Industrial Crops and Products 35 (2012) 313–316

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Short communication

NaCl plays a key role for *in vitro* micropropagation of *Salicornia brachiata*, an extreme halophyte

Mukul Joshi, Avinash Mishra, Bhavanath Jha*

Discipline of Marine Biotechnology and Ecology, Central Salt and Marine Chemicals Research Institute, Council of Scientific and Industrial Research (CSIR), G. B. Marg, Bhavnagar 364021, Gujarat, India

ARTICLE INFO

Article history:
Received 11 April 2011
Received in revised form 17 June 2011
Accepted 18 June 2011
Available online 16 July 2011

Keywords:
Halophytes
Micropropagation
Regeneration
Salicornia
Salt stress

ABSTRACT

A simple and rapid method for micropropagation of succulent, salt accumulator and extreme halophyte *Salicornia brachiata* has been established for the first time using shoot tips and nodes. Individually, BA showed significant response compared to Kn and in combinations, improved shoot proliferation was observed with BA + NAA than BA + 2,4-D, however no significant response was observed with BA + IAA. Percentage of shoot response significantly increased with NaCl treatment in the combination of BA + NAA while BA + 2,4-D + NaCl combination showed reduced shoot proliferation followed by demises of most of cultures. Efficient shoot proliferation was observed with combinations BA (8.9 μM) + NAA (5.37 μM) + NaCl (500 mM) and BA (13.3 μM) + NAA (5.37 μM) + NaCl (250 mM) indicating that NaCl is required for the micropropagation. The developed method will facilitate functional analysis of novel salt responsive gene(s) isolated from *S. brachiata* and propagation of industrially important elite accessions.

1. Introduction

Salinity is the major limiting factor influencing plant growth and productivity. High salinity and water scarcity cause ion imbalances, hyper-osmotic stress and ensuing in secondary stresses (Li et al., 2010). Halophytes have not been well studied for tissue culture due to heterozygosity and intricacy in establishing *in vitro* cultures (Uno et al., 2009). Halophytes serve as model system for investigating adaptation mechanisms to saline environment. The molecular mechanism of salt tolerance in halophytes is considerably highly developed and study of molecular mechanism will facilitate engineering of abiotic stress tolerance. Analysis of gene expression patterns of halophytic plants are one of the approach for studying stress signaling and transcriptional responses.

Salicornia brachiata is a leaf-less annual succulent, one of the most salt tolerant plants in general, capable of growing under highly saline conditions on salt marshes and can accumulate 30–40% NaCl in its dry weight (Glenn et al., 1999; Pandya et al., 2006; Sharma et al., 2010). *S. brachiata* is one of the major gene resources for stress responsive genes (Jha et al., 2009, 2011) and considered as a potential alternative crop of seawater agriculture and rehabilitation of wasteland due to its economic potential (Glenn et al., 1999; Stanley, 2008). The vegetable salt Saloni formulated from Salicornia contains low sodium and recommended for high blood pressure patients as micronutrients i.e. calcium, magnesium, iodine, etc. are naturally present in this Salicornia salt (Ghosh et al., 2005; US patent no. 6,929,809). In addition, Salicornia oil contains about 87–88% of unsaturated fatty acid and 12–13% of saturated fatty acid (Desai et al., 2006). Moreover, the oil from *S. brachiata* contains 10-undecanoic acid, used in the lubrication industries and high ester and saponification suggest its potential for industrial use (Eganathan et al., 2006). Bioprospection, gene resources, salt encrust with natural essential micronutrients and oil content make this extreme halophyte a promising candidate for industrial purposes.

We have developed EST database of salt responsive gene(s) from *S. brachiata*, of which 4.8% ESTs belonged to stress tolerant gene category while ca 29% ESTs showed no homology with any of known functional gene sequences and thus, classified as unknown or hypothetical genes (Jha et al., 2009). A large number of up-regulated ESTs with unknown putative functions make this species an interesting gene resource. Functional analysis of these unknown genes is needed for over expression in the donor plants. The understanding of molecular mechanism of high salt adaptation using gene knock-out approach requires an efficient tissue culture system of *S. brachiata*.

Abbreviations: 2,4-D, 2,4-Dichlorophenoxyacetic acid; BA, 6-Benzyladenine; IAA, Indole-3-acetic acid; IBA, Indole-3-butyric acid; Kn, Kinetin; MS, Murashige and Skoog; NAA, α-Naphthalene acetic acid; PGRs, Plant growth regulators.

* Corresponding author. Tel.: +91 278 256 7352; fax: +91 278 257 0885.
E-mail address: bjha@csmcri.org (B. Jha).

0926-6690/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.indcrop.2011.06.024

Table 1
Effect of PGRs and NaCl on micropropagation of the halophyte *Salicornia brachiata*.

PGRs		NaCl (mM)	Days after culturing				Rooting
BA (μM)	NAA (μM)		15 days	30 days	45 days	60 days	
4.44	–	–	14.4 ± 2.4	12.8 ± 3.6	23.8 ± 2.8	N	–
4.44	5.37	–	23.9 ± 2.3	12.2 ± 2.8	NT	–	–
4.44	5.37	50	28.9 ± 2.9	28.9 ± 4.1	16.1 ± 6.8	15.6 ± 7.1^{a}	R–
4.44	5.37	100	22.2 ± 2.9	20.0 ± 5.0	19.4 ± 5.6	13.9 ± 4.4^{bc}	R–
4.44	5.37	250	23.3 ± 4.1	21.2 ± 4.1	23.1 ± 5.1	21.9 ± 4.6^{de}	R–
4.44	5.37	500	26.1 ± 2.5	25.6 ± 2.6	30.0 ± 3.9	27.4 ± 5.9	R+
8.9	–	–	14.2 ± 3.8	10.6 ± 4.3	20.0 ± 3.3	N	–
8.9	5.37	–	21.7 ± 1.2	17.2 ± 2.8	NT	–	–
8.9	5.37	50	17.2 ± 2.4	23.9 ± 0.7	17.8 ± 5.8	17.8 ± 5.8^{fg}	R–
8.9	5.37	100	20.0 ± 2.2	17.5 ± 2.3	12.8 ± 3.7	12.5 ± 3.6^{hij}	R–
8.9	5.37	250	25.0 ± 2.6	25.6 ± 2.8	26.1 ± 4.3	29.4 ± 5.4	R++
8.9	**5.37**	**500**	**28.3 ± 2.2**	**30.6 ± 1.5**	**37.8 ± 2.8**	**37.8 ± 2.8**^{bdfhkimn}	**R+++**
13.3	–	–	16.1 ± 5.1	12.2 ± 4.6	23.8 ± 0.7	N	–
13.3	5.37	–	21.1 ± 1.6	18.3 ± 2.2	NT	–	–
13.3	5.37	50	18.9 ± 2.5	17.8 ± 2.1	15.0 ± 4.1	15.0 ± 4.1^{ko}	R–
13.3	5.37	100	22.5 ± 1.9	20.0 ± 3.2	21.7 ± 5.7	21.7 ± 5.7^{p}	R+
13.3	**5.37**	**250**	**31.7 ± 2.2**	**33.9 ± 3.3**	**32.8 ± 6.4**	**36.1 ± 7.0**^{iq}	**R+++**
13.3	5.37	500	29.2 ± 1.5	29.2 ± 1.5	25.0 ± 5.2	21.7 ± 4.8^{r}	R+
22.2	–	–	16.8 ± 4.3	16.2 ± 4.4	17.1 ± 5.2	–	–
22.2	5.37	–	23.2 ± 2.0	15.8 ± 2.9	NT	–	–
22.2	5.37	50	21.1 ± 2.0	19.4 ± 2.1	11.1 ± 4.3	11.1 ± 4.3^{iqs}	R–
22.2	5.37	100	26.7 ± 2.9	26.7 ± 2.9	24.4 ± 5.0	27.2 ± 6.6	R
22.2	5.37	250	23.9 ± 0.7	25.0 ± 0.8	27.2 ± 3.2	28.3 ± 3.0^{js}	R–
22.2	5.37	500	23.9 ± 2.9	25.6 ± 2.6	21.7 ± 5.7	25.6 ± 6.2	R–
44.4	–	–	17.8 ± 3.1	13.0 ± 3.0	10.0 ± 2.2	N	–
44.4	5.37	–	21.7 ± 1.9	16.7 ± 3.2	NT	–	–
44.4	5.37	50	15.0 ± 2.0	15.0 ± 2.9	14.4 ± 5.4	14.4 ± 5.4^{mt}	R–
44.4	5.37	100	24.4 ± 2.4	26.1 ± 2.5	26.1 ± 3.8	24.4 ± 5.2	R–
44.4	5.37	250	32.2 ± 1.2	38.3 ± 1.7	38.3 ± 1.7	40.6 ± 1.8^{acegjoprstu}	R–
44.4	5.37	500	26.1 ± 1.1	28.3 ± 3.2	15.0 ± 4.5	19.4 ± 4.9^{nu}	R–
BA (μM)	**2,4-D (μM)**	–					
4.44	4.52	–	15.6 ± 2.4	13.9 ± 3.1	NT	–	–
4.44	4.52	50	12.2 ± 1.5	5.6 ± 2.4	NT	–	–
4.44	4.52	100	16.7 ± 2.4	10.0 ± 2.4	NT	–	–
4.44	4.52	250	22.8 ± 3.3	20.0 ± 3.0	25.6 ± 2.8	27.2 ± 2.9^{A}	R++
4.44	4.52	500	15.6 ± 2.3	8.9 ± 2.6	NT	–	–
8.9	4.52	–	20.0 ± 1.7	13.3 ± 2.4	NT	–	–
8.9	4.52	50	13.3 ± 1.7	11.7 ± 2.9	16.7 ± 6.7	16.7 ± 6.7	R–
8.9	4.52	100	14.4 ± 1.8	6.7 ± 2.4	NT	–	–
8.9	4.52	250	25.0 ± 2.6	18.9 ± 3.6	17.2 ± 4.9	17.2 ± 4.9	R–
8.9	4.52	500	21.1 ± 3.4	18.9 ± 2.0	22.8 ± 1.7	23.9 ± 1.6^{B}	R++
22.2	4.52	–	15.6 ± 2.9	13.3 ± 2.9	NT	–	–
22.2	4.52	50	12.8 ± 2.8	13.9 ± 2.9	7.8 ± 3.2	8.9 ± 3.5^{ABC}	R–
22.2	4.52	100	13.9 ± 2.0	14.4 ± 2.8	11.7 ± 4.8	12.2 ± 4.9	R–
22.2	4.52	250	17.2 ± 2.8	15.0 ± 3.7	14.4 ± 5.0	17.2 ± 5.6	R–
22.2	4.52	500	20.6 ± 3.2	19.4 ± 4.9	20.6 ± 6.8	22.8 ± 7.3	R+
44.4	4.52	–	16.1 ± 2.0	8.9 ± 2.0	NT	–	–
44.4	4.52	50	10.0 ± 1.7	7.8 ± 2.2	NT	–	–
44.4	4.52	100	13.3 ± 1.7	14.4 ± 1.8	NT	–	–
44.4	4.52	250	15.0 ± 1.4	11.7 ± 1.1	NT	–	–
44.4	4.52	500	18.9 ± 2.0	18.9 ± 1.8	21.1 ± 1.6	22.2 ± 1.7^{C}	R–

Data is shown as percentage mean of shoot response ± SE; N: No further response or necrosis; NT: Not transferred; optimized combinations are shown in bold letters and means ± SE followed by similar letters are significantly different according to Tukey HSD at $P < 0.01$.

Salicornia has been studied for bioprospection, oils, wasteland rehabilitation, salt stress responsive genes, physiology and biochemistry (Desai et al., 2006; Eganathan et al., 2006; Stanley, 2008; Jha et al., 2009; Parida and Jha, 2010) while there are few reports on tissue culture. Previously, *in vitro* propagation of *S. bigelovii* and *S. europaea* was studied (Lee et al., 1992; Shi et al., 2006) but *in vitro* culture system of succulent halophytes was not very successful so far. Lee et al. (1992) developed *in vitro* propagation of *S. bigelovii* using shoot tip culture method but the regeneration rate was very low, however Shi et al. (2006) established a method of *S. europaea* regeneration from mature embryos. Tissue culture of succulent halophytes using mature embryo is not promising because of microscopic size and non-availability of explants throughout the year. Efficient micropropagation of succulent halophytes is not reported so far and we have successfully developed a rapid and simple micropropagation of *S. brachiata* using shoot tip and node as explants. The developed method can be the basis for the functional analysis of genes isolated from *S. brachiata* and propagation of industrially important elite accessions.

2. Materials and methods

Shoot tips and nodes of *S. brachiata* plants were cut and sterilized with 2% (v/v) sodium hypochlorite (NaOCl) solution for 15 min followed by washing 4–5 times with sterile distilled water. MS basal media (Murashige and Skoog, 1962) containing 0.8% (w/v) agar, 3% (w/v) sucrose, supplemented with different concentrations of cytokinins (BA and Kn) and auxins (2,4-D, IAA, IBA and NAA), individually or in combinations with and without different concentrations of NaCl (50, 100, 250 and 500 mM) was used. Ster-