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Comparative Study of the Response of Four Native to the Bulgarian Black Sea Coast Psammophytes to Simulated Flooding Experiments

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript. The author read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed 1) to determine and compare the effects of flooding stress, caused by storms on whole plants of four native to the Bulgarian Black Sea Coast psammophytes and how long their rhizomes can remain viable in sea water; and 2) to investigate post-immersion changes in plant biomass and allocation to above- and below-ground biomass in order to determine and compare their capacity as dune stabilizers.
Methodology: Two simulated flooding experiments were conducted. In the first experiment, whole plants were immersed in sea water for 20 days. Visible morphological changes of leaves, stems and roots are recorded and assessed in 12 parameters. In the second experiment, rhizomes were immersed in sea water, were planted and allow growing for one month before harvesting in order to establish rhizomes viability, biomass and root/shoot ratio.
Results: Conducted flooding experiments established that investigated psammophytes were very tolerant to immersion impact and salt stress. Whole plants stay viable longer than the flood with a maximum duration along the Bulgarian Black Sea Coast, and rhizomes were able to regenerate after 30 days in seawater. Statistical analysis of experimental data demonstrates that immersion in

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sea water increases rhizomes viability, biomass and allocation to root biomass, whereas other factors, such as duration of immersion and temperatures of sea water have not significant effect.

Conclusion: Investigated psammophytes show high tolerance to sea water immersion and high viability during the simulated flooding experiments. Investigated species from family Cyperaceae are less tolerant to water immersion than those from Poaceae. According to growth response, *Leymus racemosus* subsp. *sabulosus* demonstrates a high potential to be a key species for dune stabilization, followed by *Ammophila arenaria*, *Carex colchica* and *Galilea mucronata*. All psammophytes could contribute to the protection of coastal sands during storms.

Keywords: Leymus racemosus subsp. sabulosus; Ammophila arenaria; Galilea mucronata; Carex colchica; immersion tolerance; viability; dune stabilization, erosion and flooding control.

1. INTRODUCTION

Coastal areas are very fragile ecosystems that are extremely sensitive to global climate changes, sea level rise and more frequent storm surges [1]. These areas act as a buffer for inland areas and provide habitats for many rare and endangered species [2], which will be lost due to the combination of negative consequences of flooding [3,4], erosion [5] and strengthen anthropogenic impact.

Dune vegetation is formed under the combination of specific site conditions and is dominated by psammophytes [6]. These species form an extensive system of horizontal and vertical rhizomes that reduced wind speeds across the surface, trapping and holding a great amount of sand [7]. Thus, they support sand stabilization and increase the dune's ability to buffer inland areas from erosion and flooding and thereby protect from economic losses in urban areas and agrocoenosis.

Ecosystem services require searching for well adapted, native, salt-tolerant plant species with extensive root systems and studying their ability for erosion and flooding control [8]. Unlike artificial coastal stabilization structures used in coastal protection, the transplanting techniques of plant species can effectively minimize erosion and reduce storm damages with minimal negative impacts to natural ecosystems [9].

The experience of the recorded extreme meteorological events over the Bulgarian Black Sea Coast shows the high potential of the root system of native psammophytes to accumulate sands and prevent from washout [10,11]. Although, the Bulgarian Black Sea Coast is relatively protected from sea floods due to the small amplitude tides [10], some extreme meteorological events, such as unusual storms may cause flooding and erosion of dunes. Storm waves carry away sandy sediments and cause destruction of communities of dominant sand stabilizers *Leymus racemosus* (Lam.) Tzvelev subsp. *sabulosus* (M. Bieb.) and *Ammophila arenaria* (L.) Link [11]. In such cases *Carex colchica* J. Gay and in limited areas *Galilea mucronata* (L.) Parl. become major dune stabilizers and colonize territories from the other two dune pioneers.

The physiological and molecular mechanisms of tolerance to salinity stress are well studied [12,13,14], but our understanding of response is limited to halophytes and agricultural species [2]. Although, psammophytes are able to cope with some degree of exposure to salinity from sea water [15], they are vulnerable and sensitive to the impact of flooding [11]. As an invasive species in North America, New Zealand and West Australia, the buoyancy, salt tolerance and sprouting ability of *A. arenaria* are well studied [16,17]. Previous study of *L. racemosus* subsp. *sabulosus* shows high viability to the stress of flooding in short-term intervals [11]. Data about the impact of flooding to *C. ligerica* and *G. mucronata* are insufficient.

This study aims: 1) to determine and compare the effects of flooding stress, caused by storms on whole plants of four native to the Bulgarian Black Sea Coast psammophytes and how long their rhizomes can remain viable in sea water; 2) to investigate post-immersion changes in plant biomass and allocation to above- and below-ground biomass in order to determine and compare their capacity as dune stabilizers.

2. MATERIALS AND METHODS

2.1 Study Species

Galilea mucronata (L.) Parl. (syn. *Cyperus capitatus* Vand.) is a perennial plant from family Cyperaceae with creeping rhizomes, up to 50 cm high, triangular or nearly rounded stem and basal revolute leaves [18]. The species is included in the Red Data Book of the Republic of Bulgaria as endangered [19]. *Carex colchica* J. Gay (syn. *Carex ligerica* J. Gay, Cyperaceae) is herbaceous, perennial plant with 1.5–2 mm thick creeping rhizomes and shoots arising at intervals. Stems are up to 35 cm high, slender or slightly nodding. Leaves are 1–2.5 mm broad, greyish-green, shorter than stems [20]. These psammophytes are comparatively widely distributed among the fixed dune vegetation cover, occurs in the composition and structure of different phytocoenoses [21] and forms monodominant communities with high abundance and poor floristic composition [11].

Leymus racemosus (Lam.) Tzvelev subsp. *sabulosus* (M. Bieb) Tzvelev (mammoth wildrye) and *Ammophila arenaria* (L.) Link (marram grass) are erect, perennial, rhizomatous grass species from family Poaceae [22]. They are major psammophilous species which dominate sand dunes due to their biological characteristics and their communities have an important role in the formation of the natural vegetation cover of coastal sand strips along the Bulgarian Black Sea Coast.

2.2 Simulated Flooding Experiments

Whole plants and rhizomes of each investigated species are collected in April 2015 and January 2015, respectively. The simulated flooding experiments were based on experimental methods of simulating sea water floods without taking into consideration the direct mechanical effects of storm waves and were conducted in the Botany Laboratory of the Varna Museum of Natural History. All plant materials were immersed in glass tanks (40 l), filled with sea water with constant maintained temperatures (4±1ºC, 13±1ºC and 23±1ºC). The water was changed several times per day.

In the first experiment, ten whole plants were planted in washed and sterilized sand in plastic pots (90x90x80 mm) and were immersed for 20 days. Visible morphological changes of different parts of the specimens (leaves, stems, roots) are recorded and assessed in 12 parameters [11]. This test shows not only the changes and adaptations of investigated species to the stress of flooding in certain short-term intervals and the plant survival, but allows to obtain experimental results for Critical Decomposition Time (CDT), which is a crucial parameter for vulnerability assessments of flooding impacts to coastal plant communities [23]. CDT is defined as the time point at which each plant, submerged in sea water, shows signs of irreversible decomposition of vegetative organs [10] and indicates that the plants will not survive and their communities will not be able to recover after floods. CDT is a parameter that is subjectively determined on the basis of visible morphological changes and shows the smallest degree of decay of plant organs (most often the leaves).

In the second experiment, twenty rhizomes per treatment were removed every fifth day and were planted in washed and sterilized sand in plastic pots (150x150x130 mm) in the glasshouse with controlled air temperature [17]. Control rhizomes were planted directly. Rhizomes were grown with daily watering and natural daylight for one month before harvesting [11]. All plants were cleaned and oven-drying at 60℃ for 24 hours.

2.3 Statistical Analyses

Mean bud viability (MBV) was measured as the percentage of rhizome nodes that produced vegetative shoots and roots [17]. MBV as well as mean dry weight biomass per plant replicate and R:S ratio (Root mass/Shoot mass) were analyzed with one-way analysis of variance (ANOVA). Where necessary, data were transformed in order to obtain homogeneous variances.

3. RESULTS AND DISCUSSION

Different studies and experiments were conducted in order to assess and quantify possible negative consequences to coastal plant species, but most of them are focused only on studying substrate salinity and the effect of salt aerosol. These tests are not applicable to establish the response of psammophytes to flooding due to their regular exposure to sea water [11] and specific mechanisms of neutralizing salt. Therefore, in the present study direct methods were carried out in order to establish the changes and adaptations of investigated species to the stress of flooding in certain short-term intervals and the period of which rhizomes, submerged in sea water still maintain viable buds capable of producing new plants. Exposure time to sea water is considered to be the most important factor which influences the survival ability, viability and growth response of the investigated species [24].

The Black Sea is the largest anoxic water body with a specific lower average water salinity (about 18‰) and average temperature of 13ºC (average minimum of 4ºC and average maximum 23ºC) [25]. Storm events on the Black Sea Coast occur during winter and early spring when average surface sea water temperature is about 4ºC [25]. In order to study the relation between temperature and viability, two other treatments with temperatures of 13ºC (average surface sea water temperature) and 23ºC (average summer surface sea water temperature) were included in the simulated experiments.

No mortality of the specimens were recorded during the course of the first experiment. There were visible morphological changes of different parts of the specimens (leaves, stems, roots) only of six of all 12 parameters. Beginning of the decomposition of leaves of the submerged replicates started from the 140th and 144th hours for *G. mucronata* and *C. ligerica* respectively, and from 168th hours for *L. racemosus* subsp. *sabulosus* and *A. arenaria* (Table 1). According to Vergiev et al. [26] these time points could be designated as CDT for these species. These values are similar to CDT of other psammophytes [26], and are longer than the floods with a maximum duration for the Bulgarian Black Sea Coast [10,27].

On the 168th hours of the experiment, a growth of stems and root sprouts was observed (Table 1). There were no complete decomposition of stems and roots and no visible decompositions of newly grown stems, roots and rhizomes till the end of the experiment (480 hours). All investigated parameters were unrelated to water temperature, contrary to results of sea water submergence of *Crambe maritima* L., *Artemisia vulgaris* L., *Eryngium maritimum* L. Decomposition process of these species was accelerated by higher water temperatures [26].

The second experiment aims to investigate rhizomes viability and growth response of the investigated species. Results show that the rhizomes remain viable in sea water for 720 hours (maximum duration of the simulated flooding experiment). This is in agreement with previous studies of *A. arenaria*, which evaluated its rhizomes viability from 312 to 1680 hours in sea water [11,16,17].

Despite different temperatures, all treatments show identical trends of mean bud viability following time of sea water submergence (Fig. 1). MBV gradually increases and reaches its maximum at the 480th hours of sea water submergence, followed by slightly decrease till the end of the immersion (Fig. 1). All treatments have higher values than mean viability of the

Table 1. Results from simulated flooding experiment (in hours). Visible morphological changes of different parts of the specimens (leaves, stems, roots) assessed in 12 parameters. Data in shaded cells are accepted as CDT

Parameter	Plant			
	C. colchica	***G. mucronata***	***L. racemosus***	***A. arenaria***
Beginning of decomposition of leaves	144	140	168	168
Beginning of decomposition of stems	408	360	n/a	n/a
Beginning of decomposition of roots	n/a	480	n/a	n/a
Complete decomposition of leaves	468	460	480	460
Complete decomposition of stems	n/a			
Complete decomposition of roots	n/a			
Growth of stems	164	168	168	168
Growth of root sprouts	164	168	168	168
Beginning of decomposition of newly grown stems	n/a			
Beginning of decomposition of newly grown roots	n/a			
Complete decomposition of newly grown stems	n/a			
Complete decomposition of newly grown roots	n/a			

** All 12 investigated parameters were unrelated to water temperature*

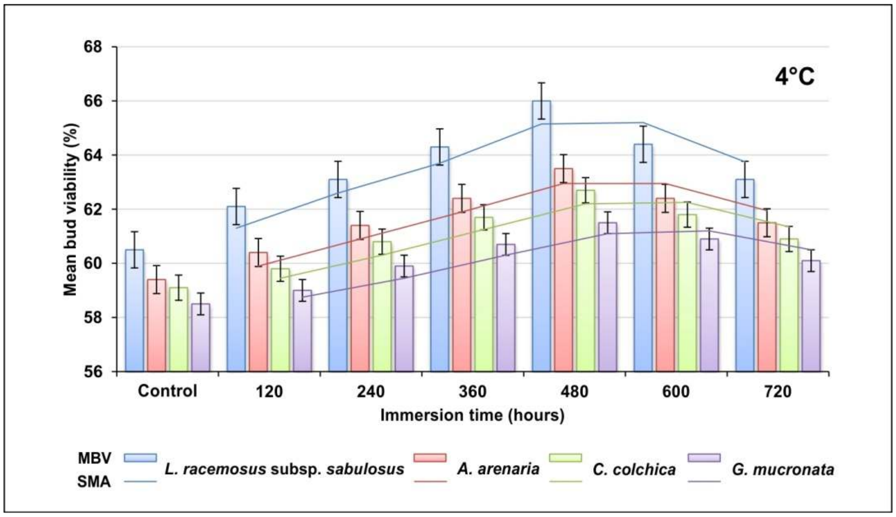

Fig. 1. Mean bud viability (MBV) following sea water immersion
MBV ± S.E.; SMA - Simple Moving Average (MBV were unrelated to water temperature. Graph presents data for 4ºC)

untreated control and appears to be enhanced by sea water ($P < 0.05$). The same trend was observed for mean bud viability of other psammophytes [11].

All treatments had a maximum rhizome bud viability of 100%, which is defined as the bud viability of the rhizome replicate with the highest bud viability for each treatment [17]. Each immersion period, even on the 720 hours of immersion, 80% of rhizomes still had at least one viable bud and 5% of rhizomes had maximum bud viability.

Different temperatures were influenced rhizomes viability in similar levels, and average differences between coolest and warmest temperatures were statistically insignificant. Replicates in all treatments demonstrated higher viability than the untreated replicates ($P = 0.038$). This is contrary to the results of study of *A. arenaria* rhizomes [28], which retained viability for longer in cooler water. So it can be concluded that the water itself as defining factor impact viability more than the temperature of water.

L. racemosus subsp. *sabulosus* demonstrates the highest values of rhizomes viability followed by other species. They can be ranked in descending order: *A. arenaria*, *C. colchica* and *G. mucronata*.

While crucial for rhizomes viability is the cumulative effect on the durability of flooding and sea water temperature, defining factor in ability of psammophytes to fix loose sandy substrates and contribute for dune stabilization is the size of their root systems [24]. In order to measure how immersion affected the root system, the mean dry weight biomass per replicate was taken as well as R:S ratio (Root mass/Shoot mass).

Dry weight biomass was increased by immersion in sea water till 480th hours (Fig. 2) and remained unchanged till the end of the experiment. The water temperature had no significant effect on the biomass of the treated groups. Replicates in all treatments demonstrate higher biomass than the control replicates (Fig. 2).

Comparison of the values of that variable shows that species from family Cyperaceae are increased their biomass less than those from Poaceae following water immersion. Increased biomass enlarges the potential of these species to be a key species for dune stabilization, and could contribute to the protection of coastal sands during storms.

R:S ratio measures plant allocation to above- and below-ground biomass [29]. This variable was not affected significantly by immersion duration ($P = 0.086$). Biomass allocation to roots in plants exposed to sea water was slightly increased. Increased water temperatures tended to decrease the R:S ratio, but the effect was insignificant (Fig. 3). Replicates in all treatments demonstrate higher biomass than the control replicates. All investigated species demonstrates similar values of this parameter.

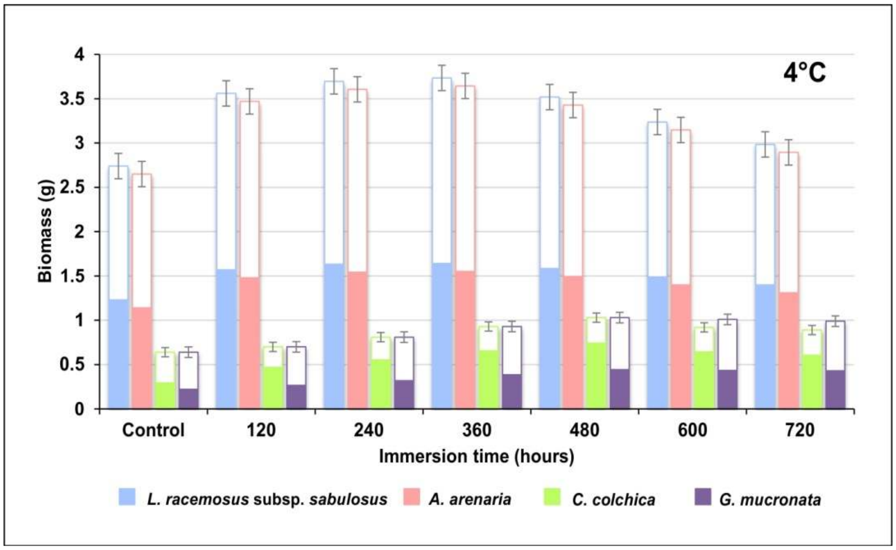

Fig. 2. Effects of sea water immersion on dry weight biomass. The shaded portion of the bars represents underground biomass; blank portion represents belowground biomass

Dry weight biomass ± S.E (Biomass were unrelated to water temperature. Graph presents data for 4ºC)

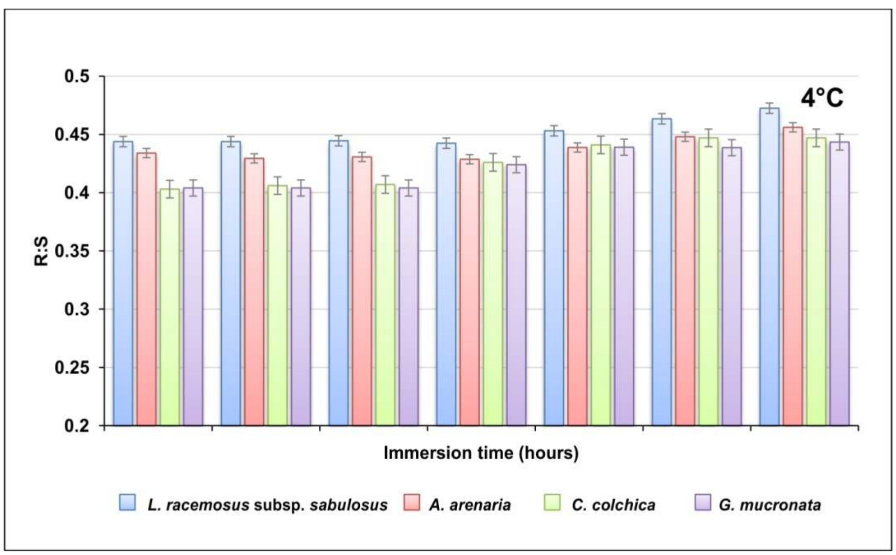

Fig. 3. Effects of sea water immersion on R:S ratio

R:S ratio ± S.E. (*R:S ratio were unrelated to water temperature*. *Graph presents data for 4ºC)*

In the present study, all four species tended to increase biomass allocation to the roots following time of sea water submergence compared to the untreated controls, but the values are not statistically significant.

4. CONCLUSION

Investigated psammophytes show high tolerance to sea water immersion and high viability during the simulated flooding experiments. Investigated species from family Cyperaceae are less tolerant to water immersion than those from Poaceae. According to growth response, *L. racemosus* subsp. *sabulosus* demonstrates the highest potential to be a key species for dune stabilization, followed by *A. arenaria*, *C. colchica* and *G. mucronata*. All psammophytes could contribute to the protection of coastal sands during storms.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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