Form 2

Dissertation Abstract

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Dissertation title	EFFECTS OF TEMPERATURE CHANGES ON STRESS-DEFENSE RELATIONSHIPS AND COMPETITIVE CAPACITY OF SUBMERGED MACROPHYTES (沈水植物のストレス耐性及び競合に対する水温変化の影響)		

Abstract

Submerged macrophytes; which are usually rooted in the bottom soil with the vegetative parts predominantly submerged play key roles in biological structure and functions of freshwater ecosystems. Therefore, diversity, density and the distribution of submerged macrophytes can be used as effective indicators for the ecosystem health. The natural environment for submerged macrophytes is composed of a complex set of abiotic factors and biotic factors. Any kind of significant deviation whether rising or falling in those abiotic factors beyond optimum levels for a period of time sufficient to cause remarkable changes in plant growth and development is generally termed as abiotic stress. Because of the sedentary nature, plants are highly sensitive to abiotic stresses and different types of morphological and physiological changes appeared according to the condition. The degree of survival in particular plant species under the effects of environmental variables depend on the way that how do plants respond to a particular stress. Generally, the plants themselves develop several defense mechanisms to cope with abiotic stresses and the type and the magnitude of those defense mechanisms depend on the plant species and the abiotic stress factor.

Temperature stresses, which include low and high temperatures are considered a major abiotic stress, which shape the structure and function of submerged macrophytes. However, comparatively lesser attention has been paid to evaluate the effects of different types of temperature stresses and detailed mechanisms behind the thremotolerability of submerged macrophytes, in spite of the commonly cited changes in morphology, growth performance, photosynthesis and cellular respiration

Sudden and gradual increases of temperature in aquatic environments play important roles in determining growth and physiological dynamics of aquatic macrophytes. However, comparatively lesser attention has been paid to identifying the different types of temperature regimes that mediate oxidative stress in submerged macrophytes. Therefore, the 1st experiment focused on comparing the effects of shock and gradual heat stresses on growth, photosynthetic attributes and oxidative damage of Elodea nuttallii by the laboratory maintained two separate experimental setups. A significant decline in shoot elongation coupled with a decline in endogenous indole acetic acid and an increase in hydrogen peroxide was observed in both temperature treatments. These effects were further accompanied by oxidative damage to photosynthetic pigments and cell membrane structures in *E. nuttallii*. Temperature-mediated oxidative stress was significantly pronounced under SHS, which induced the activation of different defensive mechanisms against reactive oxygen species, including anti-oxidant enzymes, secondary metabolites and some osmoprotectants. The present study reveals that temperature-induced oxidative damage was more severe when the temperature increase occurred suddenly. Further, heat acclimation was observed when the plant was exposed to 30˚C under GHS, although this treatment induces significant oxidative stress under 35˚C. Additionally, after the sudden application of 30˚C heat shock, *E. nuttallii* had ability to successfully overcome the stress situation by balancing the production and destruction of ROSs. But, under the application of 35˚C, the plants were able to recover from stress situations.

The 2nd experiment was designed with the hypothesis that heat stress may induce species-specific oxidative damage that determines the competitive capacity of common submerged macrophytes. We conducted two laboratory experiments to simulate mono and mixed cultures of three submerged macrophytes, *E. nuttallii*, *Potamogeton crispus* and *Vallisneria asiatica*, with the application of two heat shock treatments: 30˚C and 35˚C. The considered three species behaved different ways and species-specific variations were obtained. *E. nuttallii* showed a kind of thermotolerance to heat stress, while *P. crispus* and *V. asiatica* significantly suppressed by both magnitudes of heat stresses. The comparative results of mono cultures and mixed cultures showed that *P. crispus* had an advantage in both the control and high temperature treatments as a strong competitor in the mixed culture over the other two species. Further, the competitive capacity of *P. crispus* increased in the moderately high temperature condition compared to the control. Although *V. asiatica* was initially suppressed by competition from *P. crispus*,

with continuous warming, it surpassed the competition. In the case of *E. nuttallii*, suppression by competition further increased with increased temperature.

Low temperature or cold stress, which includes both chilling (<20˚C) stress and freezing (<0˚C) stress is another aspect of temperature. Low, but not freezing temperatures can be considered as one of the most important factors those limits the growth, development and distribution of submerged macrophytes. Generally, low-non freezing temperatures are common in nature and they can damage macrophytes severely. The species-specific variations in the effects of low-non freezing temperatures on the morphology and physiology of submerged macrophytes are in lesser attention in literature. The 3rd experiment was conducted to comparatively evaluate the low temperature induced changes and the mechanisms behind the cold acclimation of two common submerged macrophytes; *E. nuttallii* and *P. cripsus*. The low, non-lethal temperature could have the ability to induce species-specific changes in plant morphometry, photosynthetic pigments and other physiological factors of considered submerged macrophyte species. *E. nuttallii* showed a comparatively higher cold tolerance as plant growth and photosynthetic pigments increased, while oxidative stress decreased towards the low temperature treatments. The cold tolerance of *E. nuttallii* might be mediated by the enhanced production of cryo-protective compounds such as lignin which can strength the plants and the balance between ROSs and the anti-oxidative mechanisms. However, *P. crispus* was highly suppressed by low temperature at which the growth performance and photosynthetic pigments were significantly reduced by owing to the low temperature induced oxidative stress.

The synthesis and accumulation of heat shock proteins (HSPs) play a crucial role in conferring themotorerance to plants. However, there is a significant knowledge gap regarding the species specific oxidative damage induced by heat stress and the HSP mediated tolerance mechanisms for submerged macrophytes. So, in the 4th experiment, changes in growth rate, photosynthetic pigments, reactive oxygen species (ROSs), total protein and the expression of HSP 70 were studied in three common submerged macrophytes; *E. nuttallii*, *P. crispus* and *V. asiatica* subjected two different heat shock treatments; 30˚C and 35˚C. The results indicate that all considered species were affected by the heat stress induced oxidative damage in various degrees. Among the considered species, *E. nuttallii* showed a thermotolerance by increasing the growth rate and chl *a* concentration at moderate heat stress (30˚C). Further, they could have an ability to avoid the damage to the cellular proteins. By contrast, *P crispus* and *V. asiatica* were highly susceptible by the heat stress induced oxidative stress and denaturation of cellular proteins. The expression levels of HSP 70 was more pronounced in *E. nuttallii* under the both moderate and high heat stresses while the expression levels were comparatively low in *P. crispus* and *V. asiatic*a. In *V. asiatica*, the expression levels of HSP 70 increased while the total protein content decreased. In the present study, *E. nuttallii* is the most tolerant species to sudden heat stress and their tolerant mechanisms are principally mediated by the synthesis of HSP 70. But, *V. asiatica* has comparatively low tolerability to cope with high temperatures though they attempt to mitigate the stress by the production of HSP 70.

The results of the present study revealed that the changes in water temperature can make significant impacts which can be either positive or negative. Further, these species-specific impacts can have ability to induce different defensive mechanisms by which the tolerance capacity of plants is determined. Overall, the present findings divulge the significance of dynamics in water temperature on submerged macrophytes, which eventually provide prescience on interactions between submerged macrophytes and water temperature.