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Short communication

Oligosaccharide mass profiling of nutritionally important *Salicornia brachiata*, an extreme halophyte

Avinash Mishra*, Mukul Joshi, Bhavanath Jha

Discipline of Marine Biotechnology and Ecology, CSIR-Central Salt and Marine Chemicals Research Institute, G.B. Marg, Bhavnagar, Gujarat 364002, India

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ABSTRACT

Salicornia brachiata is an extreme halophyte, growing opulently in salt marshes and considered as potential alternative crop for seawater agriculture. *Salicornia* seeds are rich in protein and tender-shoots are eaten as salad greens. Total cell wall carbohydrate was isolated from seedlings, digested with β-glucanase enzyme cocktail and oligosaccharide mass profiling (OLIMP) was performed by using MALDI TOF-TOF mass spectroscopy. *Salicornia* OLIMP is represented by characteristic mass peaks *m*/*z* 477.3297–2094.4363. MS spectra exhibit xyloglucan oligosaccharide building blocks, dominated by XXXG (1084.9927 *m*/*z*). Characteristic mass peaks (*m*/*z*) of xyloglucan oligomers (XXG, XXFG, XLXG and XLFG) were also detected, which showed resemblance to the mass profile of highly nutritious plant soybean. The present study is the first report on OLIMP for any edible halophyte, so far. OLIMP supports use of *Salicornia* as a potential source of dietary supplementation. Further linkage-analysis is required to get the structural information of oligosaccharides.

1. Introduction

Naturally occurring carbohydrates including cellulose, dextran, glycogen, etc. are comprised of multiple units of single or different type of monosaccharide residues, arranged in distinct patterns; linear, in branch or both and exhibit a specific structure. Carbohydrates are structurally diverse and one of the most abundant macromolecules in the nature. Oligo and polysaccharides are either linear polymers like proteins and nucleic acids or branched structures because of the linkage of constituent monosaccharide residues. Saccharides or glycans provide complexity to proteins by glycosylation and thus influence protein folding, modulate protein function and involve in recognition of binding ligands (Zaia, 2004). Apart from the basic functions at cellular and bio-molecular level, saccharides also play a key role in food and nutritional chemistry, pharmaceuticals, biofuels and biomaterials. In plants, carbohydrate polymers act as structural and storage molecules. These are substantially large macromolecules and require chemical or enzymatic degradation for mass spectrometric analysis.

Oligosaccharide mass profiling (OLIMP) coupled with matrix assisted laser desorption ionization-time of flight-time of flight (MALDI TOF-TOF) mass spectrometry is a rapid, highly sensitive, convenient and precise technique for the analysis of diverse types of carbohydrates and carbohydrate-containing compounds (Harvey, 1999; Zaia, 2004; Persson, Sørensen, Moller, Willats, & Pauly, 2010). The technique is comparatively salt independent and avoids derivatization, which is required for most of the classical mass spectrometric techniques. It can be utilized for the study of large mass molecules and a wide range of organisms with small amount of samples (Günl, Kraemer, & Pauly, 2011; Mishra, Kavita, & Jha, 2011; Obel, Erben, Schwarz, Kühnel, Fodor, & Pauly, 2009; Ropartz et al., 2011; Westphal, Schols, Voragen, & Gruppen, 2010). Furthermore OLIMP is a preferred modern robust analytical method with high performance capability and can also be performed *in situ* with tissues or seedlings (Obel et al., 2009; Günl et al., 2011).

Salicornia brachiata Roxb. is one of the high salt-tolerant and leaf-less annual halophyte with green, jointed, succulent stems and terminal fruit-bearing spikes. This plant is capable of growing on salt marshes, accumulates 30–40% NaCl in dry weight and even requires NaCl for *in vitro* regeneration (Glenn, Brown, & Blumwald, 1999; Joshi, Mishra, & Jha, 2012). The plant is cultivated in the areas with elevated salinity and considered a potential alternative seawater crop (Glenn et al., 1999; Stanley, 2008). Several species of *Salicornia* are considered as probable source of commercial oil (Desai, Dave, & Devi, 2006; Eganathan, Subramanian, Latha, & Rao, 2006). This plant has nutritional values due to protein rich seeds and shoots are eaten as salad greens (Jha, Singh, & Mishra, 2012). Further, its potential as a naturally adapted higher plant model for abiotic stress responsive gene resources (Chaturvedi, Mishra, Tiwari, & Jha, 2012; Jha, Sharma, & Mishra, 2011) make this extreme halophyte a promising candidate with diverse applications. Considering the nutritional qualities of extreme halophyte *S. brachiata*, the present work is focused on oligosaccharide mass profiling (OLIMP) using MALDI TOF-TOF MS to demonstrate the presence of complex

* Corresponding author. Tel.: +91 278 2567760x6260; fax: +91 278 2567562.
E-mail address: avinash@csmcri.org (A. Mishra).

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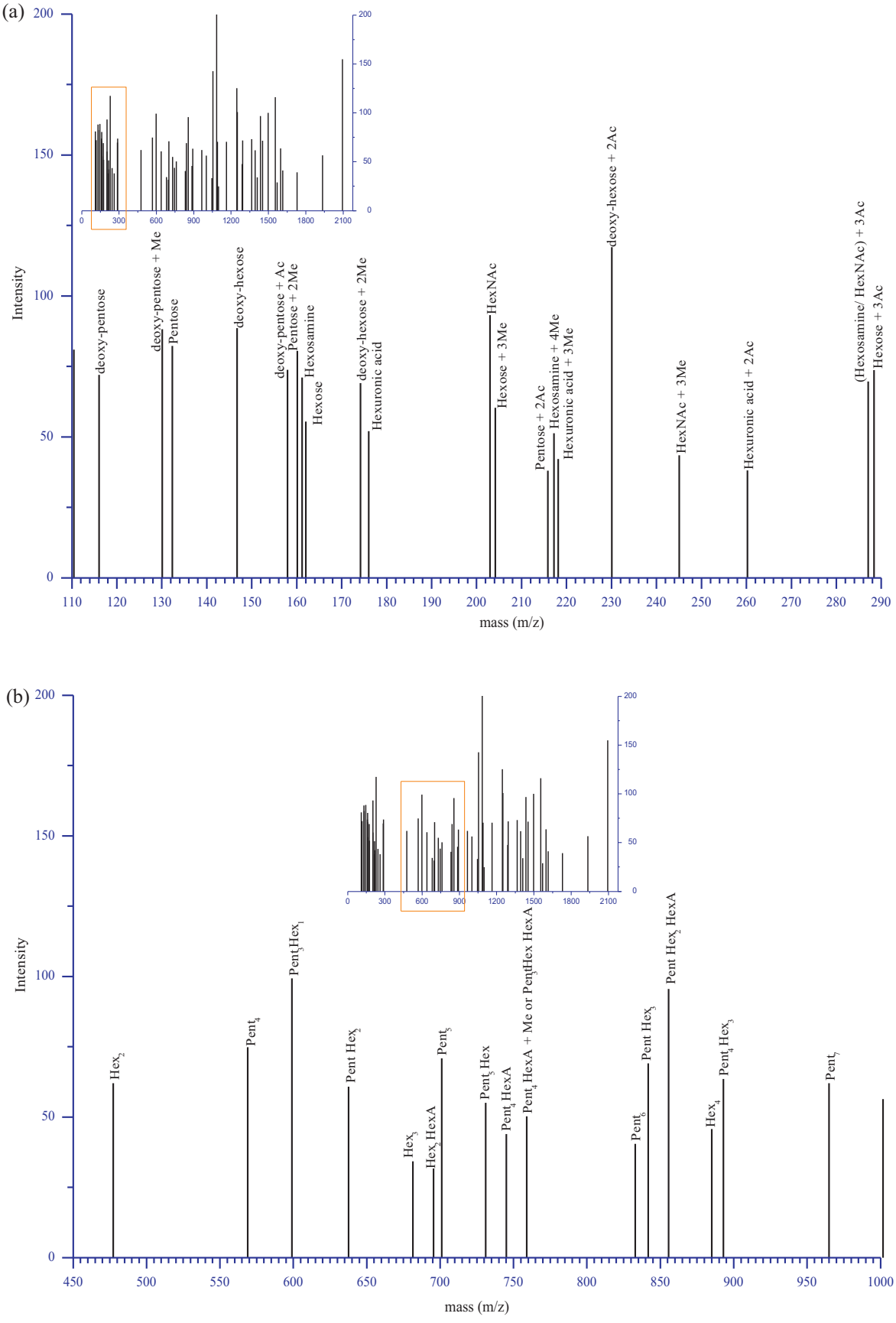

Fig. 1. MALDI-TOF-TOF mass spectra of *Salicornia brachiata* after digestion with the β-glucanase enzyme cocktail; (a) zoom of *m*/*z* 110–300, (b) zoom of *m*/*z* 450-1000 and (c) zoom of *m*/*z* 1000–2100. Peaks were marked with corresponding mono- and oligo-saccharides.

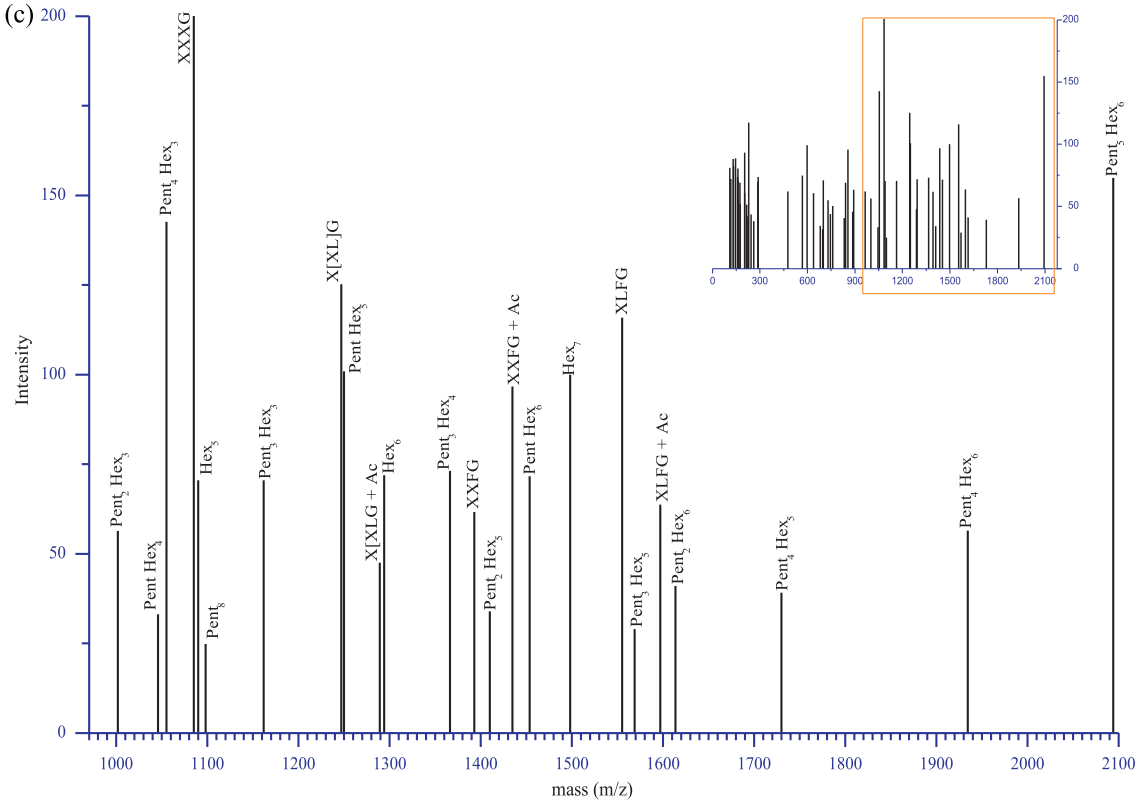

Fig. 1. (*Continued*)

free saccharides and to the best of our knowledge, this is the first report of OLIMP for any edible halophyte.

2. Materials and methods

Plant sample was prepared using the method described by Günl et al. (2011) with slight modifications. *S. brachiata* seedlings (30 mg, 21 days-old) were harvested from the pot, transferred to 1.5 ml microfuge tube and powdered in liquid nitrogen using homogenizer. A measure of 1 ml ethanol (70%, v/v) was added, vortexed thoroughly and centrifuged at 18,000 × *g* for 10 min (room temperature) in table top refrigerated centrifuge (Sigma, USA; model 3K30). Supernatant was removed carefully without disturbing pellet. Then pellet was re-suspended in 1 ml chloroform–methanol (1:1, v/v) solution, vortexed and centrifuged at 18,000 × *g* for 10 min (room temperature). Supernatant was removed and pellet was dried using concentrator (Eppendorf, Germany; model 5301). The dried pellet was added in 100 μl ammonium formate (50 mM) solution containing 2 units of β-glucanase enzyme mix (Sigma–Aldrich, Cat No. G4423; a mixture comprised of β-1→3/1→4-glucanase, xylanase, cellulase, β-glucosidase, β-xylosidase and α-L-arabinofuranosidase) for enzymatic digestion. The reaction was carried out at 37 °C for 16 h, thereafter supernatant containing released oligosaccharides was collected by centrifugation at 18,000 × *g* for 10 min (4 °C). About 10 μl supernatant containing released oligosaccharides was mixed with equal volume (10 μl) of matrix α-cyano-4-hydroxycinnamic acid [10 mg ml^{-1} (w/v) suspended in aqueous acetonitrile (50%, v/v) and trifluoro acetic acid (0.1%, v/v)].

MALDI TOF-TOF MS analysis was performed on an Applied Biosystems 4800 MALDI TOF-TOF analyzer with an intensity of 7900 Nd-YAG (neodymium-doped yttrium aluminium garnet) laser (355 nm, 200 Hz) operated in accelerated voltage (20 kV). Each spectrum was collected as an average of 1400 laser shots per spectrum in reflector mode (300 cm) with 1 kV positive MS–MS acquisition method and 1 kV positive processing method. The data was acquired with instrument setting as digitizer-Bin size 0.5 ns, input band width 500MHz, noise window width 250 and signal to noise (S/N) ratio 10. Reproducibility of the spectrum was checked from 36 (6 × 6) spot-sets and the spectra were analyzed after centroiding and de-isotoping using Data explorer software (Applied Biosystems, USA).

3. Results and discussion

The MALDI TOF-TOF mass spectroscopy was previously optimized for extracellular polysaccharides and fragmentation peaks were detected in positive ion mode only (Mishra et al., 2011). The positive ion linear and reflector mode were found suitable for the oligosaccharide and polysaccharide analysis, respectively, while fragmentation peaks were not detected in negative ion linear or reflector mode. The present analysis represents a series of mass range *m*/*z* 116.0702–2094.4363 (Supplementary Figs. S1 and S2) where mass peaks ranging from *m*/*z* 116.0702 to 288.3836 attributed to monosaccharide units and derivatives (Fig. 1a). Sugar moieties deoxy-pentose (116.0702 *m*/*z*), methylated deoxy-pentose (130.0817 *m*/*z*), pentose (132.3180 *m*/*z*), deoxy-hexose (146.7274 *m*/*z*), acetylated deoxy-pentose (157.9526 *m*/*z*), dimethylated pentose (160.1351 *m*/*z*), hexosamine (161.1732 *m*/*z*), hexose (162.0174 *m*/*z*), dimethylated deoxy-hexose (174.16 *m*/*z*), hexuronic acid (176.0043 *m*/*z*), N-acetyl hexosamine (202.9826 *m*/*z*), trimethylated hexose (204.1788 *m*/*z*), diacetylated pentose (215.8574 *m*/*z*), tetramethylated hexosamine (217.2057 *m*/*z*), trimethylated hexuronic acid (218.1584 *m*/*z*), diacetylated deoxy-hexose (230.0667 *m*/*z*), trimethylated N-acetyl hexosamine (245.0850 *m*/*z*), diacetylated hexuronic acid (260.2178 *m*/*z*), triacetylated hexosamine or triacetylated N-acetyl

hexosamine (287.1071 *m*/*z*) and triacetyl hexose (288.3836 *m*/*z*) were detected in lower mass peak range.

Mass peaks from *m*/*z* 477.3297 to 2094.4363 showed characteristic peaks of oligosaccharides (Fig. 1b and c). Mass range *m*/*z* 477.3297–964.9817 (Fig. 1b) correspond to di-hexose (477.3297 *m*/*z*), tetra-pentose (569.0047 *m*/*z*), tri-pentose hexose (599.0007 *m*/*z*), pentose di-hexose (637.6686 *m*/*z*), tri-hexose (681.4967 *m*/*z*), di-hexose hexuronic acid (695.5369 *m*/*z*), penta-pentose (701.0933 *m*/*z*), penta-pentose hexose (730.9849 *m*/*z*), tetra-pentose hexuronic acid (745.0378 *m*/*z*), methylated tetra-pentose hexuronic acid or tri-penta hexose hexuronic acid (758.9994 *m*/*z*), hexa-pentose (832.9988 *m*/*z*), pentose tri-hexose (841.7686 *m*/*z*), pentose di-hexose hexuronic acid (855.6658 *m*/*z*), tetra-hexose (885.0244 *m*/*z*), tetra-pentose tri-hexose (893.0603 *m*/*z*) and hepta-pentose (964.9817 *m*/*z*).

Mass peak range *m*/*z* 1001.7886–2094.4363 was assigned to oligosaccharides with high mass (Fig. 1c), that includes di-pentose tri-hexose (1001.7886 *m*/*z*), pentose tetra-hexose (1045.7299 *m*/*z*), tetra-pentose tri-hexose (1055.0187 *m*/*z*), penta-hexose (1089.7150 *m*/*z*), octa-pentose (1097.9965 *m*/*z*), tri-pentose tri-hexose (1161.9010 *m*/*z*), pentose penta-hexose (1249.8263 *m*/*z*), hexa-hexose (1293.9266 *m*/*z*), tri-pentose tetra-hexose (1366.1829 *m*/*z*), di-pentose penta-hexose (1409.9360 *m*/*z*), pentose hexa-hexose (1453.8546 *m*/*z*), hepta-hexose (1498.1283 *m*/*z*), tri-pentose penta-hexose (1569.0231 *m*/*z*), di-pentose hexa-hexose (1613.9067 *m*/*z*), tetra-pentose penta-hexose (1730.0580 *m*/*z*), tetra-pentose hexa-hexose (1934.3990 *m*/*z*) and penta-pentose hexa-hexose (2094.4363 *m*/*z*). Xyloglucan oligosaccharide building blocks produced from xyloglucan by the action of (1→4)-β-glucanases were identified (Fig. 1c) as XXXG (1084.9927 *m*/*z*), X[XL]G (1247.0164 *m*/*z*), acetylated X[XL]G (1289.0447 *m*/*z*), XXFG (1392.9716 *m*/*z*), acetylated XXFG (1434.9568 *m*/*z*), XLFG (1555.0087 *m*/*z*) and acetylated XLFG (1596.9789 *m*/*z*).

The plant cell wall is a complex and dynamic structure comprised of cellulose, hemicellulose, pectin, lignin, etc. OLIMP is commonly used for semi-quantitative fingerprint and depend upon the digestion of cell wall polymers (Obel et al., 2009), which revealed that *S. brachiata* cell wall contains a series monomers. Similar profile was previously observed for cell wall of *Arabidopsis* (Westphal et al., 2010). The hemicellulose xyloglucan is a characteristic of the cell wall of vascular dicots, which play an important role in cell wall structure and function. The oligosaccharide mass profiling indicated the presence of xyloglucans (XXXG, X[XL]G, XXFG and XLFG) in the cell wall of *S. brachiata*. MS spectral analysis showed the dominance of XXXG (1084.9927 *m*/*z*) over other xyloglucans. The characteristic mass peak (*m*/*z*) of poly-XXXG xyloglucan oligomers (XXG, XXXG, XXFG, XLXG, and XLFG) were detected in the cell wall of highly nutritious plant soybean (Huisman, Weel, Schols, & Voragen, 2000) and model plant tobacco (Nguema-Ona et al., 2012). Though mass spectroscopy cannot distinguish diastereomers but it indicates the number and type(s) of sugar moieties in an oligomer.

4. Conclusion

Halophyte *S. brachiata* is considered as a potential alternative crop for seawater agriculture. Its OLIMP exhibited the characteristic mass peaks (*m*/*z*) corresponding to mono- and oligo-saccharides and their derivatives. The mass profile revealed the dominance of XXXG over other oligosaccharides and therefore exhibited resemblance to the mass profile of highly nutritive soybean plant. This study reveals the nutritional potential of this plant as a latent source for dietary supplementation. The present study is the first report on oligosaccharide mass profiling (OLIMP) for any edible halophyte, so far. However, further linkage analysis is required to get the detailed structural information of oligomers.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.11.055.

References

Chaturvedi, A. K., Mishra, A., Tiwari, V., & Jha, B. (2012). Cloning and transcript analysis of type 2 metallothionein gene (*SbMT*-2) from extreme halophyte *Salicornia brachiata* and its heterologous expression in *E. coli*. *Gene*, *499*, 280–287.

Desai, P. D., Dave, A. M., & Devi, S. (2006). Alcoholysis of *Salicornia* oil using free and covalently bound lipase onto chitosan beads. *Food Chemistry*, *95*, 193–199.

Eganathan, P., Subramanian, H. M., Latha, R., & Rao, C. S. (2006). Oil analysis in seeds of *Salicornia brachiata*. *Industrial Crops and Products*, *23*, 177–179.

Glenn, E. P., Brown, J. J., & Blumwald, E. (1999). Salt tolerance and crop potential of halophytes. *Critical Reviews in Plant Sciences*, *18*, 227–255.

Günl, M., Kraemer, F. J., & Pauly, M. (2011). Oligosaccharide mass profiling (OLIMP) of cell wall polysaccharides by MALDI-TOF/MS. In Z. A. Popper (Ed.), *The plant cell wall: methods and protocols, methods in molecular biology* (pp. 43–54). USA: Humana Press.

Harvey, D. J. (1999). Matrix assisted laser desorption/ionization mass spectrometry of carbohydrates. *Mass Spectrometry Reviews*, *18*, 349–451.

Huisman, M. M. H., Weel, K. G. C., Schols, H. A., & Voragen, A. G. J. (2000). Xyloglucan from soybean (*Glycine max*) meal is composed of XXXG-type building units. *Carbohydrate Polymers*, *42*, 185–191.

Jha, B., Sharma, A., & Mishra, A. (2011). Expression of *SbGSTU* (tau class glutathione S-transferase) gene isolated from *Salicornia brachiata* in tobacco for salt tolerance. *Molecular Biology Reports*, *38*, 4823–4832.

Jha, B., Singh, N. P., & Mishra, A. (2012). Proteome profiling of seed storage proteins reveals the nutritional potential of *Salicornia brachiata* Roxb., an extreme halophyte. *Journal of Agricultural and Food Chemistry*, *60*, 4320–4326.

Joshi, M., Mishra, A., & Jha, B. (2012). NaCl plays a key role for in vitro micropropagation of *Salicornia brachiata*, an extreme halophyte. *Industrial Crops and Products*, *35*, 313–316.

Mishra, A., Kavita, K., & Jha, B. (2011). Characterization of extracellular polymeric substances produced by micro-algae *Dunaliella salina*. *Carbohydrate Polymers*, *83*, 852–857.

Nguema-Ona, E., Moore, J. P., Fagerström, A., Fangel, J. U., Willats, W. G. T., Hugo, A., et al. (2012). Profiling the main cell wall polysaccharides of tobacco leaves using high-throughput and fractionation techniques. *Carbohydrate Polymers*, *88*, 939–949.

Obel, N., Erben, V., Schwarz, T., Kühnel, S., Fodor, A., & Pauly, M. (2009). Microanalysis of plant cell wall polysaccharides. *Molecular Plant*, *2*, 922–932.

Persson, S., Sørensen, I., Moller, I., Willats, W., & Pauly, M. (2010). Dissection of plant cell walls by high-throughput methods. In P. Ulvskov (Ed.), *Annual plant reviews: plant polysaccharides, biosynthesis and bioengineering* (pp. 43–64). Oxford, UK: Wiley-Blackwell.

Ropartz, D., Bodet, P. E., Przybylski, C., Gonnet, F., Daniel, R., Fer, M., et al. (2011). Performance evaluation on a wide set of matrix-assisted laser desorption ionization matrices for the detection of oligosaccharides in a high-throughput mass spectrometric screening of carbohydrate depolymerizing enzymes. *Rapid Communications in Mass Spectrometry*, *25*, 2059–2070.

Stanley, O. D. (2008). Bioprospecting marine halophyte *Salicornia brachiata* for medical importance and salt encrusted land development. *Journal of Coastal Development*, *11*, 62–69.

Westphal, Y., Schols, H. A., Voragen, A. G. J., & Gruppen, H. (2010). MALDI-TOF MS and CE-LIF fingerprinting of plant cell wall polysaccharide digests as a screening tool for *Arabidopsis* cell wall mutants. *Journal of Agricultural and Food Chemistry*, *58*, 4644–4652.

Zaia, J. (2004). Mass spectrometry of oligosaccharides. *Mass Spectrometry Reviews*, *23*, 161–227.