

Studies on Physiological Response to Salt Stress of Eelgrass (*Zostera marina* L.)

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Abstract

In lab conditions, study on physiological response of eelgrass in different salinities (0, 10, 20, 30, 40, 50, 60). Take permeability of plasma membrane, proline content and chlorophyll content as parameters. The results show, (1) High salinity leads to high permeability of plasma membrane making intracellular material spreads to external environment which cannot maintain the normal physiological activity, (2) with the increasing of salinity, proline is accumulated in eelgrass in a large scale, which has a toxic action on eelgrass, (3) with the increasing of salinity, the content of chlorophyll descends. Comprehensive the above results, (1) the suitable salinity range for eelgrass living is 10-30, (2) the most suitable salinity for eelgrass living is about 20, (3) when the salinity is greater than 40, eelgrass cannot grow normally, (4) when the salinity is greater than 60, the plasma membrane is seriously damaged and it has a high permeability, the cells of eelgrass cannot maintain the basic physiological activity.

Keywords: Eelgrass, Salt stress, Physiological response

1. Instruction

Eelgrass meadows are highly productive components of estuarine and coastal ecosystems and support large and diverse faunal assemblages (Thayer, 1984; Heck, 1995). Eelgrass plants filter and retain nutrients from the water column (Short, 1984), provide sediment stabilization (Ward, 1984), and baffle wave energy (Fonseca, 1986), thereby reducing erosional forces and protecting adjacent shorelines (Christiansen, 1981). The study of seagrass trends and the ecosystem services they provide raises an active interest in seagrass conservation and restoration in the last two decades (Kenworthy, 2006).

A number of surveys in physical, chemical and biological factors influencing survival, growth and reproduction of eelgrass and may also affect its overall population dynamics and distribution. Light is the single most important factor for eelgrass growth and survival and the role of light has been relatively well studied (Dennison, 1986; Dennison, 1987; Olesen, 1993) as has the relationship between light availability and depth limit of eelgrass and other seagrasses (Duarte, 1991; Nielsen, 2002). The effects of salinity on eelgrass performance have, in the other hand, been less studied. Estuaries are typically affected by variations in fresh water inputs from precipitation, rivers and surface run-off. Changes in water level can also be substantial due to winds and tides (Conley, 2000) and most estuaries are therefore tend to large and sometimes rapid changes in salinity (Lartigue, 2003). Plants living under such conditions must be able to cope with a wide range of salinities. In recent years, researchers have studied the response to salt stress in Morphology, microstructure, molecular levels.

The purpose of this study is to investigate how various levels of salinity affected the cellular structure and physiology of eelgrass in order to establish the relationship between eelgrass performance and salinity. Eelgrass plants were exposed to 7 levels of salinity for one week in separate culture experiments where membrane permeability, proline content and chlorophyll content were measured as response parameters. Combined with all the response parameters, we can assure the optimum salinity for eelgrass to provide references for cultivation and restoration.

2. Methods

2.1 Preparation of Plant Materials

Individual shoots of *Z. marina* L. were collected from Huiquan Bay, Qingdao, China. Plants were carefully removed by hand to insure intact rhizome-systems and subsequently transported to laboratory in a 60L plastic box with seawater. Cultivation temperature was $15 \pm 2^\circ\text{C}$ with natural light. Seawater changed every other day.

2.2 Setup of Experiment on Salt Stress

After preculture for a week, 15 eelgrass plants were planted in each of 21 aquaria that were subjected to 7 levels of salinity (0, 10, 20, 30, 40, 50 and 60). Cultivation temperature was $15 \pm 2^\circ\text{C}$ with natural light. Seawater changed every other day. Physiological parameters (permeability of plasma membrane, proline content and chlorophyll content) were measured after a week.

On determining the levels of salinity, I referred to Nejrup L B (2008)'s setting (2.5, 5, 10, 15, 20, 25, 30 and 35), which was low in gradients, there were no obvious differences between many adjacent treatment groups. However, Ye Chunjiang's (2002) setting (30, 45 and 60) was high in gradients without treatment groups of under seawater salinity. Therefore, combined

two settings, the level of salinity were determined as above.

3. Results

3.1 Effect of Salt Stress on Permeability of Plasma Membrane of Eelgrass

It shows that permeability of plasma membrane of eelgrass varies as the salinity changes. The level of permeability of plasma membrane can reflect the damage of membrane, the higher the permeability is, the heavier the damage is. When the salinity is 0, permeability is lowest, as the raise of salinity, permeability increases which indicates that the level of damageraises. When salinity is more than 30, permeability increases significantly. When salinity is more than 50, permeability is four times of that when salinity is 0, the plasma membrane has damaged heavily.

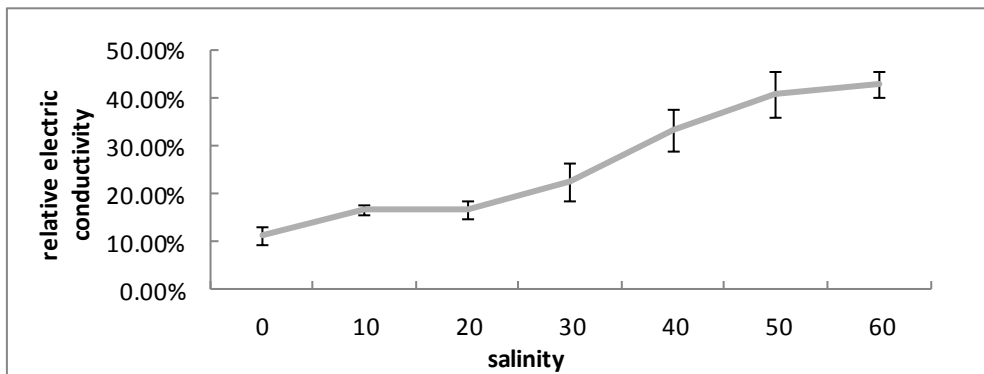


Figure1. Effect of salt stress on relative electric conductivity of eelgrass

3.2 Effect of Salt Stress on Proline Content of Eelgrass

It shows that proline content of eelgrass varies as the salinity changes. When salinity is 0, proline content is relative low, as the salinity raises, proline content increases continuously. When the salinity is 20-30, proline content reaches the highest level. Then, with the salinity goes on rising, proline content tends to go down. When the salinity reaches 60, proline content is almost 0.

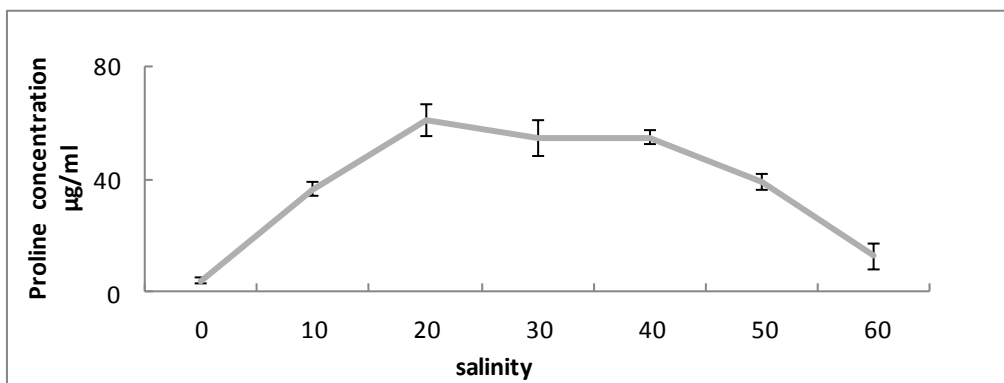


Figure 2. Effect of salt stress on proline content of eelgrass

3.3 Effect of Salt Stress on Chlorophyll Content of Eelgrass

It shows that chlorophyll content of eelgrass varies as the salinity changes. When the salinity is 0, chlorophyll content is high, as the raising of salinity, chlorophyll content tends to increase at first and then decrease. When the salinity is more than 20, chlorophyll content drops obviously. When the salinity is 60, the chlorophyll content is a half of it in the salinity of 20.

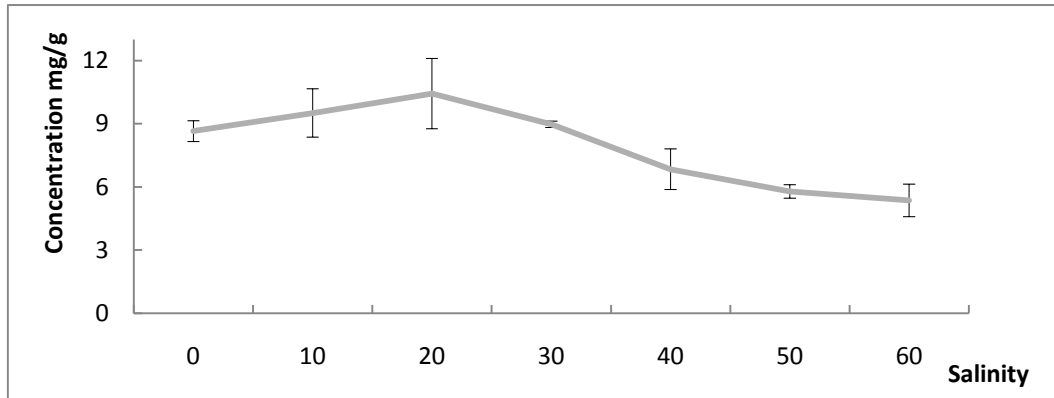


Figure 3. Effect of salt stress on chlorophyll content of eelgrass

4. Discussion

Cell membrane is the basic structure to maintain the normal physiological of cells, which is the initial structure to accept the change of the external environment (Liu, 1987). Thus, the membrane permeability is one kind of indicator response to the damage of cell membrane, which the high permeability of membrane means the high damage of cell membrane. Qiu (1999) made the conclusion that the change of cell membrane structure is the initial response of the plant to the osmotic stress when he was doing the research of osmotic stress to wheat root. In the same time, Vigh (1993) hold the view that membrane fluidity and permeability was changed by severe environment. In this experiment, we got the conclusion that the cell membrane of *Z. marina* was significantly affected by salt stress, which limited the transportation of some indispensable material, then made further influence to the growth of *Z. marina*. We also found that the damage of salt stress to *Z. marina* was deepen by the high salinity, which had the same result with Qiu (1993) and Ming (2011).

Proline is a kind of important material to regulate osmotic pressure, and it can reflect the stress tolerance ability of plants in some degree (Ming, 2011). From our experiment, we found that when the salinity wasn't very high, with increasing of salinity, the content of Proline was increased, which revealed that proline is one kind of material to regulate osmotic. However, when the salinity is beyond 40, the content of proline in cells decreased significantly, which was different from the result of Ming (2011). In her experiment, she found that the proline maintained at a high state even the salinity was 52.5. This difference might cause by the different experiment material, experiment method and different experiment environment. We can get the conclusion from our experiment that proline is really a good kind of material to regulate osmotic pressure, and the ability of regulating osmotic pressure is limited, when salinity is beyond 40, the cell membrane was damaged and a lot of prolines were eudated from the cell, which got severe damage to the cell.

Salt stress could reduce the efficiency of photosynthesis, thus inhibit the growth of plants (Muns, 1993). Ye (2002) found that the photosynthesis efficiency of *Z. marina* decreased significantly. Previous study showed that chloroplast was one of the most sensitive organelle of plant in high salt stress and photosynthesis pigments were the most basic material in chloroplast (Strogonov, 1973). The increased of salinity, the activity of some enzyme in *Z. marina* enhanced, which accelerated the degradation of photosynthesis pigments, then influenced the photosynthesis efficiency, and inhibited the growth of *Z. marina*. When the salinity was between 0 and 30, there was no significant influence to the content of chlorophyll. On the contrary, when the salinity was greater than 40, the content of chlorophyll decreased significantly. That means when the salinity was greater than 40, the *Z. marina* was hard to growth even to live. To explain this phenomenon, Barnabas held the view that in high salinity, the activity of chlorophyll enzyme enhanced, which increased the degradation of the chlorophyll. However, we have a hypothesis that the decreased of chlorophyll may have some relation to the increased of permeability of cell membrane.

5. Conclusion

Through the determination of the above physiological indexes, we got the result that the salt stress influenced the growth of *Z. marina*. High salinity can make the plasma membrane permeability increased, intracellular material outward diffusion unable to maintain normal physiological activities. The reducing of the chlorophyll content, would reduce photosynthesis, thus affected the normal growth of *Z. marina*. From the above experiment results, we make the conclusion that the appropriate salinity of the *Z. marina* growing range from 10 to 30, optimum salinity in 20. When salinity was greater than 40, it was not suitable for *Z. Marina* to growth. When the salinity is greater than 60, *Z. marina* cell membrane was severe damaged, the permeability of cell membrane was enhanced, unable to maintain the basic physiological activities of *Z. marina*.

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