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Corresponding Author	Family Name	**Mondal**
	Particle	
	Given Name	**Bidisha**
	Prefix	
	Suffix	
	Role	
	Division	
	Organization	The Neotia University
	Address	743368, Sarisha, West Bengal, India
	Email	bidisha.mondal@tnu.in

Abstract

Global agricultural productivity is regulated by soil salinity, one of the major abiotic constraints faced by the farmers, growers and breeders. The genomic, transcriptomics, proteomics and metabolomic salt profile of coastal plants could provide an insight into the mechanisms by which the differential performance is regulated in contrasting varieties of a single crop. This study proposes the construction of an Ionome suitable for the coastal saline region for sustainable food security. This study focuses on functional genomic studies of saline belt crops and meta-analysis of the information for proposed Ionome (Salt-Omic) development. In the salt-genome segment, the appropriate genes were identified and categorized covering ion-transport-genes, senescence-associated genes, molecular-chaperones, dehydration-genes. Proteome provides additional information on protein coding sequences, endogenous small molecules. The identified genes, proteins and signalling pathways could form an Ionome repository for molecular crop breeding programmes. The primary bioinformatics web source along with a customized database for several crops were found useful for identifying essential biomolecules. The study was able to assist in the formation of agri-ionome for the improvement of coastal crops. The sequential integration of agri-engineering model along with Omic details could be utilized for the construction of an explicit repository for molecular plant breeders in a way similar to AMBAB, LIS, Pulsechip or RiceMetaSys.

Keyword (separated by '-')	Ionome (Salt-omic) - Molecular crop-improvement - Food security - Breeder focused-database

Chapter 5
Development of Ionome (Salt-Omic) for the Varietal Improvement and Food Security of the Coastal Region of India

Bidisha Mondal

Abstract Global agricultural productivity is regulated by soil salinity, one of the major abiotic constraints faced by the farmers, growers and breeders. The genomic, transcriptomics, proteomics and metabolomic salt profile of coastal plants could provide an insight into the mechanisms by which the differential performance is regulated in contrasting varieties of a single crop. This study proposes the construction of an Ionome suitable for the coastal saline region for sustainable food security. This study focuses on functional genomic studies of saline belt crops and meta-analysis of the information for proposed Ionome (Salt-Omic) development. In the salt-genome segment, the appropriate genes were identified and categorized covering ion-transport-genes, senescence-associated genes, molecular-chaperones, dehydration-genes. Proteome provides additional information on protein coding sequences, endogenous small molecules. The identified genes, proteins and signalling pathways could form an Ionome repository for molecular crop breeding programmes. The primary bioinformatics web source along with a customized database for several crops were found useful for identifying essential biomolecules. The study was able to assist in the formation of agri-ionome for the improvement of coastal crops. The sequential integration of agri-engineering model along with Omic details could be utilized for the construction of an explicit repository for molecular plant breeders in a way similar to AMBAB, LIS, Pulsechip or RiceMetaSys.

Keyword Ionome (Salt-omic) · Molecular crop-improvement · Food security · Breeder focused-database

5.1 Introduction

Salinity problem creates massive impact on food security of our country. Enhanced crop productivity and area augmentation are the two focus area that could assist in sustainable food security. India requires 311 million food grains for its exceedingly

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B. Mondal (✉)
The Neotia University, 743368, Sarisha, West Bengal, India
e-mail: bidisha.mondal@tnu.in

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growing population of approximately 1.43 billion in 2030. Intensive cropping, biodiversity loss, abundant fertilizer application, ground water depletion, deforestation, increased urbanization has led to an extraordinary increase in soil salinization in more than 6.74 million ha of land in the country. Advanced soil reclamation using novel technologies and strategies recovered and increased the crop area transforming the affected 2.18 million hectares of salt affected soil (Kumar and Sharma 2020). Inclusive technologies of gypsum-based treatment of sodic soil, subsurface drainage of saline soil, phytoremediation, bio-remediation, agro-forestry, novel breeding techniques could increase food security, income elevation and rural employability. The selection, retention and promotion of advanced breeding technologies could boost crop productivity aiding food security. The economically important varieties of rice, sunflower, sesame, moong, mustard, jute, vegetables and betel leaf grown in the coastal saline belt of West Bengal, India are susceptible to salinity stress. Plants can tolerate salinity stress by processes including salt exclusion or inclusion. The most predominant form of salt stress involving NaCl causes nutrient and metabolic imbalance. Increased salt stress leads to ion toxicity affecting the water retention capacity of the plant.

The breeders face constant challenges in development of new varieties resilient to extreme abiotic stresses including salinity. In such a condition unique breeding techniques will focus on expanding the gene pool or introspection of the present gene pool to identify some potential well performers. In recent times a comprehensive bioinformatics study involving inclusive approach covering all omic information could be applied for developing dynamic breeding strategies. The inclusion of high throughput techniques of DNA/RNA sequencing, microarray data and advanced proteomics could help the breeders to enter inside the unexplored territories of molecular crop advancement programme.

In salt and salinity management a number of agri-engineering computational models were already been invented and used. Scientists have analysed water flow and salt flow processes and developed scientific and engineering models for salinity management. On the basis of scope of application two models, basin model and field-scale models were developed by soil scientists, agri-engineers and agri-informatic professionals. LEACHC, SWAP, SOWACH, HYDRUS and UNSATCHEM were largely used for analysis of water flow, salt flow and plant performance. Among these models SWAP software package involves one-dimensional vertical transport of water, solute and heat and could predict crop yield. The SWAP model was effective in irrigation application and effective yield return studies in cotton (Pan et al. 2020).

SOWACH, a third generation salinity management model was written using QBASIC and runs under DOS and WINDOWS operating system. In a test, it was proved SOWACH model could effectively identify the rooting architecture ideal for increased yield (Alahmad et al. 2019). This model in alfalfa proved that across salinity treatments, final root length density was 24% higher in high fibrous root type and accordingly herbage yield of these root type superseded the low fibrous type by 14%.

The UNSATCHEM model was able to evaluate the efficacy of gypsum administered at different depths and able to suggest the feasibility of alternative management practises. Green manuring involving calcite could reclaim sodic field with less water or without addition of gypsum was proved by software application (Amini et al. 2016). The abundant application of agri-engineering computer software for analysing saline soil was invented for saving time, expense and accuracy of the growers, farmers and stakeholders.

The apparent abundance of soil engineering models could be surpassed if information on molecular crop databases could be utilized directly in salinity research. In reality, the employment of biological database in salinity research is meagre. The vast molecular resource available in public domain could be effectively used for Ionome construction and allied research. In this aspect the application and performance of OMIC data have enormous potential. The available OMIC database related to salinity could be exploited by plant breeders and molecular biologists. The key information for plant Ionome construction could be gathered from three main public repositories. GenBank, EMBL and DDBJ along with UniProt and Swissprot could provide initial data for salinity gene and protein sequence information. The major sequence repositories includes curated collections and provide holistic information on different domains (Lizumi and Sakai 2020). The individuality of these collections are maintained by their virtue of curation as well as unique approach of presentation of biological discoveries. A General plant database search could provide the detailed idea about available plant-omic information. Additionally, sequential exploration of the customized resources could assist in plant Ionome construction. The identification of important salinity gene, protein, EST, biological pathways, conserved domains and other relevant information were discussed in this paper to emphasize the importance of crop Ionome construction. Ionome (Salt-OMIC) could assist in the speedy selection and propagation of salt tolerant and resistant crops for the rejuvenation of the coastal and inland saline belts of developing countries ultimately ensuring food security for the human population.

5.2 Materials and Methods

In the present study two approaches were taken for OMIC data collection. In the first approach all the available primary bioinformatics resource were explored for development of proposed Ionome relevant to crop improvement programme of saline belt of West Bengal, India. In this study mainly GenBank, DDBJ and EMBL, Uniprot and Swissprot repositories were searched for gathering OMIC information of the major crops grown in saline belt of West Bengal. Additionally several other customised public database for the above mentioned crops were investigated for gene and cDNA.

In second approach the open source research database Google Scholar was used for article searching using a search string of salt + omic + crop name consensus tab with only one variable (crop name). It yielded articles with a sample size of 9818. Only English articles published in last 5 years were included. The selected articles were screened on the basis of abstract and keywords. In initial selection grey literature, preprints, presentation, duplication and keynotes were excluded manually reducing the sample size to 303. In the next phase the study applied Voyant tool

Table 5.1 Selection and screening of salt-omic-based research articles

Crop	Extracted paper (*n*)	Initial selection	Selected full paper
Rice	5010	254	14
Lentil	1240	57	9
Mung	1480	62	9
Sunflower	748	74	11
Mustard	514	102	9
Sesame	384	37	10
Jute	462	117	8
Total	9818	703	70

(Sampsel 2018), an open source, web-based application for executing text mining and analysis, promoting rapid analysis of scholarly articles and interpretation of texts from the selected articles. It precisely extracted the frequencies of keywords from all selected published articles and ultimately 70 articles covering only salt-omic information were thoroughly studied and data was extracted (Table 5.1).

5.3 Results and Discussion

5.3.1 Exploitation of Computational Data Source for Ionome Construction

In the saline coastal belts of West Bengal apart from conventional rice cultivation moong, mustard, sunflower, sesame, betel leaf and jute were also cultivated. In this study, the unique dichotomous approach recorded major salt genes and proteins. A comparative study was made between the crop specific saline data source and omic associated papers. This study generated significant findings worthy to be applicable for Ionome construction for coastal saline agricultural crops of West Bengal as well as other salt affected regions of India. Omic information was extracted from reported genes and proteins to be used as candidate or reference gene in abiotic stress tolerance. In rice a Google Scholar a search using Salt + omic + rice shows 5010 papers with 22 salt genes reported in gene repositories. Collectively, 1480 omic papers were found related to moong bean though there is no reporting of any gene. In NCBI platform a database search for moong bean yields only 1 database recovery under nucleotides 2 reporting was found and that also comes under a test result performed for exclusive NCBI internal data testing. Thousand two hundred forty papers were found for lentil salt genes with 5 proteins. Whereas a search with salinity genes in soybean extracts information from 10 databases out of which 8 could yield good result in reference OMIC study of moong bean and legume breeding. In sunflower 1480 omic papers are available with only one salt gene. NCBI covers a total of 22 bioinformatics database under which 10 were selected most suitable for the collection of Ionome information. These information could be used by plant breeders for novel molecular studies with allied species or varieties and in integration could act as a valid resource for saline agricultural research. Crop specific useful salinity details under each database was recorded for salt breeding experiments. The database information of 10 cultivated saline belt crops were given in Table 5.2.

As rice is an important cereal the omic information were explored from several fields of publically accessible databases like OryzaExpress (Kudo et al. 2017), Oryzabase (Kurata et al. 2006) and RiceXPro (Sato et al. 2013). The above domains were consulted for Ionome study. Salinity related 22 genes were found to be reported in NCBI database. The reported genes mainly came from japonica subspecies of rice and the available data could be utilized for advance screening of promising rice genotypes for a new region before the commencement of field trials. In RiceMetaSys web interface differential gene expression studies integrated molecular data for rice abiotic and biotic stresses (Sandhu et al. 2017). The breeders could use the OryzaExpress or Oryzabase for the construction of primers and contribute towards rice omic study as well. These database information could be utilized by the breeders of any omic discipline in the elucidation of molecular experimental result in rice. These database information could be utilized for pan-genomic and comparative candidate salt related genetic performance analysis of different salt tolerant indigenous and hybrid rice varieties.

LIS or legume information system is a community resource utilized for legume improvement programme (Gonzales et al. 2005). The LIS contains legume mines integrating 8 individual mines covering bean mine, chickpea mine, cowpea mine, medic mine, lupin mine, soy mine, peanut mine and joint vetch mines combining genomic and expression data of the inter-mines. LIS has potential to be used for gene identification along with the primary data available in major public database. Pulsedb, a database mapping software (Humann et al. 2019) and KnowPulse a diversity based software (Sanderson et al. 2019) could be utilized for stress related classical and molecular genetic studies of pulse.

BASC is an integrated bioinformatics system for Brassica research is available for omic study of salinity tolerant and resistant brassica (Timothy et al. 2007). BASC is generated for browsing and mining of Brassica genetic, genomic and phenotypic data. Multinational Brassica Genome Sequencing Project is a collection of five distinct modules, ESTDB, Microarray, MarkerQTL, CMap and EnsEMBL. ESTDB is a microarray module hosting expressed gene sequences and related annotation derived from comparison with GenBank, UniRef and the genome sequence of Arabidopsis. In mustard 130 salinity genes were evidenced with 720 proteins and 21EST from NCBI database. The Brassica database contains 47,555 unigenes made up of 17,939 consensus and 29,616 singleton sequences requires separation of stress genes of Brassica. Additionally, raw and normalized both types of data could be extracted from BASC.

In tomato different database extracts noteworthy information for genetic expression studies of tomato. Newly developed curated, open source integrated data resource including TGRD-Tomato Genomic Resources Database (Suresh et al.

Table 5.2 Omic details of essential salinity database of major crops of saline coastal zone of West Bengal obtained from NCBI public database (NCBI 2016)

Crop	Gene	Nucleotide	Protein	EST	Bio-projects	Geo dataset	Popset	dbGap	Conserved domain	SRA
Rice	22	358	224	203	49	80	4	0	2	4
Sunflower	0	8	5	18	1	1	0	0	0	0
Moong	1	4	10	0	1	0	0	0	0	0
Lentil	0	5	5	0	1	2	0	3	1	1
Betel leaf	0	0	10	0	0	0	0	0	0	0
Mustard	130	85	720	21	1	13	0	13	0	5
Grass pea	2	462	329	0	2	529	4	0	0	12
Sesame	36	14	268	1	2	29	0	5	0	6
Jute	0	1	168	2	2	0	0	0	0	1
Soybean	11	53	30	26	8	9	1	2	1	0

2014), TFGD-Tomato Functional Genomics Database (Tranchida-Lombardo et al. 2018), KaTomicsDB-Kazuka Tomato Genomics Database (Shirasawa et al. 2013), MoToDB-Metabolome Database (Grenan 2009) and COXPathDB (Narise et al. 2017) were of prime importance for tomato omic studies. Gramene (Tello-Ruiz et al. 2021)and MaizeGDB (Lawrence et al. 2008) portal was found equally effective in comparative functional genomic studies. The portal initially provided information for grass species but later the expansion of the portal made space for dicot species also. This portal hosts omic information of 93 reference genome of different plant species and 3.9 million genes covering 122,947 families with orthologous and paralogous details (Carson et al. 2016).

5.3.2 Salt Gene and Protein for Ionome Construction

In this study integrative meta-analysis efficiently identified several promising salinity genes. Twenty-five productive salt genes were identified in this study that could act as reference gene in virtual molecular breeding design. Bioinformatics tools could aid the breeder in initial design of a new crop related study revealing paralogous or ortholog gene function. The information presented in Tables 5.3 and 5.4 could be utilized for an inclusive study of sequence information, marker assay, trait association, locus details and biochemical path analysis of salinity tolerant important crops of West Bengal. This kind of bioinformatics data was utilized in testing of salinity responsive candidate gene in tomato for varietal performance testing (J'afar et al. 2018). In another paper two drought tolerant landraces were compared using 122 candidate gene studies. The approach identified high effect SNPs, structural variants and promising heat shock proteins and Cation /H^+ antiporters. Additionally, pan-genomic studies using 753 accessions in tomato showed 4873 genes were diverse from the reference genome (Chaudhary et al. 2019). In case of betel leaf the recorded information in NCBI repository requires huge input and there is provision for omic study.

5.3.3 Signalling Pathways for Ionome Construction

To combat salt stress the plant maintains an equilibrium among carbon sink distribution, energy allocation and osmotic balance. Multiple signalling pathways become active for controlling salinity stress (Othman et al. 2017). Ionic stress signalling pathways along with osmotic stress signalling and detoxification pathways become operative in salt-stressed plants. A plethora of genes and proteins are related to this signalling cascades. SOS response, jasmonate pathway, tyrosine signalling, aquaporin regulation, ethylene synthesis, annexin mediated conductance, expression of asparagine synthetase, hydroxyl proline-rich glycoproteins, ROS and ABA related proteins expressions were vital in salt stress management. Along with the

Table 5.3 Essential genes identified for reference salt genomic studies

Gene	Crop	Gene ID	Function	References
SUV3	*Oryza sativa* Ssp. Japonica	4,334,089	Delayed senescence, ATP-dependent RNA helicase	Macovei et al. (2017)
KAT (4386)	Sunflower, rice, maize, potato	110,864,580	Cytosolic cation homoeostasis	DeLeon et al. (2015)
HKT1	Japonica rice	4,341,971	Regulation of Na toxicity under salt stress	Oda et al. (2018)
NHX	Barley	103,934,031	Na/H exchanger, Vacuolar transporter	Liangbo et al. (2018)
SOS family	Rice	4,341,015	SOS response peptidase	Rice Consortium (2003), Platten et al. (2013)
TIFY gene family	Brassicaceae	4,342,421	Jasmonate ZIM domain specific protein	Reddy et al. (2017)
CRY1b	Rice, Brassica	543,688 2,829,419	Cryptochrome 1b Melatonin biosynthesis	Hwang and Back (2021)
CDPK	Rice/Arabidopsis	819,282	Receptor kinase Ca/CaM	Reddy et al. (2017)
MAPK	Dicot/Monocot	541,618 (Maize)	Serine/threonine protein kinase, salt related oxidative stress	Kong et al. (2019)
GH3	Rice, Gossypium, Vitis	107,896,815 Gossypium	Lipid transporter protein, Tyrosine kinase signalling	Wong et al. (2019)
ERF4 OsSIRP1	Monocot, spinach, Arabidopsis	101,290,597 Triticum	Jasmonate, ethylene pathway	Zhang et al. (2020)
NAC	Wheat, rice, sorghum, sugarcane, mung	818,902 Arabidopsis	AtHB13 and JUB1 transcription regulator	Ebrahimian-Motlagh et al. (2018)
OBF1 (2021)	Maize, rice, Dendrobium	542,394 Maize	Zn finger ring domain, ocs element transcription factor	Alexandrov et al. (2009)

(continued)

Table 5.3 (continued)

Gene	Crop	Gene ID	Function	References
JcAP2/ERFs 2381	Rice, physic nut, Arabidopsis	844,348	Leucine rich repeat family, stomatal development	Tang et al. (2016)
OsFd1/Saltol	Red rice	3,974,662	Ferrodoxin 1 ETS, Na/K homoeostasis	He et al. 2020
RSS3	*Oryza sativa* Ssp. Japonica	4,350,435	Jasmonate induced gene expression	To et al. (2019), Toda et al. (2013)
OsGTv2	*Oryza sativa* Ssp. Japonica	4,330,612	Mitochondrial aldehyde dehydrogenase, Salinity adaptation	Xie et al. (2020)
AFP1,ninja family protein	Sunflower, tomato, rapeseed	110,920,831	putative ethylene responsive binding factor-associated repression, Ninja family	Badouin et al. (2017)
TaHAG1	Bread wheat. Barley, Arabidopsis	119,339,917	Modulating ROS production and salinity regulation	Zheng et al. (2021)
OsLEA	*Oryza sativa* Ssp. Japonica	4,339,745	ABA induced antioxidant stresses	Rice Consortium (2003)
OsPYL/RCAR7	*Oryza sativa* Ssp. Japonica	2,829,419	Glutaredoxin, ABA receptor	Bhatnagar et al. (2020)
TaOFP family	Triticum	3,760,030	Tillering, water stress	Wang et al. (2020)
ZmPTPN	Maize, Arabidopsis	AT5G50670	ABA signalling AsA biosynthesis	Zhang et al. (2020)
AQP (cDNA)	Chickpea, lathyrus, lentil	DY475124	Aquaporin regulation	Mantri et al. (2007)
ASNS (cDNA)	Chickpea, gram, lentil, grass pea	DY475477	Asparagine synthetase (glutamine hydrolysing)	Mantri et al. (2007)

Table 5.4 Essential proteins identified for reference proteomic studies from Uniprot repository

Protein	Accession	Length (AA)	Pathway	Crop	References
WRKY transcription factor WRKY71	Q6QHD1	348	Gibberellin signalling pathway	Rice	Zhang et al. (2020)
Jasmonate ZIM domain-containing protein 9	Q8GSI0	179	Jasmonic acid signalling pathway	Rice	Young et al. (2018)
SNF1-related protein kinase catalytic subunit alpha	Q38997	521	Serine/threonine protein kinase	Arabidopsis	Simon et al. (2018)
Bidirectional sugar transporter SWEET15	Q9FY94	292	Transmembrane transporter	Maize	Doidy et al. (2019)
Ascorbate oxidase	M4DUF2	570	Oxidoreductase, defense pathway	Rapeseed	Nudrat et al. (2017)
Mitogen activated protein kinase	ACJ31803	586	ABA signal transduction	Groundnut	Wang et al. (2016)
XERICO	KAG1363776	166	E3 ubiquitin-protein ligase	Coconut	Brugiere et al. (2017)
5MYB	ABI74688	348	Transcriptase	Cabbage	Wang et al. (2015)
JUNGBRUNNEN	Q 9SK55	375	TF, Central longevity regulator	Thalecress	Dudhate et al. (2021)
ABRC5	AAR06258	141	Ethylene responsive binding factor	Sunflower	Najafi et al. (2018)
RCAR3	5GWO_D	175	ABA receptor	Rice	Hyunmi et al. (2012)
DELLA	NP_001240948	523	Ethylene regulator	Tomato	Shohat et al. (2020)
SnRK2	AID23890	354	sucrose non-fermenting 1-related protein kinase 2	Cotton	Liu et al. (2017)
ANAC017	EFH67341	547	Transcription regulator	Thale cress	Meng et al. (2019)
AKT	AT3G49850	693	AKT kinase telomeric DNA binding protein		Xu et al. (2020)

salt genes several important salt proteins were found impressive for comparative proteomic studies. In proteomics 2D gel electrophoresis, MALDI-TOF, mass spectroscopy (Moco et al. 2006) and western blot have proved effective in analysing stress response (Shinozaki et al. 2018). Differential response of 49 proteins was noticed under aluminium stress in tomato seedlings and 40 for silicon stress in tomato (Zhou et al. 2009; Muneer et al. 2015).

5.4 Conclusions

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The web-based primary bioinformatics database provides abundant omic information for several crops assisting scientists and researchers in the construction of crop specific public or private databases. Salinity stress is regarded as one of the robust abiotic problems reducing global food productivity. The proper alleviation of salt stress requires an understanding of the activity or expression of mineral and trace elements forming an integrated database including agri-engineering-based plant modelling and multivariate omic information to provide a solution to farmers. The omic study could provide true insight into the physiological activity of plant organs, differential expression of genes and epigenetic regulations. The above study proposes the necessity of the formation of a breeder-focused standalone Ionome (Salt-OMIC) database or crop specific customised construction for ensuring global food security under ever changing environmental complications.

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Chapter 5

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