminck's surfperch as well as for the nursery grounds of fish and many other organisms in coastal environments. In addition, seagrasses play vital roles in nutrient cycling and water filtration and are as prolific as tropical rainforests in storing organic carbon known as "blue carbon" (Johannessen 2022). The most abundant seagrass species in the Northern Hemisphere is *Zostera marina* Linnaeus (eelgrass) (Howarth *et al.* 2022). Because of their ability to modify their physical, chemical, and biological environment, seagrasses are often referred to as "ecosystem engineers" (Bos *et al.* 2007).

Instead of increasing in importance, seagrass has declined over the past century for a variety of reasons (Krause-Jensen *et al.* 2021). Global seagrass degradation is accelerating due to coastal expansion, urban and agricultural runoff, untreated sewage and industrial waste discharges, rising sea surface temperatures, high temperature events, and seagrass degradation (Grech *et al.* 2012). Unfortunately, only 37% of recent seagrass restoration efforts have been effective (van Katwijk *et al.* 2016). In many areas, citizens and local governments are involved in activities such as seed dispersal and seedling planting to restore eelgrass beds; however, the low germination rate of eelgrass has become a significant problem (Marion and Orth 2010). The objective of this study was to investigate the effects of various plant growth regulators on the germination rate of eelgrass seeds under aseptic conditions, with the aim of identifying the hormonal treatments that significantly increased the germination rate and also support eelgrass regeneration activities.

Eelgrass seeds were collected from Lake Hamana in Kosai city (34°69'26.2"N 137°58'17.7"E 0 m a.s.l.). After collection the seeds were kept in the glass bottle with artificial seawater at 4°C for 64 days.

Seeds were removed from the bottle and then rinsed with sterilized artificial seawater for 1 min, followed by 70% ethanol for 30 sec, and again rinsed with sterilized artificial seawater for 1 min. The bottles were then washed with 0.1% chlorine for 1 min (3 times) and rinsed with sterilized artificial seawater for 1 min. Provasoli Enrich Seawater (PES) media was used to improve the germination of eelgrass (Provasoli 1968).

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The glass bottles were filled with culture medium by spreading sea sand to a depth of 1 cm and sterilized by autoclaving. Then the sterilized seeds were placed on the sand and the incubation was started by adding plant growth regulators (PGRs). All cultures were incubated at 20 ± 2°C (MIR-154-PJ, PHCbi, Japan).

The eelgrass seeds were collected from flower buds and stored at 4°C after the immature, green, and white seeds were removed. During this time, some seeds germinated at 4ºC. Intact seeds were further selected for the experiment by excluding those that had germinated. Under the experimental conditions (20°C), the germination rate in natural seawater was significantly lower at 8% (27/340) without using any plant growth regulators (PGRs).

Initial attempts to germinate seagrass seeds on solid media with the basic Murashige and Skoog (MS) plant salt mixture supplemented with PGRs yielded very low germination rates. To determine the optimum amount of PGRs for improving the germination rate of seagrass, different concentrations of PGRs, GA_3, auxin (2,4-D and NAA), were used in this experiment. Germination was improved up to 55% with some combinations of PGRs (Fig. 1). The highest percentage of germination (80%) was induced at concentrations of 10 µM of GA_3 and 2,4-D. In another combination of PGRs with GA_3 20 µM and NAA 10 µM a relatively low percentage (65%), whereas only GA_3 at 20 µM concentration showed 75% germination (Fig. 2). Length and number of leaves and roots showed the same trend as germination rate. The highest germination rates at 10 µM GA_3 and 2,4-D resulted in the most elongated leaves (50.5%) and roots (142.4%) (Figs 1D, E and 3). After germination, the seedlings grew rapidly, reaching 3-4 cm in one week (Fig. 1D). Within one month, they had grown to more than 8 cm, whereas untreated plants were 5.8 cm (Fig. 1E).

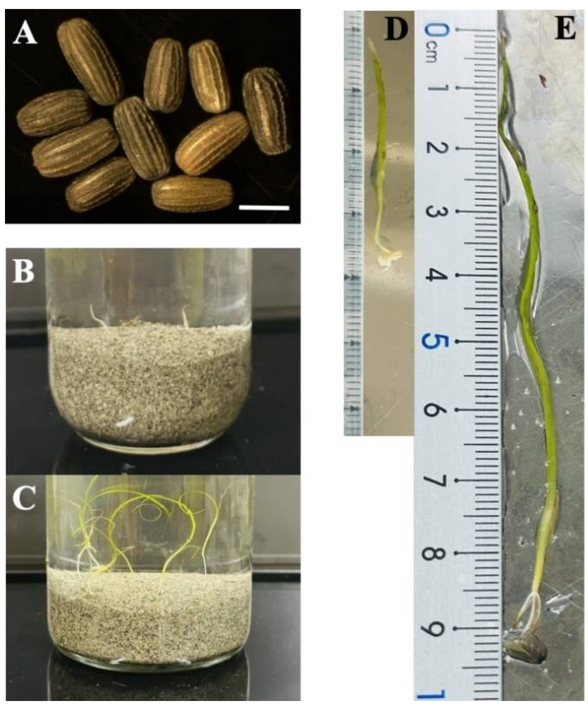

Fig. 1. Germination of eelgrass seed with plant growth regulators. A: Mature eelgrass seeds, B: Germination induced in normal seawater, C: Germination induced in PES medium with PGRs (10 µM GA_3 and 2,4-D), D: Representative photo of the seedling grown after 1 week and 1 month (E).

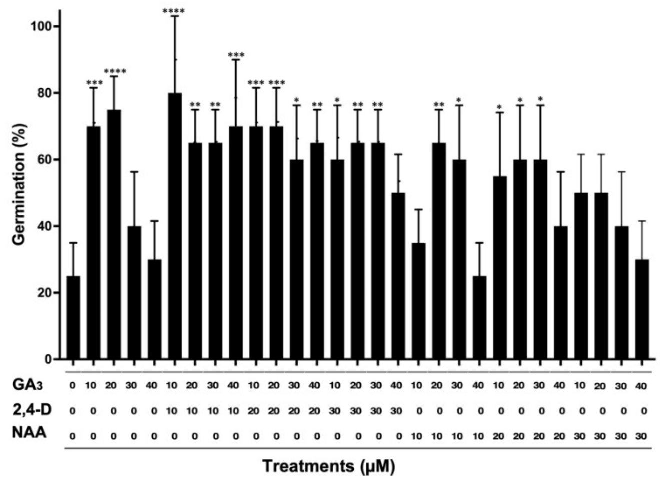

Fig. 2. Percentage of seed germination of eelgrass in PES media with different concentrations of GA_3, 2,4-D and NAA. n = 4,* $P \leq 0.05$, ** $P \leq 0.001$, *** $P \leq 0.0001$, **** $P \leq 0.00001$.

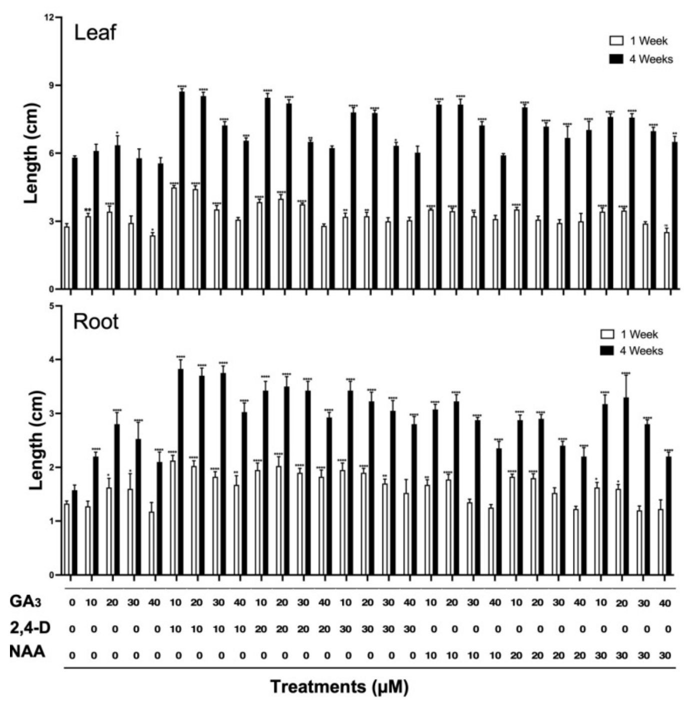

Fig. 3. Seedling growth of eelgrass in PES media with different concentrations of GA_3, 2,4-D and NAA. n=4, * $P\leq 0.05$, ** $P\leq 0.001$, *** $P\leq 0.0001$, **** $P \leq 0.00001$.

In the present experiment, germination of eelgrass seeds was successfully achieved by using PES media and combinations of PGRs. It was found that the good combination of PGRs for eelgrass germination was GA_3 and 2,4-D.

Eelgrass is an important seagrass in coastal communities. The necessary conditions to accelerate the germination rate of eelgrass seeds by 10 times (from 8 to 80%) were identified. Restoration of eelgrass beds can be more efficiently promoted by planting seedlings that have germinated under artificial conditions, rather than by directly spraying seeds. Achieving a higher germination rate will enhance the eelgrass planting activity worldwide.

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