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Microscopic Analysis of the Structure and Function of Silica Storing Cells in *Arundo donax* Leaves

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With diminishing supplies of fossil fuels, research is increasingly focused on renewable energy sources. Perennial grasses are highly promising alternative sources of energy, and one in particular, *Arundo donax,* has several features that especially suit it for this use (Fig. 1a). This unique plant has an elevated biomass yield when irrigated with waste water [1], and it is a major agent for environmental remediation having a propensity for absorbing pollutants/toxins from the soil. Silica has been reported to reduce abiotic stress (e.g., salt stress) in certain crop plants, but the mechanism for its action is not well understood [2]. Leaf structure in *Arundo donax* is peculiar in that clusters of silica cells join in continuous strands situated between the typical parallel veins, and whatever mechanism is in effect, silica may play a role in salt tolerance. The investigation reported here was directed toward a characterization of salt tolerance and its relationship to silica accumulation in *Arundo donax.*

To determine the role of the silica cells under salt stress, salt tolerant and non-salt tolerant ecotypes (designated as 'Gt' and 'Bl' and collected from a high salinity and non-saline environment respectively) were grown on 0%, 0.5% and 1% NaCl in a hydroponic environment. After two months, samples were prepared for Environmental SEM and bright-field microscopy studies. Leaf cross sections were prepared with a Hydro-microtome (Fig.1b) a new instrument for cutting fresh sections of roots, stems, and leaves. The Hydro-microtome is a unique fresh-section microtome that disallows the sample sections to be exposed to air during the process, thus protecting the structural integrity of the sample [3]. The presence and the amount of silica in these cells were compared between the two ecotypes by Tescan Vega3 SBU Variable Pressure and FEI Quanta 200 SEM outfitted with Energy Dispersive X-ray Spectroscopy (EDX) analysis. Bright-field microscopy was used to examine the structure of these cells.

Growth was reduced in both ecotypes on 0.5% and 1% NaCl but was less reduced in 'Gt' ecotype. The strands of silica storage cells situated between the vascular bundles are flanked by adjacent rows of photosynthetic mesophyll (Fig 2a,c). In cross section, each cluster consists of 3-5 colorless cells of unequal size but all larger than cells of the adjacent mesophyll (Fig. 2b, 3a,b). In the 'Bl' ecotype, silica concentration in the storage cells was approximately 20% higher in plants grown in a 1% saline environment than in those grown in a saline-free environment. By contrast, in the 'Gt' ecotype silica concentration was 3 times higher in a 0% than in a 1% saline environment (Fig. 3c).

Our previous studies showed that salt tolerant ecotypes exclude sodium ions from stems and leaves**.** EDS (Energy Dispersive X-ray Spectroscopy) analysis shows that silica accumulation is greater in the non-salt tolerant ecotype under salt stress than in the salt tolerant ecotype not under salt stress. Data suggest that in the 'Gt' ecotype silica concentration is significantly decreased in plants grown in a 1% salt environment but not in those grown in a saline-free environment. Whether and by what mechanism silica influences salt tolerance in *Arundo donax* requires further investigation.

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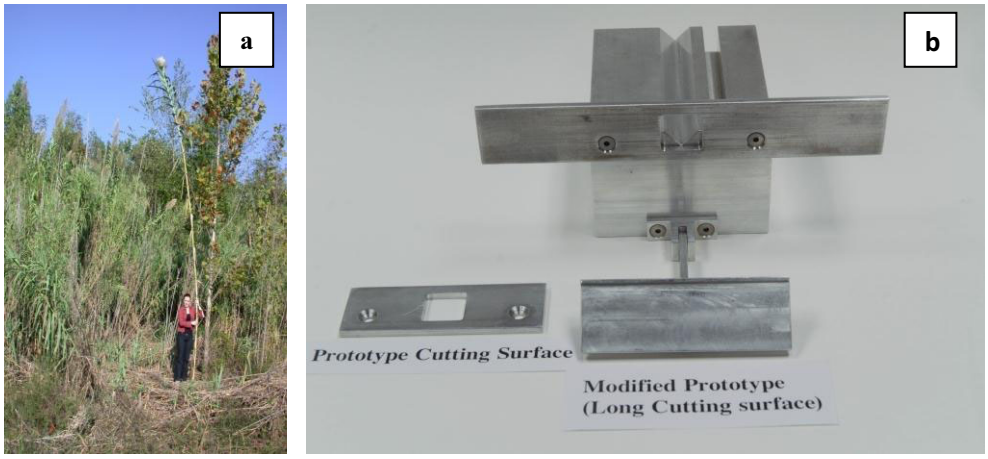

Figure 1. Field image of *Arundo donax* (a). Image of the Hydro microtome (b).

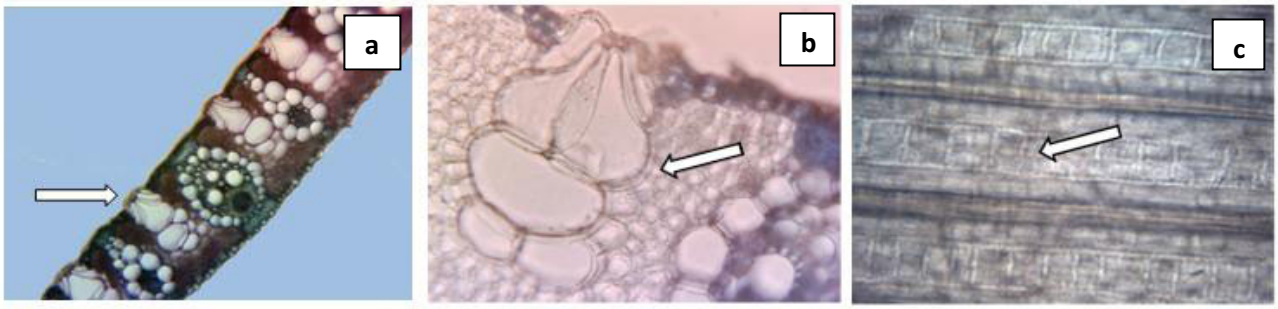

Figure 2. Bright field microscope image of cross section (a,b) and optical sagittal section (c) of *Arundo donax* leaves. (Arrows showing silica cells)

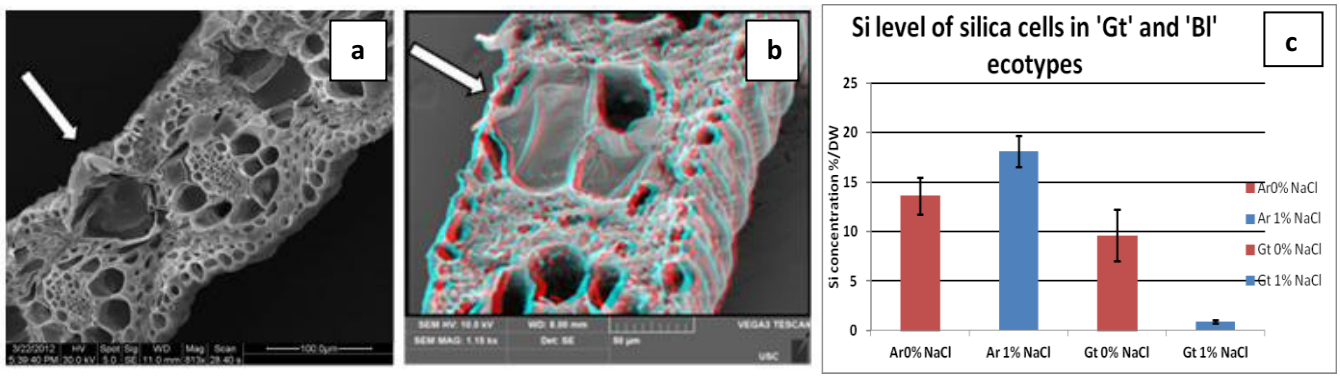

Figure 3. SEM images of longitudinal section of *Arundo donax* leaf sections (a,b). EDS analysis of Si levels in silica storage cells from 'Gt' and 'Bl' plants grown on 0% , 1% NaCl (c). Note: 3b is a 3 dimensional image

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