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SHORT COMMUNICATION

Composition and localization of mucilage under short term NaCl stress in *Salicornia brachiata* Roxb. and *Suaeda maritima* (L.) Dumort.

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The present study reports the localization and characterization of mucilage contents in two extreme halophytes, ***Salicornia brachiata*** Roxb. and ***Suaeda maritima*** (L.) Dumort. under short term NaCl stress. Halophytes that have the capacity to accumulate and exclude salt can be effectively utilized in salt affected area and a source of essential natural products. Plants under study were grown in hydroponic culture medium with or without NaCl as mucilage is thought to play a role in salinity tolerance. The study evaluated structural localization of mucilage to reveal their cellular characteristic in the presence or absence of salt and analyzed mucilage using gas chromatography. The major monosaccharides observed in ***S. brachiata*** were fucose, arabinose, mannose, galactose and glucose whereas in ***S. maritima,*** fucose, mannose, galactose and glucose were the major components. It was observed that ***S. brachiata*** and ***S. maritima*** showed highest crude mucilage of 14.8±0.98 and 12.5±1.4 %, respectively. The studied halophytes can be used as the potential source of mucilage by using saline soil.

Keywords: Cells, Halophyte, Monosaccharide, NaCl stress, Staining.

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Introduction

Many succulent and non-succulent plants produce releasable mucilage in their tissues, which is a type of soluble fiber having viscous nature. The mucilage may function as a healing agent, casually or in the practice of traditional-folk or conventional medicine[1]. It is found in various parts of the plants where it functions primarily as reserve energy material[2]. It is also produced by seeds of many plants and has an important role in the germination; upon contact with water, its volume increases and maintains a layer of moisture around the seed thereby facilitating germination. Mucilage in plants plays a significant role in the storage of water and food as well as thickening of membranes. The natural plant based materials are gaining importance because they are renewable and if cultivated or harvested in a sustainable manner, they can provide a constant supply of raw materials[3]. The plant based polymers have been studied for their application in different pharmaceutical dosage forms like matrix controlled system, film coating agents, buccal films, microspheres, nanoparticles, viscous liquid formulations like ophthalmic solutions and suspensions[4-6]. Mucilage has been used efficiently for many years in the food and pharmaceutical industries[7]. Achene mucilage presumably plays an important role in the life cycle of ***Artemisia shaerocephala*** by adding germination in osmotically and saline stressful habits of the scold desert environment[8].

The high water binding capacity of mucilage plays a crucial role in the drought resistance of certain plant species. Mucilage is also involved in moisture uptake from the environment and plays an important role in the water supply to the apical part of the salt tolerant species and increase mucilage content in salt stress plant[9]. However, Golezani ***et al***[10] studied the seed and mucilage yield of ***Isabgol*** (***Plantago ovata*** Forsk.) under salinity stress and concluded that seed and mucilage yield per plant decreases with increasing salinity. Since these are the two different hypothesis regarding the salt stress effect on mucilage content of the plants, it is crucial to study this type of phenomenon in salt accumulating plants. Mucilage in the root apex can also be protective against the Aluminum (Al) toxicity[11]. Geng ***et al***[11], observed that presence of mucilage does protect the root apex from Al toxicity by immobilizing Al in high molecular weight polysaccharides. Due to their sustaining capacities, binding and gelling properties mucilage has been proposed as a most valuable material to the modulating drug delivery[12]. Since mucilage has great ecological importance and wide range of application, it is important to investigate different plant species as new sources of mucilage content and composition of

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constituent monosaccharide's in the mucilage. Also halophytes will not compete with conventional agricultural crops for valuable resources like fertile soil and fresh water. Thus, the present study examined the mucilage contents and its localization in highly salt tolerant plant species i.e. *Salicornia brachiata* Roxb. and *Suaeda maritima* (L.) Dumort. Study involves short salt stress treatment on the chemical composition and abundance of crude mucilage extract.

Materials and Methods

Plant materials and culture condition

Seeds of *S. brachiata* and *S. maritima* were collected from the field (Victor Port, Near Mahua, Bhavnagar, Gujarat) at N 20° 58.312' latitude and E 071° 33.55' longitude. The plants were identified based on a monograph on Indian halophytes[13]. Seeds were germinated in the pot containing garden soil for about two months. The healthy seedlings were harvested and grown in Hoagland's nutrient medium. The plants were allowed to acclimatize for a week in the hydroponic under 12 h photoperiod. The nutrient solution comprised of the following chemicals (in mM): 2.0 KNO_3, 1.7 $Ca(NO_3)_2$, 1.0 KH_2PO_4, 0.5 NH_4NO_3, 0.5 $MgSO_4$, and (in µM) 17.8 Na_2SO_4, 11.3 H_3BO_3, 1.6 $MnSO_4$, 1.0 $ZnSO_4$, 0.3 $CuSO_4$, 0.03 $(NH_4)_6Mo_7O_{24}$ and 14.4 µM Fe-EDDHA[9]. After one week, the plants were treated with NaCl concentration (0, 50, 100 and 150 mM). The medium was changed every alternative day and after the 10th day, plants were harvested for localization and crude mucilage analysis.

Localization of mucilage

Mucilage staining experiment was carried out with the fresh stem and leaves. The transverse section (TS) of about 10µm thickness was cut by microtome (MT-3, Nippon, Japan). Aqueous solution (0.5 %) of Alcian blue ($C_{56}H_{68}Cl_4CuN_{16}S_4$) 8GX (Himedia, India) at pH 3 has been used for the localization of mucilage in the plants sections[14]. Alcian blue is a basic dye most often used to stain acidic mucosubstances[15]. Tissues were tested for the presence of mucilaginous substances by staining with alcian blue[16]. The sections were incubated in 0.5 % of the alcian blue solution for about 2 h, after that the section was washed with distilled water to remove excess stains for examination. The TS were examined with Axioimager M1 microscope (Carl Zeiss) and photographed with an AxioCamHR camera on the same microscope in air and under immersion oil for higher magnification.

Analysis of crude mucilage

The shoot part of the plants were collected and washed 2-3 times with deionized water to remove the debris and oven dried at 60 °C for 48 h. Dried shoots were powdered with morter and pestle and passed through 250 µm sieve. The method followed for extraction of crude mucilage was as per Ghanem *et al*[9]. One g powder with 100 mL of ion free water was kept for continuous stirring at room temperature for about 40 h. Centrifugation was carried out at 20,000 g for 10 min at 4 °C, supernatant was removed and stored at 4°C. The remaining pellets were again re-extracted with water following the same procedure three times. Finally, the aqueous solutions were combined and concentrated to 30 mL by evaporation (Rotavapor, Buchi). This 30 mL solution was poured into 120 mL of a mixture of 96 % ethanol and 1 % acetic acid. The precipitate obtained was washed with cold ethanol and lyophilized (Virtis freeze mobile) and the dry weight of the mucilage was determined.

The neutral monosaccharides in the crude mucilage were analyzed by Gas chromatography (GC- 2010, Shimadzu) following Foster *et al*[17] after conversion of hydrolysates into alditol acetates. Weak acid hydrolysis of the crude mucilage was performed by adding 250 µL 2M trifluoroacetic acid (TFA) to 2 mg of crude mucilage into 2 mL of the glass tube. The cap was tightened and incubated for 90 min at 121 °C in a heating block. Cooled and centrifuged the tube at 10,000 rpm for 10 min. About 100 µL of acetic supernatant containing monosaccharide was transferred to fresh glass tube and TFA in the tube was evaporated under gentle stream of air in a concentrator (MD 200, Sample concentrator, China). About 300 µL of 2-propanol was added to the tube, vortex and evaporated at 25 °C three times. The first step of the alditol acetate derivatization procedure involved reduction of the monosaccharides to their corresponding alditols. For this purpose 200 µL of sodium borohydrate solution was added to the dried sample and the glass vial was kept at room temperature for 1.5 h. The solution was neutralized by adding 150 µL of glacial acetic acid. For acetylation of alditols, 50 µL each of acetic anhydride and of pyridine was added, vortex and incubated for 20 min at 121 °C in a heating block. Sample was

cooled and the reagent was evaporated under gentle stream at room temperature. Finally the alditol acetate was extracted in ethyl acetate by adding 500 µL of ethyl acetate and 2 mL of water, the tube was centrifuged at 2000 rpm for five minutes to get the clear layers of ethyl acetate on top and water on bottom. About 50 µL of the ethyl acetate layer was diluted with acetone in GCMS vial. One µL sample was injected into GC equipped with BP-225 column (25m × 0.25mm × 0.25µm). Helium was used as a carrier gas at constant flow rate of 1 mL/min and the injector temperature was set at 230 °C.

Results and Discussion

Localization and composition of mucilage

The shoot T.S. of *S. brachiata* was round in shape. The epidermal cells were large and formed single layer ring, both under control (Plate 1a) and salt stress (Plate 2a) plants showed intense blue color and presence of blue precipitate on the surface of epidermal cells. Clifford *et al.*[14] also reported that upper epidermis contain large amount of mucilage in *Ziziphus* species. The single layer of green densely arranged epidermis palisade tissue also showed to contain large quantity of mucilage in stress plant whereas palisade tissue appear green. The findings of this study corroborate with the results of earlier reports for spongy mesophyll layer of *Hemizonia luzifolia*[18]. Below palisade layer, several layers of blue coloured parenchymatous cells (also known as water storage tissue), were also observed in stress as well as control plant. The last layer of parenchymatous tissue was thin walled and elongated endodermal cells. After epidermal cells, a single layer of epicycle was present which stained relatively less in blue color as compared to the layer of upper epidermal cells. In the central region, collateral vascular bundles were arranged in a circle with well-developed sclerenchyma tissue. These regions of the plants were full of blue color indicating presence of mucopolysaccharides (Plate 2b). The results are in agreement with the observations for *Hibiscus schizopetalus*[19].

The leaf T.S. of *S. maritima* was elliptical in shape. The epidermal cells were large and single layer with thick cuticle. The isolateral palisade tissue was elongated and arranged densely with 1-2 layers. The blue color granules could be seen on the walls of epidermal and palisade cells (Plate 1b and 2c), both in control as well as NaCl treated plants. Similar observation was reported in *Ziziphus* species[14]. The vascular bundles were in the central region surrounded by water storage parenchymatous cells with blue color appearance representing mucopolysaccharides. The stem T.S. had single layer of epidermis and was covered with thin cuticle and blue color granules (Plate 2d). The external part of the stem cortex was differentiated into palisade and collenchymas with no blue color precipitation. Below this layer, thin walled parenchymatous tissues were arranged. The central region consisted of many vascular bundles arranged in ring. The pith contains large parenchymatous cells with no central cavity. All parenchymatous cells showed blue color precipitation on its wall in control (Plate 1c) as well as NaCl treated plant. No difference was observed for the localization of mucilage under control or salt-treated plants of *S. brachiata* and *S. maritima.*

A positive correlation between mucilage content and salt tolerance has been reported where mucilage content continuously increased in response to various concentration of salt treatment in *Kosteletzkya virginica*[9]. However, present study showed no change in mucilage concentration either in control or salt

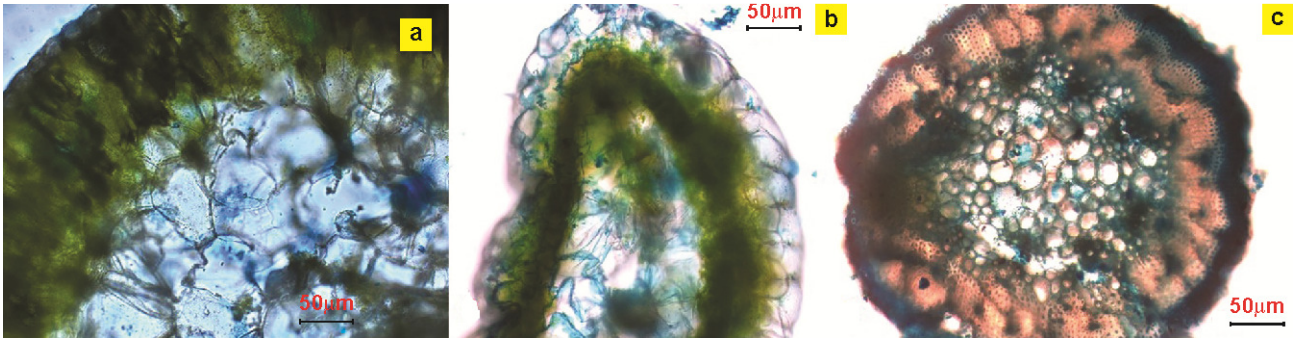

Plate 1—Localization of mucilage (control plant). a) Blue precipitate on the surface of epidermal cells and palisade tissue appear green (*Salicornia brachiata* stem), b) Blue precipitate on the surface of epidermal cells and palisade tissue shows green appearance (*Suaeda maritima* leaf) and c) Blue colour appear on collateral vascular bundles with sclerenchyma tissue (*S. maritima* stem).

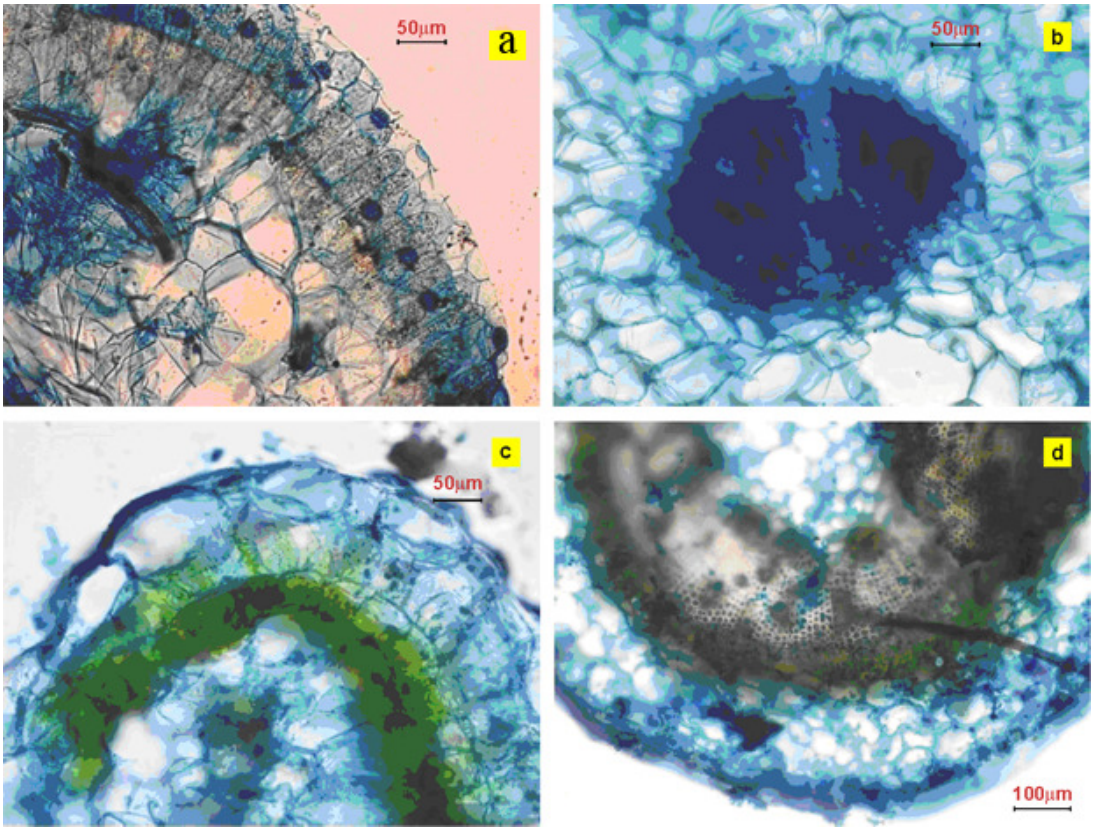

Plate 2—Localization of mucilage (stress plant). a) Blue precipitate on the surface of epidermal cells and palisade tissue *(Salicornia brachiata* stem), b) Blue colour in collateral vascular bundles with sclerenchyma tissue (*S. brachiata* stem), c) Blue precipitate on the surface of epidermal cells and palisade tissue (*Suaeda maritima* leaf) and d) Blue colour in collateral vascular bundles with sclerenchyma tissue (*S. maritima* stem)

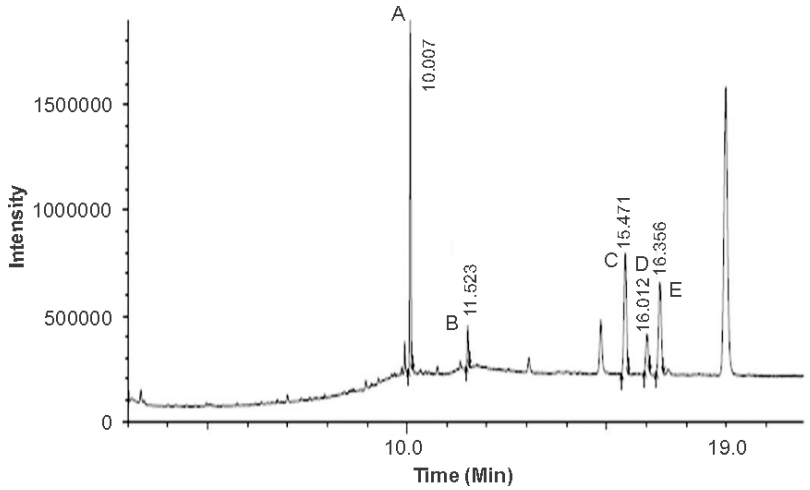

Fig. 1—GCMS analysis of neutral monosaccharides in *Salicornia brachiata*. Peak identified are a) Fucose, b) Arabinose, c) Manose, d) Galactose and e) Glucose.

stress condition for both halophytes. The major neutral monosaccharide found in *S. brachiata* was fucose, arabinose, mannose, galactose and glucose (Fig. 1), whereas in *S. maritima* fucose, mannose, galactose and glucose (Fig. 2) were the major constituents. Similar results were reported by Sanandiya and Siddanta[20] in *S. brachiata* except the fucose, which was detected only in ammonium oxylate extract of the root. High percentage of rhamnose was reported in salt exposed diatoms[21],

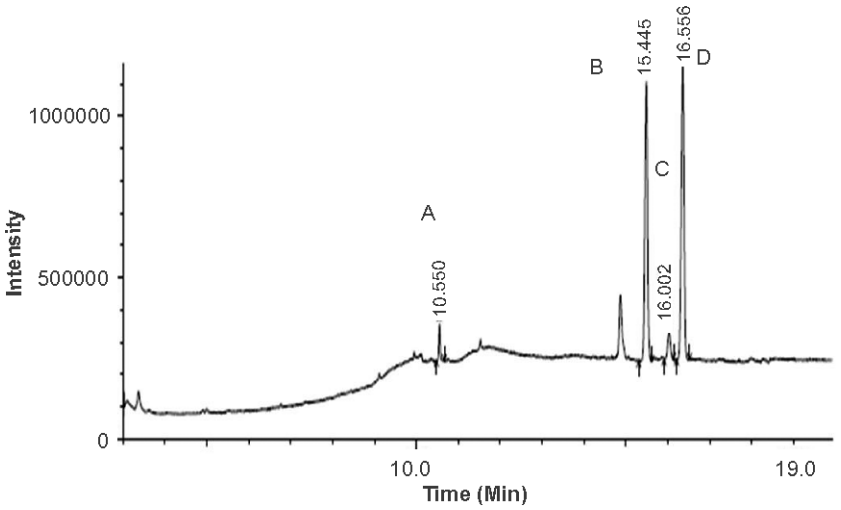

Fig. 2—GCMS analysis of neutral monosaccharides in *Suaeda maritime*. Peak identified are a) Fucose, b) Manose, c) Galactose and d) Glucose

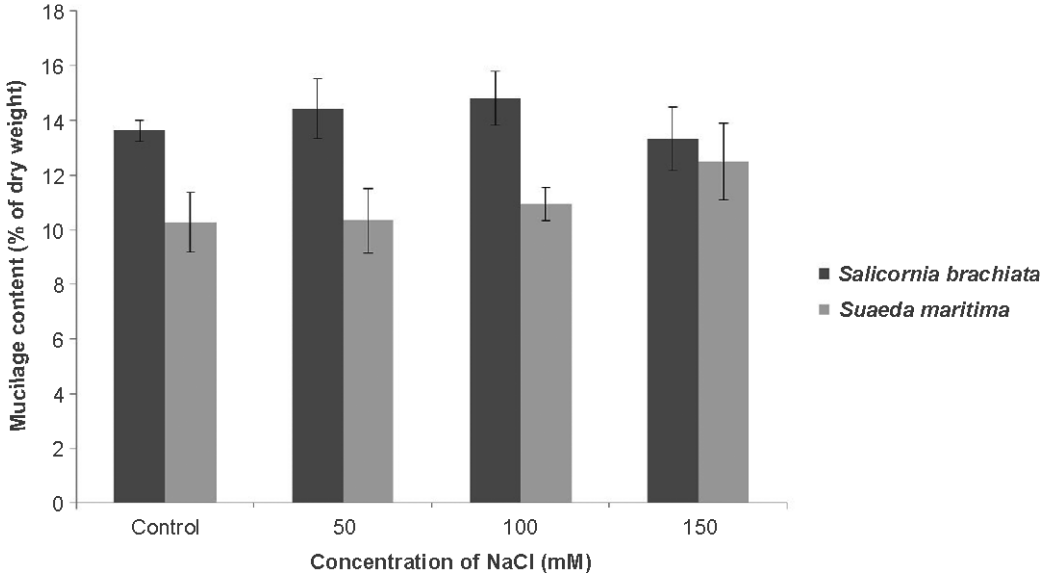

Fig. 3—Mucilage contents (% dry weight) in *Salicornia brachiata* and *Suaeda maritima* exposed for 10 days with or without concentration of NaCl

whereas in the present study, rhamnose was not found indicating that there was no momentous effect of salt stress on polysaccharides. Ebrahimzadeh *et al.*[22] studied the mucilage content and its sugar composition in leaflet of 15 species of *Astragalus* from Iran. The study concluded that glucose was the major monosaccharides in 12 species; however, galactose was shown to be a major constituent in 3 species. Similar findings were observed in the present study. In this study, *S. brachiata* and *S. maritima,* showed highest crude mucilage of 14.8±0.98 and 12.5±1.4 %, respectively at 150 mM NaCl concentration (Fig. 3); however, no significant difference was observed between control and treated plants. The crude mucilage content may vary among different plant species. For instance, mucilage content of different *Astragalus* species ranges between 3.44 to 23.56 % dry weight in a study by Ebrahimzadeh *et al*[22]. Among them, *Astragalus eugenii* exhibited highest mucilage content (23.56 %), whereas lowest dry weight (3.44 %) was reported in *A. alyssoides*. *Hibiscus rosasinensis* contains 17 % of dry mucilage, which is a superior tablet disintegrating agent[23]. On the other hand, only 9.17 % of crude mucilage has been reported in *Hibiscus esculentus*[24]. The genus *Hibiscus* has been shown as most diverse group for the crude mucilage content. The present study showed no significant difference in the dry weight of

mucilage in control and treated plants. On the contrary, Ghanem *et al*[9] showed that progressive increase of salt concentration also increases the mucilage content and helps in fixation of Na^+ ions. The present study did not show any difference in the involvement of Na fixation of polysaccharide as it did not shows any remarkable difference in its dry weight (%), both in control as well as in NaCl treated plants. Similar results were obtained by Golezani *et al*[10] concluding that mucilage percentage was not significantly affected by salinity stress in Isabgol (*P. ovata*). Further research is required to find the potential uses of this mucilage.

Conclusion

The composition of neutral monosaccharides in *S. brachiata* and *S. maritima* show excellent quantity of mucilage as compared to other plants. To the best of the author's knowledge, single report is available on localization and composition of mucilage in halophytes. *S. maritima* has been screened for the first time for localization and composition of mucilage under short term salt stress. The main monosaccharide constituents in *S. brachiata* were fucose, arabinose, mannose, galactose and glucose, whereas in *S. maritima* fucose, mannose, galactose and glucose were the major constituents. *S. brachiata* and *S. maritima* showed highest crude mucilage of 14.8±0.98 and 12.5±1.4 %, respectively. These halophytes may be potential source of mucilage grown in salt affected areas. Further research is required to characterize and to determine large scale use of the mucilage.

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