

Detection of EST-SSR and miRNA in cDNA library from *Suaeda salsa* in Salt Stress Conditions with Computational Approaches

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Abstract: *Suaeda salsa* L. is a leaf succulent C₃ euhalophyte. Its seeds contain about 40% oil, rich in unsaturated fatty acids. *S. salsa* can tolerate coastal seawater salinity. The high cytosolic Na⁺ concentration led to metabolic reactions would be inhibited. Currently, using in-silico approach through mining expressed sequences tags (EST) have become an effectively for developing molecular analysis *in vitro*. In this research, we decided to use the ESTs data in the Library of *S. salsa*, a good image for genome surgery halophyte plant. Results showed that 255 contigs and 344 singletons determined from 1011 ESTs. Gene ontology revealed 11 functional groups; cell wall, cytoskeleton, membrane transport, cell division, cell defence, chromosome metabolism, signaling components, gene expression and RNA metabolism, protein metabolism and sorting, photosynthesis, cell metabolism and cell development. In study with computational approaches several miRNA predicted in ESTs that can be used for regulation of gene transcription. Additionally, simple sequence repeats (SSRs) were also identified contributing to genes in salt stress. These genes can be used for plant breeding in crops for tolerance to salinity stress.

Key words: Computational • miRNA • Salt stress • SSR • *Suaeda salsa*

INTRODUCTION

Abiotic stresses such as salinity is affected on plant metabolism and disrupted the growth of plants [1]. Salt tolerance is a complex characteristic that includes a network of genes involved in various mechanisms that Consistently expressed. Unlike crops, halophyte with advanced characteristics that can be developed saline environments to manage and adapt to the them [2, 3]. To survive stress conditions, plants respond to changes in various aspects of the its metabolism. The mechanisms of plants during stress and help improve the conditions of stress modifications in conditions of stress include changes in the properties of molecular, cellular and phenotypic [4, 5]. Proteins are the major players in many cellular events. Proteins recipient of signals are the first proteins that involved in salinity [6, 7]. These proteins include: receptors in plasma membrane or cytoplasm, Ca²⁺ signaling protein such as calmodulin (CaM) and calreticulin (CRT), proteins of kinase cascade and ethylene receptors, receptor protein kinases (RPKs) [8-11].

14-3-3 family proteins are positives regulators of H⁺-ATPase activity response to stress [12]. The primary metabolism increases in salinity. alteration in carbohydrates and energy metabolism under salinity stress can be related to the high abundance of enzymes involved in glycolysis, TCA cycle, electron transport chain and ATP synthesis [13]. Modulating ion channel activity as an important strategy to be handled in the transport channel, transport by ABC transporters during salt stress plays an important role in wheat [14, 15]. addition to several related membrane proteins such as annexin and membrane-bound steroids are also to balance the ionic gradients membrane play a role in salinity [14, 16]. Abundance of superoxide dismutase, catalase pathway, glutathione S-transferase isoforms, peroxidase isoforms, peroxiredoxin and thioredoxin levels showed that key ROS scavenger during salt stress [17]. In recovery of salinity, the organization of the cytoskeleton and cell wall components of the cell is altered for modifying of turgor cells. cytoskeletons components such as actin, tubulin, actin-binding proteins

(ABPs), kinesin motor [18], myosin and xyloglucan endotransglycosylase(XET) hydrolases altered abundance during recovery from salinity stress [11]. Different strategies of genomic sequence analysis expressed as methods for identifying genes and proteins important to them in terms of the environmental changes or stress. So we will be able to identify genes and proteins related to them, especially in the case of entities which complete genome information is not available. Molecular markers play an important role in many aspects of plant breeding, such as identification of the genes responsible for desirable traits. Molecular markers have been widely used to map important genes and assist with the breeding of plant. Compared with other types of molecular markers, SSRs have many advantages,, EST-SSRs are derived from expressed sequences, which are more evolutionary conserved than noncoding sequences; therefore, EST-SSR markers have a relatively high transferability. Research shows that Halophytic can be a valuable genetic resources of crops are crucial for improving and adaptation. *Suaeda* is a genus of plants also known as seepweeds and seablites. Most species are confined to saline or alkaline soil habitats. There are about 110 species in the *Suaeda* genus (www.wikipedia). In this study, we decided to use of the expression sequences tags available in the database for a *Suaeda salsa*, there is a clear picture of the situation in terms of salinity provide a plant genome and to us through a series of proteins involved in plant response to salinity stress conditions for gene regulation in stress conditions and molecular markers for To increase efficiency of plants in salt stress conditions using the transmission and detection of genes involved in these processes.

MATERIALS AND METHODS

Sequence source of *S. salsa*: 1011 EST sequences of the library *S. salsa* was received of the database NCBI (www.ncbi.nlm.nih.gov).

Assembly ESTs Sequences from *S. salsa*: Remove vector sequences, duplicates and organelles (mitochondria and chloroplasts) and assembled the sequences obtained, using the online service Egassembler. Vector sequence in order to identify pollution and their length and quality of service using by bioinformatics Egassembler (www.egassembler.hgc.jp). After removal of vector sequences, repetitive sequences, chloroplast and mitochondrial sequences were removed [19].

Gene Ontology and Blast X search Sequences for Identify Hits of *S. salsa*: Searching BlastX for all unigene (contig and Singleton) with the maximum E-Value NCBI sequences. BLASTX results were used to determine the functional groups. The Gene Ontology project provides an ontology of defined terms representing gene product properties. The ontology covers three domains: cellular component, molecular function, biological process. Gene ontology for ESTs from trichomes glandular determine with uniprot server addressed www.uniprot.org.

Important Proteins That Detected in Gene Ontology of *S. salsa*: Using by results of gene ontology for EST from *S.salsa*, ESTs that encoded proteins for responses to salt stress (Table 1). *Prediction of miRNAs for Est sequences from S. salsa with modeling in Arabidopsis thaliana*:

For these prediction used all contigs and singletons sequences. Results predicted with online service addressed <http://plantgrn.noble.org>.

Frequency and Distribution of EST-SSRs Found in dbEST Sequences: Potential SSRs (simple sequence repeats) markers were detected using the tool of SSRIT (Simple Sequence Repeat Identification Tool) online with online service addressed <http://archive.gramene.org/db/markers/ssrtool>. The minimum repeat unit was defined as five for dinucleotides, tri-nucleotides and four for tetra-, penta- and hexa-nucleotides. To predict the position of SSRs with respect to coding regions.

RESULTS AND DISCUSSION

The analysis was performed on the sequence of *S. salsa* in the family Chenopodiaceae, the results were obtained in Table 1, according was introduced to the total number of EST (1011 EST), 255 contigs and 344 Singleton and a total unigene 799. searching BlastX for all unigene (contig and Singleton) with the maximum E-Value NCBI sequences.

Proteins grouped in cell wall, cytoskeleton, membrane transport, cell division, cell defence, chromosome metabolism, signaling components, gene expression and RNA metabolism, protein metabolism and sorting, photosynthesis, Cell metabolism, cell development, showed in Fig. 4.

Gene ontology for ESTs of *S. salsa* under salt stress showed proteins important for response to salt stress, showed in Table 2.

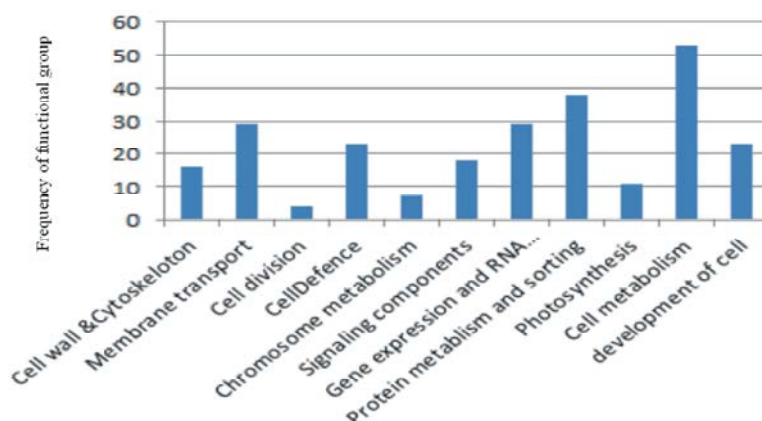
Fig. 4: Functional group determined for ESTs from *S.salsa* in salt stress.

Table 1: Results of Remove vector sequences, duplicates and organelles (mitochondria and chloroplasts) and assembled with Egassembler

Total EST number	Total length of the genome covered(bp)	Number of contig	Number of singlone
11011	428918bp	255	344

Table 2: proteins related to salt stress in *S.salsa* that revealed by gene ontology

Cell wall	Metabolism
UDP-glucuronic acid decarboxylase 2	Mannitol dehydrogenase
Ankyrin repeat-containing protein	Methionine synthase
Beta-actin	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase-like protein
Probable arabinosyltransferase ARAD1	UDP-N-acetylglucosamine diphosphorylase 1
Cinnamoyl-CoA reductase 1	zinc dependent protease
Subtilisin-like protease	Subtilisin-like protease
Callose synthase 9	Myo-inositol-1-phosphate synthase
Probable xyloglucan glycosyltransferase 5	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 9
Laminin subunit alpha-2	adenylate kinase 2
Cellulose synthase A catalytic subunit 1	Aspartate aminotransferase
Expansin-A6	Sucrose nonfermenting 4-like protein
Endoglucanase 6	choline monooxygenase
GPI-anchored protein PB15E9.01c	mitogen-activated protein kinase kinase
Oligopeptide transporter 7-like	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase
Probable beta-1,4-xylosyltransferase IRX14	Organic cation/carnitine transporter 7-like protein
Cytoskeleton	Phosphoethanolamine N-methyltransferase
Ras-related C3 botulinum toxin substrate 2	Cinnamate-4-hydroxylase 2
Membrane transport	Pyruvate kinase isozyme G, chloroplastic
V-type proton ATPase subunit H	Putative 3,4-dihydroxy-2-butanone kinase
Rho GDP-dissociation inhibitor 1	GDSL esterase/lipase APG-like protein
major intrinsic protein	Succinate dehydrogenase 1-2
Plastidic phosphate translocator-like protein1	Selenoprotein O
Transmembrane 9 superfamily member 1-like	Sorbitol dehydrogenase
Glutamate/malate translocator	4-coumarate--CoA ligase
GTP-binding protein SAR1A	Cytochrome P450 98A2
V-type proton ATPase subunit C	Probable leucine-rich repeat receptor-like protein kinase At1g35710
Protein transport protein Sec61 subunit alpha-like 1	S-adenosylmethionine synthase 2
Protein translocase subunit SecY	fumarylacetoacetase
Protein argonaute 4	Protein FAR1-RELATED SEQUENCE 3
Charged multivesicular body protein 5	fructose-1,6-bisphosphate aldolase
Protein NRT1/ PTR FAMILY 3.1	Malate dehydrogenase, cytoplasmic
Putative multidrug resistance pump	Embryogenesis-associated protein EMB8
carotenoid cleavage dioxygenase	Glycine decarboxylase P subunit
THO complex subunit 1	Lycopene epsilon cyclase

Table 2: Continued

Cell wall	Metabolism
Protein transport protein SEC23	carotenoid cleavage dioxygenase
Exocyst complex component EXO70A1	RAB6-interacting golgin
Protein RFT1 homolog	Granule-bound starch synthase 1
Peroxisomal membrane protein PMP22	Lysophospholipid acyltransferase LPEAT2
Guanosine nucleotide diphosphate dissociation inhibitor 2	Uridine kinase-like protein 3
Sodium/hydrogen exchanger	Glutamate--glyoxylate aminotransferase 2
plastidic phosphate translocator-like protein1	Serine hydroxymethyltransferase 4
vacuolar protein sorting-associated protein 35A-like	Insulinase family protein isoform 1
CTD nuclear envelope phosphatase 1 homolog	phospholipase D alpha 1-like
ras GTPase-activating protein-binding protein 1-like	Probable polyamine oxidase 2
Sodium/calcium exchanger 1	lycopene epsilon-cyclase
Metal tolerance protein 1	methyltransferase PMT20
Protein NRT1/ PTR FAMILY 3.1	Glucose-6-phosphate 1-dehydrogenase
Cell division	S-adenosyl-l-methionine synthase
transformation/transcription domain-associated protein-like	Beta-galactosidase 8
mitogen-activated protein kinase kinase	Ankyrin repeat domain-containing protein 2-like
COP9 signalosome complex subunit 1	Serine/threonine-protein phosphatase PP2A catalytic
cyclin-dependent kinase F-4	Pyridoxamine 5-phosphate oxidase
	development of cell
Chromosome metabolism	LONGIFOLIA 1
Histone H1-like	Serine hydroxymethyltransferase 4
Protein argonaute 4	Protein SUPPRESSOR OF FRI 4
Histone-lysine N-methyltransferase ATX5	Myb family transcription factor
Sister chromatid cohesion 1 protein 4	Protein TOPLESS
transformation/transcription domain-associated protein-like	choline monooxygenase
Calpain-type cysteine protease DEK1	mitogen-activated protein kinase kinase
TBC1 domain family member 22B	Protein GIGANTEA
Signaling components	Mitogen-activated protein kinase kinase kinase YODA
Rho GDP-dissociation inhibitor 1	Magnesium chelatase subunit family protein
Protein TOPLESS	Heat shock 70 kDa protein
Non-structural protein 1	Embryogenesis-associated protein EMB8
mitogen-activated protein kinase kinase	Monocopper oxidase-like protein SKU5
Regulator of nonsense transcripts UPF3	Delta-1-pyrroline-5-carboxylate synthase B
Auxin-responsive protein IAA16	Protein FAR1-RELATED SEQUENCE 3
Calreticulin	Calpain-type cysteine protease DEK1
carotenoid cleavage dioxygenase	ELMO domain-containing protein A
CBL-interacting protein kinase 15	Charged multivesicular body protein 5
Protein SGT1 homolog B	Phosphoethanolamine N-methyltransferase
V-type proton ATPase subunit C	Sorbitol dehydrogenase
EIN3-binding F-box protein 1	Trafficking protein particle complex subunit 9
Histidine kinase	Cyclin-dependent kinases regulatory subunit 1
Mitogen-activated protein kinase kinase kinase YODA	Zinc finger E-box-binding homeobox 1
Protein kinase MK5	Gene expression and RNA metabolism
ras GTPase-activating protein-binding protein 1-like	cleavage and polyadenylation specificity factor subunit1
Ras-related C3 botulinum toxin substrate 2	Pre-rRNA-processing protein TSR1 homolog
Splicing factor U2AF 65 kDa subunit	DNA-dependent RNA polymerase
Protein metabolism and sorting	Myb family transcription factor
Serine/threonine-protein phosphatase PP2A catalytic	Poly(rC)-binding protein
E3 ubiquitin-protein ligase UPL2	Non-structural protein 1
5-methyltetrahydropteroyltrimethylglutamate--homocysteine methyltransferase	Regulator of nonsense transcripts UPF3
Lysine--tRNA ligase	Polyadenylate-binding protein RBP47
Eukaryotic translation initiation factor 2 subunit beta-like protein	Auxin-responsive protein IAA16
E3 ubiquitin-protein ligase RGLG2-like protein	Protein argonaute 4
Polyubiquitin	Growth-regulating factor 2
26S proteasome non-ATPase regulatory subunit 3	THO complex subunit 1
mitogen-activated protein kinase kinase	14-3-3 protein 7

Table 2: Continued

Cell wall	Metabolism
Protein SGT1 homolog B	Splicing factor 3A subunit 3
EIN3-binding F-box protein 1	CLK4-associating serine/arginine rich protein
E3 ubiquitin-protein ligase UPL3	Phosphoglycerate kinase
Protein translocase subunit SecY	Protein TOPLESS
Protein argonaute 4	Scarecrow-like protein 8
E3 ubiquitin-protein ligase MBR2	nuclear transcription factor Y subunit A-1-like
Puromycin-sensitive aminopeptidase	Serine/threonine-protein kinase HT1
translation initiation factor eIF4G	Zinc finger E-box-binding homeobox 1
E3 ubiquitin-protein ligase Arkadia	transcription factor jumonji domain-containing protein
Protein kinase MK5	Zinc-finger type transcription factor WRKY1
ubiquitin-40S ribosomal proteinS27a	ubiquitin-40S ribosomal proteinS27a
CTD nuclear envelope phosphatase 1 homolog	zinc finger CCCH domain-containing protein 24-like
18.1 kDa class I heat shock protein	Nucleolar RNA helicase 2
Elongation factor 1-alpha	Multisubstrate pseudouridine synthase 7
Thiol protease	DNA-dependent RNA polymerase
E3 ubiquitin-protein ligase HUWE1	Paired amphipathic helix protein Sin3-like 3
Polyubiquitin family protein	Defence
Ubiquitin-conjugating enzyme E2 28	Ras-related C3 botulinum toxin substrate 2
Eukaryotic translation initiation factor 2 subunit 2	Cytosolic ascorbate peroxidase
vacuolar protein sorting-associated protein 35A-like	Lysine-tRNA ligase
Ubiquitin-associated /TS-N domain-containing protein	mitogen-activated protein kinase kinase
Rho GDP-dissociation inhibitor 1	Regulator of nonsense transcripts UPF3
Heat shock 70 kDa protein	Protein SGT1 homolog B
Eukaryotic translation initiation factor 3 subunit D	Protein GIGANTEA
protein phosphatase 2C 52	Polyadenylate-binding protein RBP47
Elongation factor 1 alpha-like protein	Protein argonaute 4
Eukaryotic translation initiation factor 4A	Charged multivesicular body protein 5
E3 ubiquitin-protein ligase ARI8	Ascorbate peroxidase
Elongation factor 1-alpha	Cellulose synthase A catalytic subunit 1
Photosynthesis	Puromycin-sensitive aminopeptidase
Chloroplast light-harvesting complex II protein Lhcbm3	V-type proton ATPase subunit H
Phosphoribulokinase	Catalase
Glycerate dehydrogenase HPR, peroxisomal	Heat shock 70 kDa protein
NADP-malate dehydrogenase	3 pollen allergen
Lycopene epsilon cyclase	Aldo-keto reductase family 4 member C9
Magnesium chelatase subunit family protein	Protein kinase MK5
serine--glyoxylate aminotransferase	Malate dehydrogenase, cytoplasmic
O2 evolving complex 33kD family protein	Disease resistance response protein 206
Glutamate--glyoxylate aminotransferase 2	Deoxynucleoside triphosphate triphosphohydrolase SAMHD1 homolog
Peroxiredoxin Q, chloroplastic	glutathione peroxidase 2
rubisco small subunit	

Table 3: miRNA detected in ESTs from *S. salsa* with modeling in *Arabidopsis thaliana*.

miRNA detected	Unigenes	Gene regulates with miRNA	Biological process
ath-miR5021	contig16	copper-transporting ATPase RAN1-like	Ethylene signaling pathway
ath-miR5021	contig21	ultraviolet-B receptor	Sensory transduction
ath-miR5658	contig32	lycopene epsilon-cyclase	Secondary metabolites
ath-miR835-5p	contig55	glucose-6-phosphate/phosphate translocator 1	Sugar transport, Transport
ath-miR5658	contig68	glutamate-glyoxylate aminotransferase 2	response to hypoxia
ath-miR173-5p	contig100	glucose-6-phosphate 1-dehydrogenase, cytoplasmic isoform 2-like	Carbohydrate metabolism, Glucose metabolism
ath-miR5021	contig 121	CTD nuclear envelope phosphatase 1 homolog	modulate the lipid composition of nuclear and/or endoplasmic reticulum membranes and be required for proper nuclear membrane morphology and/or dynamics

Table 3: Continued

miRNA detected	Unigenes	Gene regulates with miRNA	Biological process
ath-miR5658	contig 175	cysteine proteinase RD19	response to osmotic stress response to salt stress
ath-miR833a-5p	contig185	thiamine thiazole synthase	roles in adaptation to various stress conditions and in DNA damage tolerance.
ath-miR5021	contig197	protein TPRXL	involved in early embryonic development.
ath-miR5658	contig208	transcription factor GTE2	Transcription, Transcription regulation
ath-miR5658	contig 194	RAN GTPase-activating protein	nucleocytoplasmic transport, response to salt stress
ath-miR5658	contig209	vulgaris BAG family molecular chaperone regulator 7	Apoptosis, Stress response
ath-miR5653	contig217	transferring glycosyl groups	transferase,
ath-miR159c	contig 234	superoxide dismutase [Cu-Zn]	response to salt stress
ath-miR5659	contig246	ubiquinol oxidase	Carotenoid biosynthesis, Electron transport, Respiratory chain, Transport
ath-miR173-3p	singlone BE240973	phospholipase D p1	Lipid degradation, Lipid metabolism
ath-miR1886.1	singlone	BE859262	Plays a role in RNA transcription or processing during stress
ath-miR400	singloneAW982120	mitogen-activated protein kinase	Plant defence, Stress response
ath-miR414	singloneJZ712431	sister chromatid cohesion 1 protein	Cell cycle, Chromosome partition, Meiosis
ath-miR5021	singlone. BE240978	eukaryotic translation initiation factor isoform 4G-1	Protein biosynthesis, Translation regulation
ath-miR5021	singloneAW991022	endo-1,3(4)-beta-glucanase 2	carbohydrate metabolic process, defense response
ath-miR5021	singloneAW991041	glycerol-3-phosphate dehydrogenase	Lipid metabolism
ath-miR5658	singloneAW991039	glutamyl-tRNA(Gln) amidotransferase subunit A	Protein biosynthesis
ath-miR5658	singloneBE231413	60S ribosomal protein L2	translation
ath-miR842	singloneBE644596	DEAD-box ATP-dependent RNA helicase	Nonsense-mediated mRNA decay, Ribosome biogenesis, rRNA processing
ath-miR847	singloneBE656737	thioredoxin	cell redox homeostasis

Table 4: EST-SSRs found in contigs from ESTs sequences from *S. salsa*

Unigene	Motif repeat	Gene related to EST-SSR
Contig14	ct, cag, tct	lipoprotein receptor-related protein 1 (LRP1)
Contig16	tc, ag, ctt	copper-transporting ATPase RAN1
Contig35	tc,ag, attg	ferredoxin-dependent glutamate synthase
Contig41	tc,ga	cysteine proteinase RD21
Contig43,56	ttc, tc	E3 ubiquitin-protein ligase
Contig50	ga,tct	pentatricopeptide repeat-containing protein
Contig64	tc, acc	complex subunit D
Contig82	ct, cac	containing/glycosyltransferase-related
Contig88	cca, caa	inactive rhomboid protein 1-like
Contig104-	tc,cca, catt, tcaa	polyadenylate-binding protein RBP45
Contig126	ta,ga	endoplasmic reticulum-intermediate
Contig147	ct,tc	S-adenosyl-L-methionine synthase 2,
Contig175	tc, tca	cysteine proteinase RD19
Contig186	ga,ctg	GPI-anchored protein
Contig188	tc, ct	30S ribosomal protein S17
Contig201	tc,tca	catalase (CAT1)
Contig208	tc,tc, ga,atc	transcription factor GTE2
Contig209	ct,tca	BAG family molecular chaperone regulator 7
Contig213	caa, cat	ethylene-responsive transcription factor 4

Several hundred miRNAs have been identified in plants by computational and experimental approaches. But so far the species has not been reported either in the laboratory or computational identification of miRNA in *S. salsa* species.

Predicted small RNA mediated gene regulation in process that related to regulation of energy cell, signaling pathway for ethylene, sensory transduction, transport sugar, nucleocytoplasmic transport, transcription regulation, cell cycle and adaptation to DNA damage in salt stress. These prediction to gain insight into miRNAs

and regulatory functions important in salt stress using computational approach. EST-SSR markers identified for EST salt stress condition is shown in Table 4.

Gene markers such as: Ethylene-responsive transcription factor RAP2-4, Probably acts as a transcriptional activator and binds to the GCC-box pathogenesis-related promoter element. May be involved in the regulation of gene expression by stress factors and by components of stress signal transduction pathways; Rhomboid-like protein 17, Probable rhomboid-type serine protease that catalyzes intramembrane proteolysis; BAG

family molecular chaperone regulator 7, Co-chaperone that regulates diverse cellular pathways, such as programmed cell death and stress responses. Necessary for the proper maintenance of the unfolded protein response (UPR) during heat and cold tolerance, Cysteine proteinase RD19a; response to salt stress; Copper-transporting ATPase RAN1, involved in copper import into the cell, essential for ethylene signaling, which requires copper. Acts by delivering copper to create functional hormone receptors. Being of these markers help to breeder for fit selection in salt stress condition. Salt tolerance is a complex characteristic that includes a network of genes involved in various mechanisms that consistently expressed. Unlike crops, halophyte with advanced characteristics that can be developed Saline environments to manage and adapt to the them Further functional analyses on genes identified from the ESTs of *S. salsa* will provide more information about the molecular mechanism that is involved in the evolution and development salt stress. Research shows that halophytic plant can be a valuable genetic resources of crops are crucial for improving and adaptation.

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