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Root Phenotype Identification in *Spartina (Poaceae)* by Means of Marker-Controlled Watershed Segmentation of Computer Tomography (CT)-Scan Images

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Plant root phenotypes develop in response to abiotic and biotic factors, such as nutrient availability, abiotic stressors (e.g. waterlogging, drought), life history traits, livestock grazing and competition, and different root phenotypes may play a key role for rhizosphere processes. In this study, we use medical computer tomography (CT)scanning to visualize the root structure of intertidal salt marsh grasses (Spartina spp, Poaceae) investigating the role of phenotypic plasticity and genomic differences. Spartina can adapt to waterlogged soils with extensive aerenchyma development enhancing oxygen supply to root cells. Large differences in belowground biomass has been observed among closely related Spartina grasses as well as among clones of the same Spartina taxon from different habitats. However, little is currently known about differences in the 3D structure and aerenchyma volume among species and habitats. We hypothesize that plants from closely related Spartina taxa show different root phenotypes in response to comparable abiotic conditions. Medical computer tomography (CT)-scanning facilitate non-destructive 3D-analysis of the root-system in *Spartina* down to the size of coarse-roots (≥ 1 mm in diameter). Marker-controlled watershed segmentation was used to distinguish coarse-roots and rhizomes from soil particles in CT-scan images. We applied our method to samples from monospecific Spartina stands in native soil, i.e. the F1-hybrid Spartina x townsendii, its genome duplicated descendent Spartina anglica, and its parental taxa Spartina maritima and Spartina alterniflora. The F1-hybrid and its genome duplicated descendant share the same genome with low genetic variability. In consequence, differences in root morphology should reflect phenotypic plasticity rather than genomic differences. The plants for this study were collected along the European Wadden Sea and the north-west French Atlantic coast in different habitats characterized by tides, high salinity and periodic waterlogging. Our study demonstrates that marker-controlled watershed analysis of CT-scan images is a suitable method to describe the 3D-structure of Spartina roots, and we present visualizations of root system reconstructions portraying root phenotypes among different Spartina species at different soil depth. We report on differences in root volume, aerenchyma volume and root surface area at different soil depths.