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INTEGRATED MULTI-OMICS AND MICROBIOME APPROACHES FOR BIOPROSPECTING STRESS-INDUCED SECONDARY METABOLITES IN HALOPHYTES

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ABSTRACT
Halophytes, which are chemically rich yet largely unexplored in plant bioprospecting, possess specialized mechanisms that allow them to complete their life cycle under high salinity conditions (≥ 200 mM NaCl). These species constitute approximately 1% to 2% of the world's flora and are distributed across coastal, mangrove and saline agricultural regions. Upon consistent ionic and osmotic stress, these plants accumulate secondary metabolites like alkaloids, flavonoids, terpenoids, phenolics and sulphated polysaccharides. With global salinization projected at 2050, over 50% of world's cultivable land may be affected by soil salinization. Therefore, halophytes represent an ecologically important and pharmacologically unexplored source of bioactive metabolites rather than a traditional natural products pipeline. Unlike previous reviews focusing on salt tolerance physiology, this review integrates molecular regulation, multi-omics, microbiome interactions and synthetic biology into a single framework for translational bioprospecting. The molecular framework of salt stress-induced secondary metabolite biosynthesis, epigenomics, transcriptomics, proteomics, metabolomics and fluxomics evidence, and halophytes-associated microbiome as a source for bioprospecting, biotechnological strategies for exploiting the secondary metabolites, such as in vitro tissue culture, hairy root culture, genetic and metabolic engineering. Several research gaps have been identified, including the lack of standardized protocols, a shortage of in vivo validations, and fragmented multi-omics integration.

KEYWORDS: Halophytes, Salt stress, Plant Microbiome Interactions, Multi-omics, Secondary Metabolites, Bioprospecting, Metabolic Engineering

INTRODUCTION
Soil salinization is a critical abiotic stress that severely threatens global food security. Approximately 1.1 billion hectares worldwide are affected by salinity [1, 2]. Approximately 50% of agricultural land will be affected by soil salinity by 2050 upon climate change [3, 4]. Bioprospecting is the field of exploring for novel genes, metabolites and enzymes which possess medicinal and commercial value. In the fields of pharmaceuticals, nutraceuticals, and stress-resilient agriculture, bioprospecting plays a number of crucial roles. In recent years, researchers have focused on ecosystems that can survive in harsh environments due to their distinct metabolic diversity and adaptive mechanisms [5]. Out of all the organisms, halophytes have drawn the most attention from scientists because they can withstand salinity and produce metabolites that respond to stress [6].
Halophytes are plants that can tolerate soil or water that is highly salinized (≥200 mM NaCl) without experiencing any changes in yield [7]. According to Flowers and Colmer (2008), the majority of halophytes are members of the Poaceae, Tamaricaceae, Plumbaginaceae, and Chenopodiaceae families. Halophytes are perfect for salt-tolerant sources because, in contrast to glycophytes, they have some specific molecular adaptations to extreme salt stress [8]. According to Shamsutdinov et al. (2017), the continent of Asia has the highest percentage of halophytes (15%), followed by North America and Australia [9].
In addition, halophyte associated microbiomes contribute significantly to the stress adaptation by synthesizing their own secondary metabolites. Due to their contribution in saline tolerance, they may also serve as resource for bioactive secondary metabolites, which can be used for bioprospecting as well [10]. The regulatory networks connecting salt stress, transcription factor activation, and metabolic pathway upregulation have recently been untangled by the integration of multi-omics, allowing for the targeted exploitation of halophyte chemistry [11]. Although several reviews have addressed the halophyte physiology, salt tolerance mechanism, and individual omics with microbiome interactions, biotechnology application with relevant to exploitation of stress induced secondary metabolite. This review discusses the framework linking stress induced secondary metabolite production and emerging synthetic biology approaches to improve the sustainable bioprospecting and

pharmaceutical translation. Furthermore, it addresses the research gaps and future directions for translating the halophyte resource to large scale pharmaceutical development.

Decoding the Halophytic Defense Arsenal: A Multi-omics Perspective:

Halophytes inhabit environments characterized by high salinity, osmotic imbalance, and oxidative stress; unlike glycophytes, they possess active defense mechanisms that integrate signalling pathways, transcriptional regulation, and specialized metabolite synthesis. This defense mechanism, known as the "halophyte defense arsenal," is being deciphered using multi-omics techniques like transcriptomic, metabolomics, and microbiome-based methods. Epigenetic modifications regulate transcription, which determines protein and its activity. These proteins catalyse metabolic reactions which ultimately reconstitute the metabolome. Fluxomics extends this framework by quantifying the dynamic movement of metabolites through interconnected biochemical pathways under salt stress.

The recent advancement in high-throughput sequencing and mass spectrometry reveals the interconnection of regulatory networks that govern salt tolerance, instead of isolated genes or metabolites. Thus, halophytes undergo coordinated adaptive mechanisms to survive under salt stress.

Epigenomics

Epigenomics focuses on alteration in chromatin architecture like DNA methylation and histone modifications which regulates gene expression without changing the DNA sequences [12]. In *Chloris virgata,* tissue specific and anion specific DNA methylation variation helps the plant in salt tolerance [13]. DNA methylation is predominant epigenomic variation, which is highly linked to high salinity and plant stress memory [12]. Epigenetic mechanisms provide halophytes with high plasticity, which enables both short-term physiological adjustment and long-term transgenerational adaptation to salinity [12, 13].

Transcriptomics

The transcriptome analysis aids in the detection and measurement of changes in the level of gene expression related to salt stress. Transcriptome studies reveals that halophytes respond to salt stress through coordinated regulation of downstream molecular response involves ion transport, osmotic adjustment, antioxidant defense and secondary metabolite biosynthesis [14, 15].

Transcriptome analyses of the marine halophytes, *Avicennia officinalis, Avicennia marina, Suaeda maritima, Salicornia brachiata, Sesuvium portulacastrum, Urochondra setulosa and Zoysia macrostachya,* have shown a large-scale of transcriptional reprogramming during salt stress adaptation. Differential gene expression analysis indicates the activation of some genes related to ion transport, osmotic homeostasis, Calcium-mediated signalling pathways and regulation of phytochemicals. Collectively, these indicate that stress adaptation is a coordinated interplay between multiple pathways rather than individual responses [16-21]. The Weighted Gene Co-expression Network Analysis (WGCNA) study from *Avicennia marina* shows the tissue specific gene clusters, which helps in identifying unique modules for leaf epidermis versus pneumatophores [18, 21]. Early salt sensing mechanisms initiate downstream signal transduction cascades that regulate stress adaptation processes and upregulate calcium sensors such as calmodulins, calcineurin B-like proteins and CBL-interacting kinase [17]. Transporter protein genes, Na+/H+ antiporter and aquaporin, are all expressed simultaneously. This shows that ionic balance in cells and water regulation are active under saline conditions [16]. The pathways (defense) and secondary metabolite production from the families of WRKY, NAC, MYB, ERF, bZIP protein, LEA protein, NLP7 TF, and bHLH TF family are associated with the salt perception mechanisms by upregulated or downregulated transcription factors [15, 17, 19, 22]. The transcription factors like WRKY, NAC and DREB are highly conserved across the halophytic species but MYB is species specific and widely diverse. And the transcriptional factor NAC is a promising target for engineering. The transcriptional regulation of certain metabolites for stress conditions, like phenylpropanoid biosynthesis, glycinebetaine biosynthesis, trehalose metabolism, flavonoid biosynthesis, cytochrome P450, and antioxidant enzymes, is primarily involved in the upregulation of genes [16, 17]. Furthermore, deep sequencing has enabled the identification of stress-responsive miRNA genes that modulate signal transduction and transcriptional regulation under high salinity conditions [23, 24] (Table 1). Figure 1 depicts the overall response of halophyte to salt stress. The Co-expression studies reveals the integrated modules of miRNA – mRNA, which reveals miRNA are co-expressed with other genes from MAPK signalling pathway [23]. And strong negative correlation is identified between amino acids and the organic acids in *Haloxylon salicornicum* [25]. In spite of significant progress, the transcriptomics studies are limited by differences in sequencing platforms, experimental conditions, stress treatments and pipelines annotations making direct comparisons across species challenging. Future research should integrate comparative transcriptomics along with metabolomics and functional validation, which will be useful for identification of conserved regulatory modules which can be exploited for crop improvement, drug discovery and product discovery.

Proteomics

Proteomics identifies the complete set of proteins expressed in sample, which offers insights to functional executors of salt tolerant mechanisms [5]. These proteins in halophyte involves in various classes including photosynthesis, redox homeostasis and signal transduction [5]. For instance, wild halophytic rice cultivars shows enhanced level of proteins which stabilize the photosystems and synthesis of osmolytes. The profiling may reveal modifications like ubiquitination, which regulates the cell wall integrity during salt stress [26]. This will minimize the cell damage and reactive oxygen species maintaining [5]. The proteomics analysis shows the complex regulatory networks which mediates the high salt resistances in halophytes [5, 26].

Table 1 Integrated Transcriptomic and metabolomic evidence of halophyte upon salt stress

S. No.	Species	Identified Metabolites	Core Metabolic Pathways	Response to Salinity	Stress Adaptation Strategy	Reference
1.	*Atriplex griffithii*	Myo-inositol, glyceric acid, ribonic acid, methionine, aspartic acid, glutamic acid, L-threonine, tryptophan	Amino acid metabolism, carbohydrate metabolism, TCA cycle	High levels of myo-inositol, ribonic acid, and methionine	Osmotic regulation via myo-inositol accumulation; maintenance of cell turgor pressure through soluble sugars	[27]
2.	*Atriplex halimus*	Sucrose, proline, glycinebetaine	Osmotic adjustment and ion regulation	Significant increase in sucrose content under salt stress; accumulation of glycinebetaine	Transport of salts to vesiculated hairs on leaf surfaces; osmotic adjustment via solutes	[28]
3.	*Atriplex nummularia*	Malic acid, citric acid, succinic acid, 2-ketoglutaric acid, fructose, turanose, talose, arabinose	TCA cycle, carbohydrate metabolism, glyoxylate and dicarboxylate metabolism, arginine biosynthesis	Increased levels of organic acids (malic, citric) and specific sugars (fructose, turanose)	Metabolic reprogramming; maintaining high osmotic potential; accumulation of soluble sugars as osmoprotectants	[27]
4.	*Avicennia marina*	Metallothioneins (MT-2, MT-3), dehydrins, glutathione S-transferase (GST), thioredoxin (TRX), laccases, putrescine, spermidine, spermine, carotenoids, cysteine, betaine, proline	Lipid biosynthetic and degradation pathways, mitochondrial betaoxidation, hydroxygeraniol metabolism, polyamine biosynthesis, phytohormone regulation, photosynthetic pigment synthesis, sulphur metabolism	High expression of metallothioneins and dehydrins; active salt efflux; increased endogenous polyamines and cytokinins when associated with Streptomyces mutabilis ; reduced leaf area and biomass	Ultrafiltration in roots; salt glands on leaf surfaces; endophytic association with PGPA; maintenance of K+ homeostasis; induction of H2S and cysteine biosynthesis	[18, 21, 29]
5.	*Avicennia marina subsp. australasica*	Putrescine, aminoadipic acid, α-ketoglutarate, soluble sugars (glucose), phenolic acids (syringic and sinapic acids)	TCA cycle, galactose metabolism, starch and sucrose metabolism	Phenotypic associations between growth and metabolome; reduction in α-ketoglutarate; accumulation of soluble sugars	Synthesis of stress-ameliorating metabolites; osmotic adjustment via increased soluble sugar content	[30]
6.	*Avicennia officinalis*	Proline, glycinebetaine, polyols, sugars, sodium, chloride, ethylene, auxin (IAA), ABA	Ca2+ signalling, trehalose biosynthesis	Flavonoid biosynthesis, Ethylene & Auxin signalling –	Salt exclusion at roots via hydrophobic barriers; osmotic adjustment; ROS	[17]

				upregulated, ABA signalling-downregulated,	scavenging via flavonoids and antioxidant enzymes	
7.	*Halogeton glomeratus*	Flavones, flavonols, glucosinolates, isoquinolines, amino acids (proline, arginine), unsaturated fatty acids (linoleic acid), sucrose, trehalose	2-oxocarboxylic acid metabolism, Aminoacyl-tRNA biosynthesis, Phenylpropanoid biosynthesis, ATP binding cassette (ABC) transporters, glycerophospholipid metabolism	Hyper accumulation of salts in succulent leaves; extensive alteration in the metabolome among different organs	Osmotic adjustment; membrane lipid modification; toxin avoidance via ABC transporters	[31]
8.	*Haloxylon salicornicum*	Galactaric acid, aspartic acid, threonine, glutamate, leucine, arginine, tyramine, lysine alanine, Phenylalanine	Citrate cycle (TCA cycle), starch and sucrose metabolism, phenylalanine metabolism, amino sugar and nucleotide sugar metabolism	Upregulation of organic acids and sugars; downregulation of most amino acids except alanine and phenylalanine	High TCA cycle activity for energy supply; accumulation of low molecular weight osmoregulators; ABA-mediated stress signalling	[25]
9.	*Puccinellia tenuiflora*	Potassium (K+), sucrose, flavonoids, amino acids	Pentose phosphate pathway (PPP), flavonoid biosynthesis, sucrose biosynthesis,	Enhanced root K+ concentration; maintained low shoot Na+; upregulation of HAK, HKT1:3, NHX, and V-H+-ATPase genes	Gene family expansion for K+ uptake and osmotic adjustment; positive selection of K+ channel genes	[32]
10.	*Salicornia brachiata*	Soluble sugar, proline, amino acids, Na+, K+, Ca2+	SOS (Salt Overly Sensitive) pathway (SbSOS1)	Grows in 0.1–2.0 M NaCl; salt accumulation up to 40% dry weight; constitutive SOS1 expression	Active Na+ efflux via plasma membrane Na+/H+ antiporter (SOS1); Na+ loading into xylem; maintenance of high K+/Na+ ratio	[33]
11.	*Salicornia europaea*	Amino acids (L-proline, L-cysteine), total flavonoids, ascorbic acid, carotenoids, total phenols, lipids (LPE, LPC), saccharides (sucrose, glucose, turanose, melibiose, galactinol, fructose 1-phosphate), organic	Carbon fixation, nitrogen metabolism, amino acid metabolism, carbohydrate metabolism, glycolysis, flavone and flavonol biosynthesis, phenylpropanoid biosynthesis, TCA cycle, glutathione metabolism	Stimulated growth at 50–100 mM NaCl; survived up to 800 mM NaCl; increased tissue succulence; upregulation of trigonelline and specific saccharides	Protection of membranes and photosystem II; osmotic adjustment via soluble sugars, proline, and alkaloids; energy conservation via suppressed TCA cycle; suberin biosynthesis in roots	[28, 34, 35]

		acids (3-methyl-2-oxobutanoic acid), phenolic acids (p-coumaric, ferulic, protocatechuic acids), alkaloids (trigonelline, betanin), 2-propenyl (sinigrin)				
12.	*Salvadora persica*	Proline, methionine, phenylalanine, phosphonic acid, citric acid, galactaric acid, mandelic acid, caffeic acid, shikimic acid, gulonic acid, D-xylose, D-mannose, D-tagatose, D-mannitol, ABA, jasmonic acid, stearic acid, benzyl amine, serine, norvaline, threonic acid, glucose, sucrose, galactinol, glycerol, 2-ketoglutaric acid, glycine, betaine	Amino acid biosynthesis, energy metabolism, carbohydrate metabolism, glycolysis, gluconeogenesis, pyruvate metabolism, TCA cycle, inositol phosphate metabolism, galactose metabolism, alkaloid biosynthesis, shikimate pathway, butanoate metabolism, phenylalanine metabolism, ABA signalling, ion transport	Significant metabolic shifts (56 metabolites); upregulation of glucose, sucrose, mannitol, phenylalanine, and proline; downregulation of caffeic and citric acids; TCA cycle downregulation; ABA-mediated stomatal closure	Osmotic adjustment via accumulation of sugars and sugar alcohols (myo-inositol); ROS scavenging via phenolic compounds; antitranspirant activity via ABA/JA synergism; use of 2-ketoglutaric acid as a metabolic junction	[27, 28, 36]
13.	*Sonneratia apetala*	Phenylpropanoids, basic endochitinases B (CHIBs)	Plant hormone signal transduction, MAPK signalling, phenylpropanoid biosynthesis, phenylalanine metabolism	Upregulation of WRKY22; significant alteration in HD-Zip, SnRK2, and PP2C expression	miRNA-mediated regulation of target genes (Sap-miR393a); hormone cross-talk (DELLA proteins)	[23]
14.	*Suaeda fruticosa*	Glycinebetaine, glutathione, calcium ions, potassium ions, sodium, chloride	Nitrogen metabolism, histidine metabolism, lysine degradation, glycerolipid metabolism, linoleic acid metabolism, photosynthesis (PSII), glutathione metabolism	Sequestration of salts in leaves; upregulation of GST, APX, and photosynthetic genes at 300 mM NaCl	Sequestration of ions in shoot vacuoles; synthesis of glycinebetaine; maintenance of K+/Na+ ratio; antioxidant enzyme trigger	[37]
15.	*Suaeda japonica*	Na+, soluble sugars, proline	Starch and sucrose metabolism, proline biosynthesis, glutathione metabolism, ABA	Optimal growth at 100 mM NaCl; increased Na+ accumulation primarily in leaves	Ion compartmentalisation in vacuoles (NHX1, NHX4); Na+ extrusion (SOS1); osmotic regulation; LEA protein protection	[38]

			signalling, peroxisome pathway			
16.	*Suaeda maritima*	Glycinebetaine, ethanolamine, polyamines, proline, tyrosine, flavonoids, phenolics	G protein and phosphoinositide signalling, cell wall and carbohydrate metabolism, TCA cycle, glycolysis, miRNA-guided gene regulation	Upregulation of NAC and HD-Zip transcription factors; upregulation of PM-H+ATPase and Na+/H+ antiporters; maintained photosynthetic electron transport rate	Cell wall restructuring (lignification); modification of membrane lipids; Na+ sequestration; post-transcriptional regulation via salt-responsive miRNAs	[16, 24]
17.	*Suaeda rigida*	Proline, catalase, peroxidase	Signal transduction, ABC transporters, cell wall metabolism, carbohydrate metabolism	Upregulation of protein phosphatase 2C genes; cytochrome P450 induction	Na+ and Cl- compartmentalization; succulence buildup; ABA signalling activation; cell wall modification (expansins)	[39]
18.	*Suaeda salsa*	Flavonoids (quercetin, isorhamnetin, naringenin), alkaloids, coumarins, amino acids (proline, aspartic acid, asparagine, tryptophan, cystine), nucleotides, organic acids (succinic acid), lipids (α -linolenic acid), branched chain amino acids, glucosamine, maltose, D-sucrose	Amino acid metabolism, carbohydrate metabolism, secondary metabolism pathways, TCA cycle, glycolysis, flavonoid biosynthesis, phenylpropanoid biosynthesis	Upregulation of proline, aspartic acid, and soluble sugars; reconstitution of metabolic homeostasis; upregulation of ion transporters (SsNHX1, SsSOS1)	Osmotic adjustment through soluble sugar and amino acid accumulation; substrate for stress-induced protein synthesis; ROS scavenging via enzymatic and non-enzymatic components	[34, 40]
19.	*Urochondra setulosa*	Proline, glycinebetaine, trehalose	Photosynthesis (RUBISCO), MAPK signalling, ascorbate-glutathione cycle, transmembrane transport	Upregulation of RUBISCO and ferredoxin; induction of Na+/H+ exchangers and ABC transporters	Ion homeostasis via vacuolar sequestration; accumulation of compatible solutes; maintenance of cell wall plasticity	[19]
20.	*Zoysia japonica*	Na+, K+	Auxin and ABA signal transduction, MAPK signalling, phenylpropanoid biosynthesis	Salt-tolerant accessions (Z011) maintain high K+/Na+ ratios under 350 mM NaCl	Selective salt secretion via leaf salt glands; reduction of Na+ transport from roots to leaves; increased root K+ absorption	[15]

21.	*Zoysia macrostachya*	Soluble sugar, proline, soluble protein, Na+, Ca2+, K+, Cl-	Plant hormone signal transduction (Auxin, Gibberellin, ABA), gluconeogenesis, glycolysis, photosynthesis	Increased salt secretion; significant increase in SOD, POD, and CAT activities; accumulation of osmoregulatory substances	Ion exclusion via bicellular salt glands; osmotic adjustment; DELLA protein accumulation	[20]

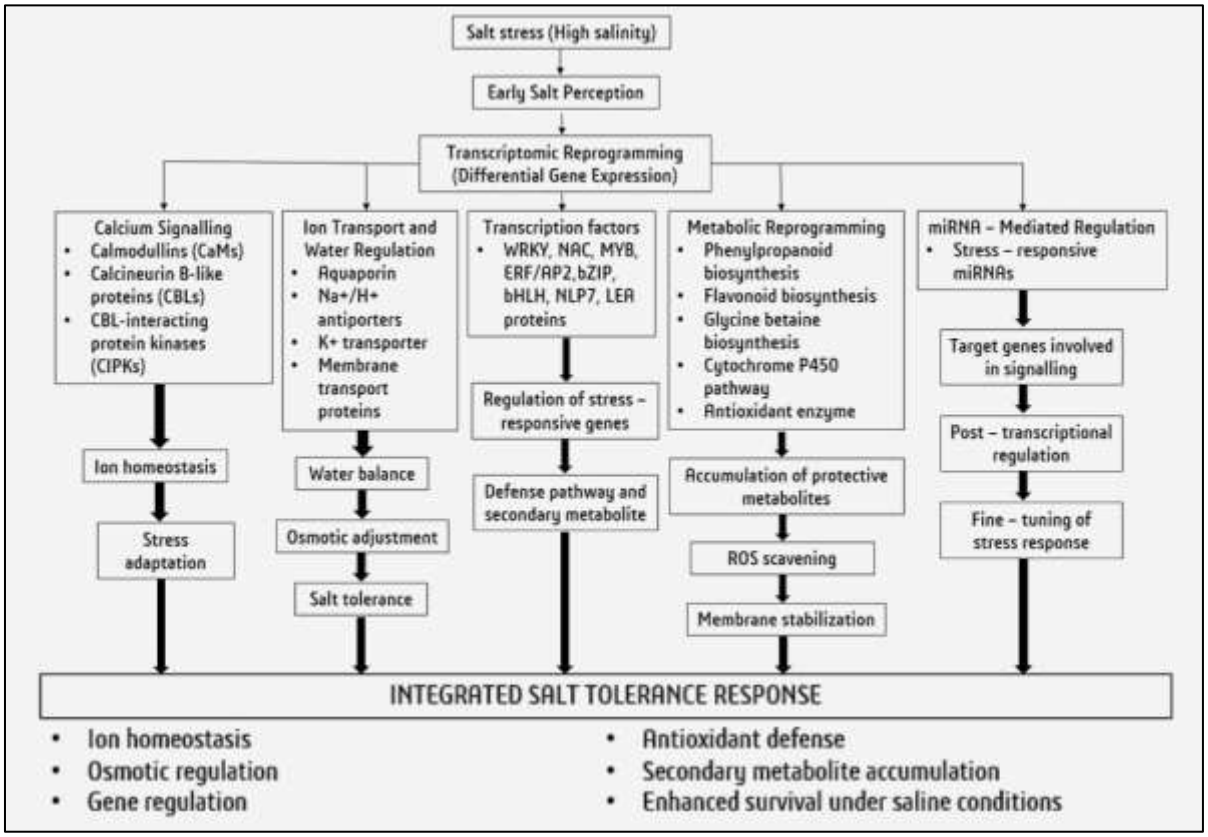

Fig. 1 Integrated transcriptomic cascade of halophytes upon salt stress, illustrating response to salt stress, calcium mediated signalling and activation of transcription factors, regulation of ion transports, osmotic adjustment, antioxidant defense and downstream secondary metabolite biosynthesis.

Metabolomics

The transcriptome shows only the regulatory mechanisms, but metabolomics provides a direct representation of pathways by which plant accumulates secondary metabolites. Metabolomics enables the comprehensive identification and quantification of metabolites present in plants. This serves as a link between phenotype and genotype. This identifies the substances that cause salt stress adaptation in halophytes [5, 34]. Accumulation of compatible solutes such as Proline, glycinebetaine, alcohols, and sugars accumulate in plants as a result of certain metabolite reactions [25, 36]. For example, D-(+)-glucose and L-proline accumulate as specific responsible metabolites in *Salicornia europaea* under salt stress. Additionally, there is a significant build-up of quercetin and kaempferol derivatives in roots and shoots as a defense against oxidative damage [35]. Similarly, by adjusting the tricarboxylic acid (TCA) cycle, the halophyte *Salvadora persica* maintains an uninterrupted energy supply for osmolytes [36]. And also for maintaining the stability and fluidity in plasma membrane, the increase of unsaturated fatty acid – α linolenic acid by lipid remodelling [40]. *Suaeda salsa* shows variation in lipid compounds to protect photosystem II and cell integrity in high salinity [34, 40]. A study on rhizosphere of *Suaeda salsa* reveals, metabolites like oleamide and 5'-methylthioadenisine (MTA) were associated with recruiting salt-tolerant microbes, which enhances plant resilience through symbiosis [41]. However, *Salicornia europaea* has shown increased trigonelline content, an alkaloid that may serve as a key metabolite in the defense against salt stress [34]. The genome scale metabolic networking facilitates reconstruction of metabolic pathways using AI assisted prediction [21, 30]. The AI-assisted pathway prediction is also being used to integrate multi-omics data, and to accelerate the discovery of loci which controls the stress responsive metabolites by developing algorithms [42]. Even though metabolomics identifies several compounds, several limitations remain. Many studies have focused on the targets which are already known, even though untargeted focussed analysis and cross species comparison are relatively low. Further, many metabolites lack functional validation for their accumulation to pharmacological activity. Integrated metabolomics profiling with transcriptome, microbiome analysis and pathway prediction using machine learning will accelerate the identification of novel metabolites for drug development and other commercial products.

Fluxomics and Ion homeostasis

Fluxomics in halophytes focuses on the dynamic movement of ions and water to maintain cellular homeostasis under salt stress [5]. The specific protein synthesis and osmotically active metabolites are responsible for controlling ion and water flux [5]. Advanced study shows root apex proton fluxes are the central to how plants

acclimate to soil salinity [43]. The fluxes are mediated by high affinity potassium transporters and vacuolar ATPases which compensates the toxic accumulation of sodium ions [5].

Halophyte microbiome interaction: Bioprospecting and the architecture of stress resilience

The concept, plant functions as integrated system together with associated microbes has changed our understanding of the stress adaptation. The endophytic bacteria, fungi and rhizosphere microbes contributes to nutrient acquisition, stress mitigation and the production of stress induced secondary metabolites.

Rather than functioning individually, combination of both plant and microorganisms gives dynamic interactions to metabolic exchange, chemical signalling. Understanding these interactions is mandatory for identifying new pharmaceuticals leads.

Endophytic and rhizosphere microbiome as the source of bioprospecting

Halophyte-associated microbes act as a source of new bioactive natural products, which are very useful in the pharmaceutical and agricultural industries [44]. Endophytic bacteria isolated from *Salicornia brachiata* produce surfactins and cyclic lipopeptides with antimicrobial properties [44]. These microorganisms produce polyketides, terpenoids, and alkaloids, which possess significant pharmaceuticals properties like antioxidant, antidiabetic, and anticancer [45]. Some rhizospheres and endophytic bacteria produce methyl jasmonate and 2,4-Diacetylphloroglucinal (DAPG), which are important defense compounds [46]. Even some halophilic bacteria produce polyextremophillic enzymes, such as xylanases and laccases, which are extremely useful in the textile and pharmaceutical industries [47]. Further research reveals that hidden metabolites with unique pharmacological characteristics can be found by activating biosynthetic gene clusters (BGCs) [48].

Plant – microbiome crosstalk: Host plant as architect

One hypothesis questions whether the microbiome or the host plant is the real architect for the synthesis of novel metabolites under salt stress is up for debate [49]. Some claim that the host plant is the real architect because it causes the root exudates to change in a way that attracts particular microbes [50]. When under salt stress, the halophytes undergoes through a "cry for help" mechanism that alters the metabolic profile of root exudates and causes the secretion of sugars, phenolics, citramalic acid and organic acids [41, 51]. The transcriptomic response is altered by the activation of genes such as SOS1, NHX1, and NCED, which results in the selective recruitment of microbes [52, 53]. And the quorum sensing is also interfered by the root exudates, which leads to formation of biofilm, virulence and secondary metabolite production [50]. Furthermore, plants regulate the microbiome to make sure that only microorganisms that sequester sodium or supply nutrients are attracted [53, 54]. According to this perspective, the microbes merely take up the space provided by the host plants in order to survive [41, 53]. From this perspective, microbes function as recruited partners, the ecological roles are determined by the host derived chemical signals. Therefore plants acts as the principal architect under saline condition by directing the microbiome composition and metabolic interaction.

Plant – microbiome crosstalk: Microbiome as architect

On the other hand, microbiomes are referred to as true architects because they possess special metabolic machinery and biosynthetic gene clusters (BGCs) necessary for the synthesis of complex stress-relieving metabolites that plants are unable to produce on their own [4, 48]. Extensive genomic research reveals the existence of large BGCs such as PKS and NRPS systems, which are in charge of assembling substances like alkaloids and surfactins [45, 48]. *Salinivibrio costicola*, an endophytic bacterium, produces polyhydroxybutyrate (PHB) derivatives, which build up in *Suaeda maritima* tissues and provide energy and osmotic protection [49]. Therefore, the microbiome as considered as plant's "second genome," providing functional capacity for plant survival under extreme condition [47, 49]. According to this viewpoint, the microbiome provides metabolites that help plants overcome their own constraints [48, 49]. This perspective gives the important implication of bioprospecting, because it searches for the novel bioactive compounds beyond plant tissue.

The synergistic architecture of holobiont

Recent evidence supports a new model, in which neither plant nor the microbiome functions independently. Instead, the both halophytes and associated microbes operates as integrated holobiont which is bidirectional signalling. Ultimately, the novel metabolites are observed from the results of combined architecture not as individual [49]. A dynamic chemical ecology is created by the bidirectional crosstalk between microbial BGC and plant exudates, whereby the microbiome provides unique metabolic output while the plant provides sensory input [45, 53]. The halotolerant plant growth promoting rhizobacteria emits microbial volatile organic compounds (VOCs) which facilitate long distance communication within rhizosphere and also this contributes in upregulation of plant growth promoting genes [55]. This synergetic interaction make sure the survival of entire symbiotic holobiont upon salt stress, as known plant specific recruitment of tolerant microbes or accumulation of microbial PHB in halophytes is shown in (Figure 2) [49, 50]. The microbiome engineering, Synthetic microbial communities (SynComs) and microbiome transplantation will improves the salt tolerance by introducing the human designed microbial consortia inoculant and transferring the entire microbial communities from stress tolerant host to susceptible hosts [53]. Identifying this mutual relationship may be future bioprospecting and development of saline tolerant crops [54, 55]. And future use of microbiome engineering, integration of metagenomics, metatranscriptomics, metabolomics and synthetic community is essential for getting the maximum potential of halophyte holobiome.

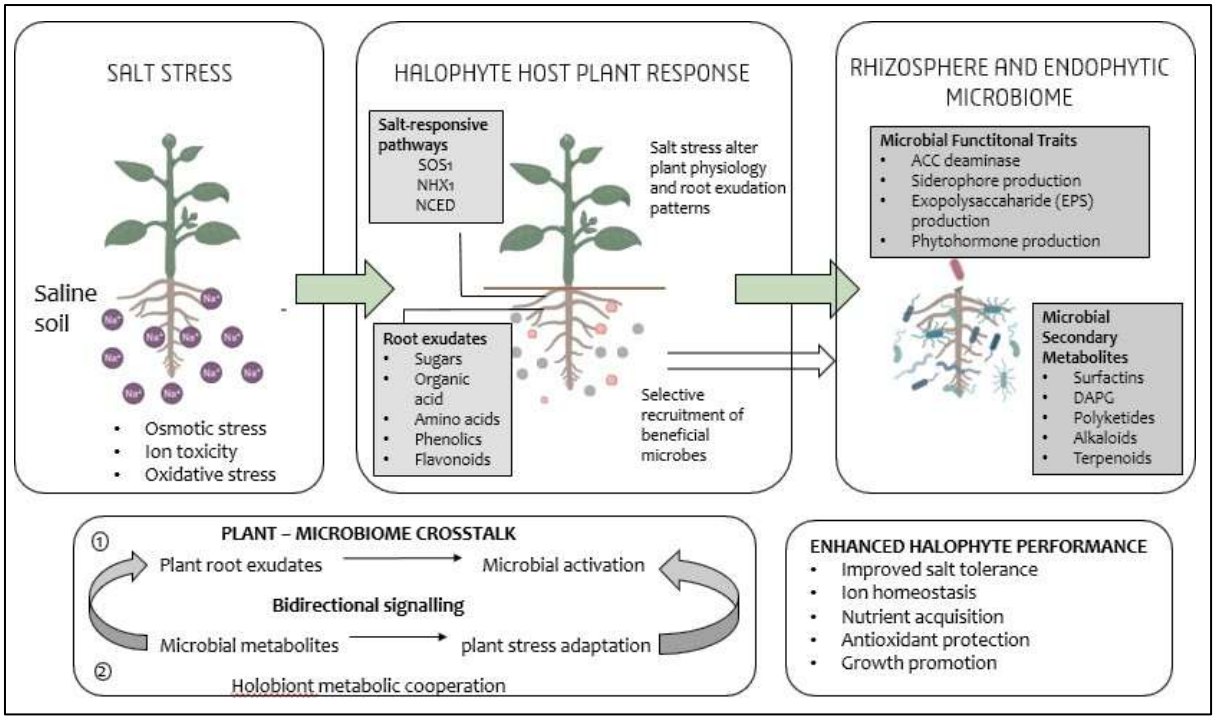

Fig. 2 Synergistic plant–microbiome interactions in halophyte under salt stress, highlighting root exudate–mediated recruitment, microbial metabolite production and coordinated stress adaptation

Influence of change in transcriptome to metabolite accumulation via microbiome mediated regulation

The halotolerant microorganisms act as the modulators, which triggers the upregulation of host stress responsive genes and metabolite pathways [56]. The microbes involves in level of expression of genes related to growth. Like change in Indole Acetic Acid (IAA) expression which helps in salinity damage mitigation [56]. Some polyamines produced by the microbes, involves in the genes expression. This transcriptional shift leads to enhanced accumulation of biomass and photosynthetic pigments [29]. By integrating the microbes influenced signalling activates the specialized defensive genes. Example, In *Avicennia marina*, this integration is involved in sulphur metabolism upregulation, which leads to accumulation of cysteine and hydrogen sulphide [18, 21]. Overall the combined transcriptome change will leads to synthesis and accumulation of compatible solutes and antioxidant secondary metabolites, which helps in protection of cellular homeostasis [5, 25].

Synthetic biology: Exploration of Halophyte genetic frameworks for crop salinity tolerance

Global food security is seriously threatened by soil salinization, which also reduces glycophyte productivity [57]. By focusing on particular responsive promoters, secondary metabolites, and genes as sources for salt tolerance beyond conventional breeding through genetic engineering and metabolic engineering, which leads to the use of such secondary metabolites in the field of bioprospecting, investigating halophytes using synthetic biology approaches helps overcome this [58].

Halophyte promoters as the genetic basis for expression that is induced by stress

The main challenge in genetic engineering is obtaining stress specific gene expression, without any deviation in growth and yield. Because halophytes have a highly effective transcriptional regulatory network, their spatiotemporal gene regulation can be precisely studied [57]. For example, the AISAP from *Aeluropus littoralis* and TsVP1 from *Thellungiella halophila* show the stress-responsive cis regulatory element can act directly on both spatially and temporally controlled gene expression under salinity [59, 60]. Other promising promoters include *Salicornia brachiata* SbGSTU and SbpAPX, which are repressor and enhancer binding sites that alter expression in response to osmotic and ionic imbalances [61].

Glycophytic crops using halophytic genes as a source of salt tolerance

The superior salt tolerance mechanism of halophytes is their capacity to maintain low cytosolic Na+ concentrations through effective ion exclusion and compartmentalization. To enhance ion homeostasis, certain genes, including SOS1 (plasma membrane Na+/H+ antiporter), NHX (vacuolar Na+/H+ exchanger), and HKT (high affinity potassium transporter), have been introduced into glycophytic crops [57]. In transgenic rice, the genes AgNHX1 and SsNHX1 from *Atriplex gmelini* and *Suaeda salsa* demonstrated a notable increase in sodium sequestration, the SbSOS1 gene from *Salicornia brachiata* mediates sodium transport and ion homeostasis [33]. *Salicornia brachiata* novel genes, like SbUSP (universal stress protein) and SbSRP (salt responsive protein), open up new possibilities for strengthening crop systems' resilience [57].

These studies collectively indicate the successful development of salt tolerance via engineering and it requires combined responses from various pathways involved in ion regulation, osmoprotection and stress signalling.

Synthetic biology: using metabolite engineering to create salt-tolerant secondary metabolite genes and salt-responsive promoters

Metabolic engineering extends the synthetic biology exceeding the ion homeostasis by targeting the biochemical pathways which responsible for compatible solutes synthesis and antioxidant metabolite synthesis. By producing secondary metabolites and compatible solutes, metabolic engineering techniques affect the halophyte pathway, which promotes osmotic adjustment and ROS scavenging [57]. Genes like betaine aldehyde dehydrogenase (BADH) and choline monooxygenase (CMO) from *Suaeda, Atriplex,* and *Salicornia* are used for accumulation of glycinebetaine via metabolite engineering to maintain the protein and to safeguard the photosynthetic mechanism under salinity [57]. And, by manipulating the flavonoid biosynthesis pathway and shikimate biosynthesis pathway genes results in enhanced antioxidant production and decreased oxidative damage upon salt stress [23, 25]. These metabolic pathways operate within broader regulatory networks rather than functioning independently, which highlights the importance of system level engineering strategy which multiple biosynthesis modules.

Salt tolerance transcription factors

The transcription factor can be used in simultaneously regulating several downstream response genes. By attaching to the cis regulating elements in promoters, the transcription factors will function as switches, controlling the intricate networks of stress-responsive genes [62]. The overexpression of transcription factor SbMYB15 from *Salicornia brachiata* responds to salt tolerance [63]. *Salicornia brachiata* and *Suaeda salsa* contain the Dehydration Responsive Element Binding (DREB) transcription factors SsDREB2A and SsDREB respectively [62, 64]. Because of lack in PEST sequence in SbDREB2A, it remains functional and stable upon overexpression in tobacco [62]. Because halophytes' transcriptional factor DREB is ABA independent, it is simpler to use in synthetic biology without interfering with endogenous hormone-mediated growth and development [64]. And it is the excellent potential target for engineering. Additionally, under hyperionic and hyperosmotic stress, these factors in transgenic crops exhibit improved germination, water use efficiency (WUE), and chlorophyll content [62]. Collectively, these findings reveal the importance of manipulating transcriptional regulators producing broader improvements than modifying individual structural genes.

Genetic engineering for precise resilience

The development of salt-tolerant glycophytes is actively advanced via the transfer and engineering of halophyte-derived genetic resources [58]. The emerging evidence shows the successful utilization of regulators from various halophytes like SsNLP7, SbMYB15, SbASR-1, SBSI-1, AoCLCf, SsNRT1, AmSOS3, SsDHN, SsRCA, which contribute to ion transport antioxidant defense photosynthetic maintenance and osmotic regulation for salt stress adaptation [22, 63, 65-71]. Compared to the constitutive CaMV 35S promoter, the endogenous AMGT1P33 promoter from *Avicennia marina* shows more effective tissue-specific gene expression [72]. Similarly, organ-specific gene expression for salt stress is demonstrated by the promoter PrAISAP from *Aeluropus littoralis* [59]. According to Kuang et. al. (2022), CRISPR/CAS9-mediated gene editing in sea barley (*Hordeum marinum*) provides a novel platform for functional research and targeted genetic improvement for major cereals [58] (Table 2).

The other advanced synthetic biology tool may also be used for synthetic biology applications. The CRISPR activation (CRISPRa) is used to upregulate the natural defense mechanisms without including any foreign genes. The CRISPR interference (CRISPRi) will silencing the mechanism which hinders the salt tolerant mechanism. Base editing and prime editing will allows us to improve the salt tolerance level by altering the nucleotides. And AI assisted enzyme engineering helps to predict and make precise engineering by integrating the machine learning models and multi omics data.

In spite of the advances, only few studies translated the laboratory finding to field applications. The future research should focus on multigene engineering, target selections based on multi-omics dataset, and should use advanced synthetic biology tools. These integrated strategies may improve the resilience of conventional crops by facilitating the exploitation of halophyte based genetic sources for agricultural biotechnology.

Biotechnological strategies for halophyte metabolite exploitation

Halophytes produce diverse secondary metabolites under salt stress, such as terpenes, phenols and alkaloids, which plays pivotal roles in defense mechanism [73]. Secondary metabolites are essential for interactions with the environment, adaptations, and defense mechanisms against salinity [74]. Custodio et al (2022) demonstrated that *Polygonum maritimum L.* adapts to saline conditions by producing bioactive flavonoids with anti-inflammatory, anti-oxidant, and neuroprotective properties, such as myricetin and quercetin glycoside. Due to seasonal variations, regional variations, challenges in standardizing large-scale production, and overexploitation of natural populations, the natural extraction of such metabolites is quite challenging [75]. Instead of functioning as individual technologies, tissue culture, hairy root culture, metabolic engineering and bioreactor based production should be used as complementary strategies which improves the metabolic production meanwhile decreasing the pressure on halophytic populations.

Table 2 Synthetic biology approaches using halophyte genetic resources for enhancing crop salt tolerance

S. No.	Halophyte Source	Genetic Resource (Promoters/Genes/TFs)	Synthetic Biology Strategy	Function in Halophyte	Reference
1.	*Aeluropus littoralis*	AlSAP promoter (stress-inducible, organ-specific)	Use as stress-inducible promoter to drive salt-tolerance genes only under stress, reducing yield drag	Drives organ-specific, high expression under abiotic stress	[59]
2.	*Atriplex gmelini*	AgNHX1 (vacuolar Na+/H+ exchangers)	Transgenic expression in crops to increase vacuolar sequestration; combine with tissue-specific promoters	Sequester Na+ into vacuoles to protect cytosol, maintain ion homeostasis	[76]
3.	*Hordeum marinum*	HKT transporters; HmSOS1 (salt-tolerance alleles)	Use CRISPR/Cas9 to edit orthologs in cereals or introgress favourable alleles to improve K+/Na+ ratio	Retrieve Na+ from xylem, regulate K+/Na+ homeostasis and translocation	[58]
4.	*Hordeum marinum*	High-quality reference genome and mapped salt-tolerance loci	Combine PacBio-style genomes with CRISPR to validate causal alleles, design precision edits for cereals	Provides allele-level mapping and functional validation (e.g., HmSOS1 essential for Na+ exclusion)	[58]
5.	*Salicornia brachiata*	SbGSTU promoter	Modular promoter engineering to tune spatial/temporal expression of ROS-scavenging and ion-homeostasis genes	Contains enhancer/repressor motifs responsive to ionic and osmotic cues	[61]
6.	*Salicornia brachiata*	SbSOS1 (plasma membrane Na+/H+ antiporter)	Overexpress or edit orthologs in crops; use halophyte promoter control or CRISPR to enhance Na+ efflux and safe translocation	Exports Na+ for exclusion/translocation; maintains cytosolic Na+ balance	[33]

7.	*Salicornia brachiata; Suaeda salsa*	DREB TFs (SbDREB2A, SsDREB — ABA-independent)	Use as ABA-independent controllers in synthetic circuits; overexpress or integrate to upregulate stress-responsive networks without hormonal trade-offs	Regulate broad stress-response genes; stable variants (lacking PEST) resist degradation and sustain activity	[62, 64]
8.	*Sonneratia apetala*	Polypropanoid biosynthesis	Redirect metabolic flux or overexpress pathway enzymes to boost antioxidants	Scavenge ROS, protect cellular components during salt-induced oxidative stress	[23]
9.	*Suaeda, Atriplex*	CMO, BADH (compatible solute biosynthesis enzymes)	Metabolic engineering to produce glycinebetaine in crops, improving osmotic adjustment and protecting photosystems	Synthesize compatible solutes that stabilize proteins and membranes under salt stress	[57]
10.	*Thellungiella halophila*	TsVP1 130 bp cis-acting element (stress-responsive)	Insert cis-element into synthetic or native promoters to create salt-responsive expression modules	Enhances downstream gene expression specifically under salt stress	[60]

In – vitro tissue culture

True-to-type plants with the necessary secondary metabolites can be obtained using in vitro tissue culture without interference from environmental fluctuations [77]. Many halophyte species demonstrates, that this approach is feasible. For example, micro propagation of sea knotgrass (*Polygonum maritimum*) from nodes gives reliable yield of standardized plant material for commercial production [77]. Additionally, 2 mg/L of 6-BA along with 0.5 mg/L of 2,4-D results in 100% callus induction from *Tetragonia tetragonoides*, New Zealand spinach [52]. Cell suspension cultures improve the scalability offers consistent nutrient availability and homogeneous growth conditions [75]. Tissue culture techniques provide high, disease-free yields in a short amount of time and space [78]. Long-term cultures of undifferentiated cells may result in genetic and biosynthetic instability [73]. Therefore, optimized culture conditions and regular monitoring of genetic and metabolic stability is essential for large scale production.

Hairy root culture

Hairy root culture is one of the most suitable techniques for producing secondary metabolites because of its rapid growth. *Agrobacterium rhizogenes* is used to induce this method by infecting plant tissue [79]. Because hairy root culture (HRC) can endure genetic stability even in hormone-free media, it is suitable for extracting metabolites of stress-responsive halophytes [79]. High growth rates and steady production levels in response to elicitors are provided by hairy root culture [73]. When compared to conventional tissue culture, hairy root cultures provide yield increased productivity and enhanced reproducibility. Nevertheless, large-scale production in bioreactors is extremely difficult due to their complex morphology, which can cause incorrect nutrient transfer and make them susceptible to shear stress [75, 79]. Addressing these limitations, metabolic engineering will be most suitable for industrial application.

Metabolic engineering for enhanced production of secondary metabolite

The metabolic engineering provides opportunity to improve in both aspects of quantity and diversity by manipulating biosynthetic pathways and regulatory networks. This entails modifying transcription factors or introducing foreign genes into biosynthetic pathways in order to manipulate genetic or regulatory mechanisms within plants in order to obtain the necessary metabolite [80]. For instance, *Glycyrrhiza inflata* accumulates bioactive flavonoids due to the overexpression of the transcription factor AtMYB12 [81]. CRISPR/CAS9 has even been used to alter the metabolic pathway or eliminate negative regulators [79]. This metabolite engineering technique is very helpful for producing foreign metabolites with high commercial value and for target-specific modifications [80]. Additionally, this approach is technically difficult and necessitates a deep understanding of intricate biosynthetic pathways [80]. Future research should focus on integration of transcriptomic and metabolomics analysis in order to identify the key regulators which regulates the stress induced metabolite biosynthesis.

Scale – up strategies and Industrial perspectives

The transition from laboratory scale cultures to large scale industrial production is one of the main challenges in halophyte biotechnology. Among these, the best techniques for increasing the amount of stress-induced secondary metabolites are hairy root culture and cell suspension culture [73]. Because hairy root culture produces stable results at salinity, it is feasible to scale up production. Additionally, bioreactors such as stirred tanks, airlifts, and nutrient mist designs are suitable for large-scale industrial production because they produce sterile, consistent micro environmental conditions and automated controls instead of cultivation processes [75]. Even though reactor design optimization, nutrient supply, downstream extraction processes must be standardized to maximize metabolite yield and overall system productivity.

Collectively, integrating all strategies like plant tissue culture, hairy root culture, and metabolic engineering reveals a comprehensive framework for exploitation of halophyte based secondary metabolites. Continuous development of such techniques is essential to translate the laboratory discoveries into commercial products.

Challenges, research gaps and future prospects

The primary disadvantage of halophyte bioprospecting is the absence of a standard protocol for metabolite reporting, bioassay, extraction, and sampling. As a result, there are significant differences in the metabolite profile between studies conducted on the same species in relation to various geographic locations, sample collection seasons, plant growth stages, and salinity levels. Thus, the variability may be decreased by adhering to standard protocols with appropriate criteria. Many bioprospecting studies are conducted in vitro but lack in vivo validation, which would clearly state the pharmacological properties of such a sample in various live models. The metabolite engineering and genetic engineering strategies show improved production of secondary metabolite in other medicinal plants, but it is lacking in halophytic plants. Applying these methods on halophytes may result in increased production of the necessary metabolite for large-scale drug production once pharmacological properties are established. Some rhizosphere and endophytic microbiome are synergistically associated with halophytes, these also possess few pharmacological properties which are unexplored. And few microorganisms are uncultivable under laboratory conditions, in such case the studying using metagenomics can be quite useful. The metagenomics analysis helps in improving the metabolic function of rhizobiome [82]. Researchers should focus on such microbiome in field of bioprospecting. The application of combined transcriptomics and untargeted metabolomics will help to identify the unexplored metabolite and to map their respective pathway. The trained machine learning with metabolomics dataset, will predict the bioactive drug with higher accuracy rate, which may lead to filtering the known compounds and identifying the novel compounds.

Overall, In future bioprospecting will go beyond descriptive studies towards multidisciplinary research framework. Standardized protocols, integration of multi-omics, advanced genetic and metabolic engineering, advanced synthetic biology techniques, microbiome integrated drug discovery and AI driven analysis will collectively proposes the maximum potential for pharmaceutical innovation and sustainable biotechnology.

Author contributions

All the authors contributed to the study conception and design. Material preparation and first draft of the manuscript were prepared by Sethunathan Sampath. All the authors commented on the previous version of manuscript. All the authors read and approved the final manuscript.

DECLARATIONS

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval

Not applicable for the present research work as no human and/or animal participation was involved.

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Table 1: Integrated Transcriptomic and metabolomic evidence of halophyte upon salt stress

S. No.	Species	Identified Metabolites	Core Metabolic Pathways	Response to Salinity	Stress Adaptation Strategy	Reference
1.	*Atriplex griffithii*	Myo-inositol, glyceric acid, ribonic acid, methionine, aspartic acid, glutamic acid, L-threonine, tryptophan	Amino acid metabolism, carbohydrate metabolism, TCA cycle	High levels of myo-inositol, ribonic acid, and methionine	Osmotic regulation via myo-inositol accumulation; maintenance of cell turgor pressure through soluble sugars	[27]
2.	*Atriplex halimus*	Sucrose, proline, glycinebetaine	Osmotic adjustment and ion regulation	Significant increase in sucrose content under salt stress; accumulation of glycinebetaine	Transport of salts to vesiculated hairs on leaf surfaces; osmotic adjustment via solutes	[28]
3.	*Atriplex nummularia*	Malic acid, citric acid, succinic acid, 2-ketoglutaric acid, fructose, turanose, talose, arabinose	TCA cycle, carbohydrate metabolism, glyoxylate and dicarboxylate metabolism, arginine biosynthesis	Increased levels of organic acids (malic, citric) and specific sugars (fructose, turanose)	Metabolic reprogramming; maintaining high osmotic potential; accumulation of soluble sugars as osmoprotectants	[27]
4.	*Avicennia marina*	Metallothioneins (MT-2, MT-3), dehydrins, glutathione S-transferase (GST), thioredoxin (TRX), laccases, putrescine, spermidine, spermine, carotenoids, cysteine, betaine, proline	Lipid biosynthetic and degradation pathways, mitochondrial betaoxidation, hydroxygeraniol metabolism, polyamine biosynthesis, phytohormone regulation, photosynthetic pigment synthesis, sulphur metabolism	High expression of metallothioneins and dehydrins; active salt efflux; increased endogenous polyamines and cytokinins when associated with Streptomyces mutabilis ; reduced leaf area and biomass	Ultrafiltration in roots; salt glands on leaf surfaces; endophytic association with PGPA; maintenance of K+ homeostasis; induction of H2S and cysteine biosynthesis	[18, 21, 29]
5.	*Avicennia marina subsp. australasica*	Putrescine, aminoadipic acid, α-ketoglutarate, soluble sugars (glucose), phenolic acids (syringic and sinapic acids)	TCA cycle, galactose metabolism, starch and sucrose metabolism	Phenotypic associations between growth and metabolome; reduction in α-ketoglutarate; accumulation of soluble sugars	Synthesis of stress-ameliorating metabolites; osmotic adjustment via increased soluble sugar content	[30]
6.	*Avicennia officinalis*	Proline, glycinebetaine, polyols, sugars, sodium, chloride, ethylene, auxin (IAA), ABA	Ca2+ signalling, trehalose biosynthesis	Flavonoid biosynthesis, Ethylene & Auxin signalling – upregulated, ABA signalling-downregulated,	Salt exclusion at roots via hydrophobic barriers; osmotic adjustment; ROS scavenging via flavonoids and antioxidant enzymes	[17]
7.	*Halogeton glomeratus*	Flavones, flavonols, glucosinolates, isoquinolines, amino acids (proline, arginine), unsaturated fatty acids (linoleic acid), sucrose, trehalose	2-oxocarboxylic acid metabolism, Aminoacyl-tRNA biosynthesis, Phenylpropanoid biosynthesis, ATP binding cassette (ABC) transporters,	Hyper accumulation of salts in succulent leaves; extensive alteration in the metabolome among different organs	Osmotic adjustment; membrane lipid modification; toxin avoidance via ABC transporters	[31]

			glycerophospholipid metabolism			
8.	*Haloxylon salicornicum*	Galactaric acid, aspartic acid, threonine, glutamate, leucine, arginine, tyramine, lysine alanine, Phenylalanine	Citrate cycle (TCA cycle), starch and sucrose metabolism, phenylalanine metabolism, amino sugar and nucleotide sugar metabolism	Upregulation of organic acids and sugars; downregulation of most amino acids except alanine and phenylalanine	High TCA cycle activity for energy supply; accumulation of low molecular weight osmoregulators; ABA-mediated stress signalling	[25]
9.	*Puccinellia tenuiflora*	Potassium (K+), sucrose, flavonoids, amino acids	Pentose phosphate pathway (PPP), flavonoid biosynthesis, sucrose biosynthesis,	Enhanced root K+ concentration; maintained low shoot Na+; upregulation of HAK, HKT1:3, NHX, and V-H+-ATPase genes	Gene family expansion for K+ uptake and osmotic adjustment; positive selection of K+ channel genes	[32]
10.	*Salicornia brachiata*	Soluble sugar, proline, amino acids, Na+, K+, Ca2+	SOS (Salt Overly Sensitive) pathway (SbSOS1)	Grows in 0.1–2.0 M NaCl; salt accumulation up to 40% dry weight; constitutive SOS1 expression	Active Na+ efflux via plasma membrane Na+/H+ antiporter (SOS1); Na+ loading into xylem; maintenance of high K+/Na+ ratio	[33]
11.	*Salicornia europaea*	Amino acids (L-proline, L-cysteine), total flavonoids, ascorbic acid, carotenoids, total phenols, lipids (LPE, LPC), saccharides (sucrose, glucose, turanose, melibiose, galactinol, fructose 1-phosphate), organic acids (3-methyl-2-oxobutanoic acid), phenolic acids (p-coumaric, ferulic, protocatechuic acids), alkaloids (trigonelline, betanin), 2-propenyl (sinigrin)	Carbon fixation, nitrogen metabolism, amino acid metabolism, carbohydrate metabolism, glycolysis, flavone and flavonol biosynthesis, phenylpropanoid biosynthesis, TCA cycle, glutathione metabolism	Stimulated growth at 50–100 mM NaCl; survived up to 800 mM NaCl; increased tissue succulence; upregulation of trigonelline and specific saccharides	Protection of membranes and photosystem II; osmotic adjustment via soluble sugars, proline, and alkaloids; energy conservation via suppressed TCA cycle; suberin biosynthesis in roots	[28, 34, 35]
12.	*Salvadora persica*	Proline, methionine, phenylalanine, phosphonic acid, citric acid, galactaric acid, mandelic acid, caffeic acid, shikimic acid, gulonic acid, D-xylose, D-mannose, D-tagatose, D-mannitol, ABA, jasmonic acid, stearic acid, benzyl amine, serine, norvaline, threonic acid, glucose, sucrose, galactinol, glycerol, 2-ketoglutaric acid, glycine, betaine	Amino acid biosynthesis, energy metabolism, carbohydrate metabolism, glycolysis, gluconeogenesis, pyruvate metabolism, TCA cycle, inositol phosphate metabolism, galactose metabolism, alkaloid biosynthesis, shikimate pathway, butanoate metabolism, phenylalanine	Significant metabolic shifts (56 metabolites); upregulation of glucose, sucrose, mannitol, phenylalanine, and proline; downregulation of caffeic and citric acids; TCA cycle downregulation; ABA-mediated stomatal closure	Osmotic adjustment via accumulation of sugars and sugar alcohols (myo-inositol); ROS scavenging via phenolic compounds; antitranspirant activity via ABA/JA synergism; use of 2-ketoglutaric acid as a metabolic junction	[27, 28, 36]

			metabolism, ABA signalling, ion transport			
13.	*Sonneratia apetala*	Phenylpropanoids, basic endochitinases B (CHIBs)	Plant hormone signal transduction, MAPK signalling, phenylpropanoid biosynthesis, phenylalanine metabolism	Upregulation of WRKY22; significant alteration in HD-Zip, SnRK2, and PP2C expression	miRNA-mediated regulation of target genes (Sap-miR393a); hormone cross-talk (DELLA proteins)	[23]
14.	*Suaeda fruticosa*	Glycinebetaine, glutathione, calcium ions, potassium ions, sodium, chloride	Nitrogen metabolism, histidine metabolism, lysine degradation, glycerolipid metabolism, linoleic acid metabolism, photosynthesis (PSII), glutathione metabolism	Sequestration of salts in leaves; upregulation of GST, APX, and photosynthetic genes at 300 mM NaCl	Sequestration of ions in shoot vacuoles; synthesis of glycinebetaine; maintenance of K+/Na+ ratio; antioxidant enzyme trigger	[37]
15.	*Suaeda japonica*	Na+, soluble sugars, proline	Starch and sucrose metabolism, proline biosynthesis, glutathione metabolism, ABA signalling, peroxisome pathway	Optimal growth at 100 mM NaCl; increased Na+ accumulation primarily in leaves	Ion compartmentalisation in vacuoles (NHX1, NHX4); Na+ extrusion (SOS1); osmotic regulation; LEA protein protection	[38]
16.	*Suaeda maritima*	Glycinebetaine, ethanolamine, polyamines, proline, tyrosine, flavonoids, phenolics	G protein and phosphoinositide signalling, cell wall and carbohydrate metabolism, TCA cycle, glycolysis, miRNA-guided gene regulation	Upregulation of NAC and HD-Zip transcription factors; upregulation of PM-H+ATPase and Na+/H+ antiporters; maintained photosynthetic electron transport rate	Cell wall restructuring (lignification); modification of membrane lipids; Na+ sequestration; post-transcriptional regulation via salt-responsive miRNAs	[16, 24]
17.	*Suaeda rigida*	Proline, catalase, peroxidase	Signal transduction, ABC transporters, cell wall metabolism, carbohydrate metabolism	Upregulation of protein phosphatase 2C genes; cytochrome P450 induction	Na+ and Cl- compartmentalization; succulence buildup; ABA signalling activation; cell wall modification (expansins)	[39]
18.	*Suaeda salsa*	Flavonoids (quercetin, isorhamnetin, naringenin), alkaloids, coumarins, amino acids (proline, aspartic acid, asparagine, tryptophan, cystine), nucleotides, organic acids (succinic acid), lipids (α -linolenic acid), branched chain amino acids, glucosamine, maltose, D-sucrose	Amino acid metabolism, carbohydrate metabolism, secondary metabolism pathways, TCA cycle, glycolysis, flavonoid biosynthesis, phenylpropanoid biosynthesis	Upregulation of proline, aspartic acid, and soluble sugars; reconstitution of metabolic homeostasis; upregulation of ion transporters (SsNHX1, SsSOS1)	Osmotic adjustment through soluble sugar and amino acid accumulation; substrate for stress-induced protein synthesis; ROS scavenging via enzymatic and non-enzymatic components	[34, 40]
19.	*Urochondra setulosa*	Proline, glycinebetaine, trehalose	Photosynthesis (RUBISCO), MAPK signalling, ascorbate-	Upregulation of RUBISCO and ferredoxin;	Ion homeostasis via vacuolar sequestration; accumulation of	[19]

			glutathione cycle, transmembrane transport	induction of Na+/H+ exchangers and ABC transporters	compatible solutes; maintenance of cell wall plasticity	
20.	*Zoysia japonica*	Na+, K+	Auxin and ABA signal transduction, MAPK signalling, phenylpropanoid biosynthesis	Salt-tolerant accessions (Z011) maintain high K+/Na+ ratios under 350 mM NaCl	Selective salt secretion via leaf salt glands; reduction of Na+ transport from roots to leaves; increased root K+ absorption	[15]
21.	*Zoysia macrostachya*	Soluble sugar, proline, soluble protein, Na+, Ca2+, K+, Cl-	Plant hormone signal transduction (Auxin, Gibberellin, ABA), gluconeogenesis, glycolysis, photosynthesis	Increased salt secretion; significant increase in SOD, POD, and CAT activities; accumulation of osmoregulatory substances	Ion exclusion via bicellular salt glands; osmotic adjustment; DELLA protein accumulation	[20]

Table 2: Synthetic biology approaches using halophyte genetic resources for enhancing crop salt tolerance

S. No.	Halophyte Source	Genetic Resource (Promoters/Genes/TFs)	Synthetic Biology Strategy	Function in Halophyte	Reference
1.	*Aeluropus littoralis*	AlSAP promoter (stress-inducible, organ-specific)	Use as stress-inducible promoter to drive salt-tolerance genes only under stress, reducing yield drag	Drives organ-specific, high expression under abiotic stress	[59]
2.	*Atriplex gmelini*	AgNHX1 (vacuolar Na+/H+ exchangers)	Transgenic expression in crops to increase vacuolar sequestration; combine with tissue-specific promoters	Sequester Na+ into vacuoles to protect cytosol, maintain ion homeostasis	[76]
3.	*Hordeum marinum*	HKT transporters; HmSOS1 (salt-tolerance alleles)	Use CRISPR/Cas9 to edit orthologs in cereals or introgress favourable alleles to improve K+/Na+ ratio	Retrieve Na+ from xylem, regulate K+/Na+ homeostasis and translocation	[58]
4.	*Hordeum marinum*	High-quality reference genome and mapped salt-tolerance loci	Combine PacBio-style genomes with CRISPR to validate causal alleles, design precision edits for cereals	Provides allele-level mapping and functional validation (e.g., HmSOS1 essential for Na+ exclusion)	[58]
5.	*Salicornia brachiata*	SbGSTU promoter	Modular promoter engineering to tune spatial/temporal expression of ROS-scavenging and ion-homeostasis genes	Contains enhancer/repressor motifs responsive to ionic and osmotic cues	[61]
6.	*Salicornia brachiata*	SbSOS1 (plasma membrane Na+/H+ antiporter)	Overexpress or edit orthologs in crops; use halophyte promoter control or CRISPR to enhance Na+ efflux and safe translocation	Exports Na+ for exclusion/translocation; maintains cytosolic Na+ balance	[33]

7.	*Salicornia brachiata; Suaeda salsa*	DREB TFs (SbDREB2A, SsDREB — ABA-independent)	Use as ABA-independent controllers in synthetic circuits; overexpress or integrate to upregulate stress-responsive networks without hormonal trade-offs	Regulate broad stress-response genes; stable variants (lacking PEST) resist degradation and sustain activity	[62, 64]
8.	*Sonneratia apetala*	Polypropanoid biosynthesis	Redirect metabolic flux or overexpress pathway enzymes to boost antioxidants	Scavenge ROS, protect cellular components during salt-induced oxidative stress	[23]
9.	*Suaeda, Atriplex*	CMO, BADH (compatible solute biosynthesis enzymes)	Metabolic engineering to produce glycinebetaine in crops, improving osmotic adjustment and protecting photosystems	Synthesize compatible solutes that stabilize proteins and membranes under salt stress	[57]
10.	*Thellungiella halophila*	TsVP1 130 bp cis-acting element (stress-responsive)	Insert cis-element into synthetic or native promoters to create salt-responsive expression modules	Enhances downstream gene expression specifically under salt stress	[60]

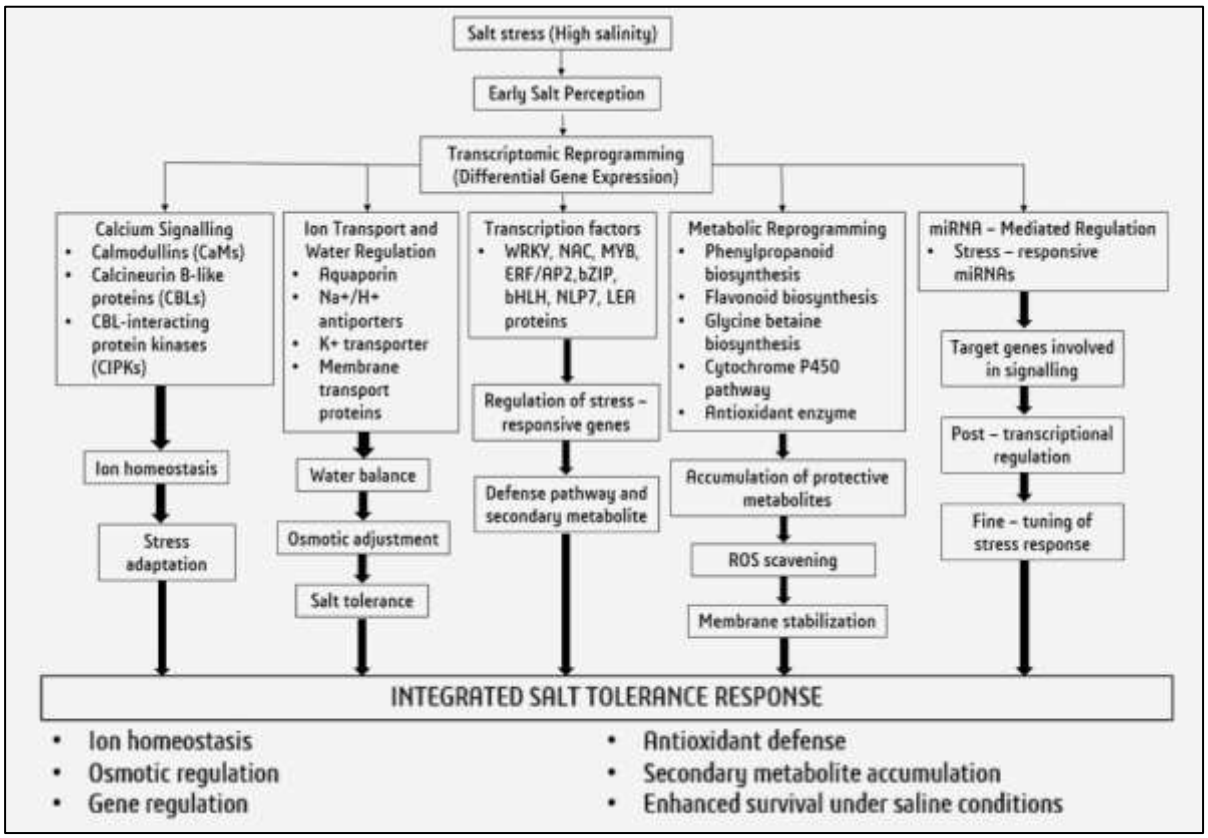

Fig. 1 Integrated transcriptomic cascade of halophytes upon salt stress, illustrating response to salt stress, calcium mediated signalling and activation of transcription factors, regulation of ion transports, osmotic adjustment, antioxidant defense and downstream secondary metabolite biosynthesis.

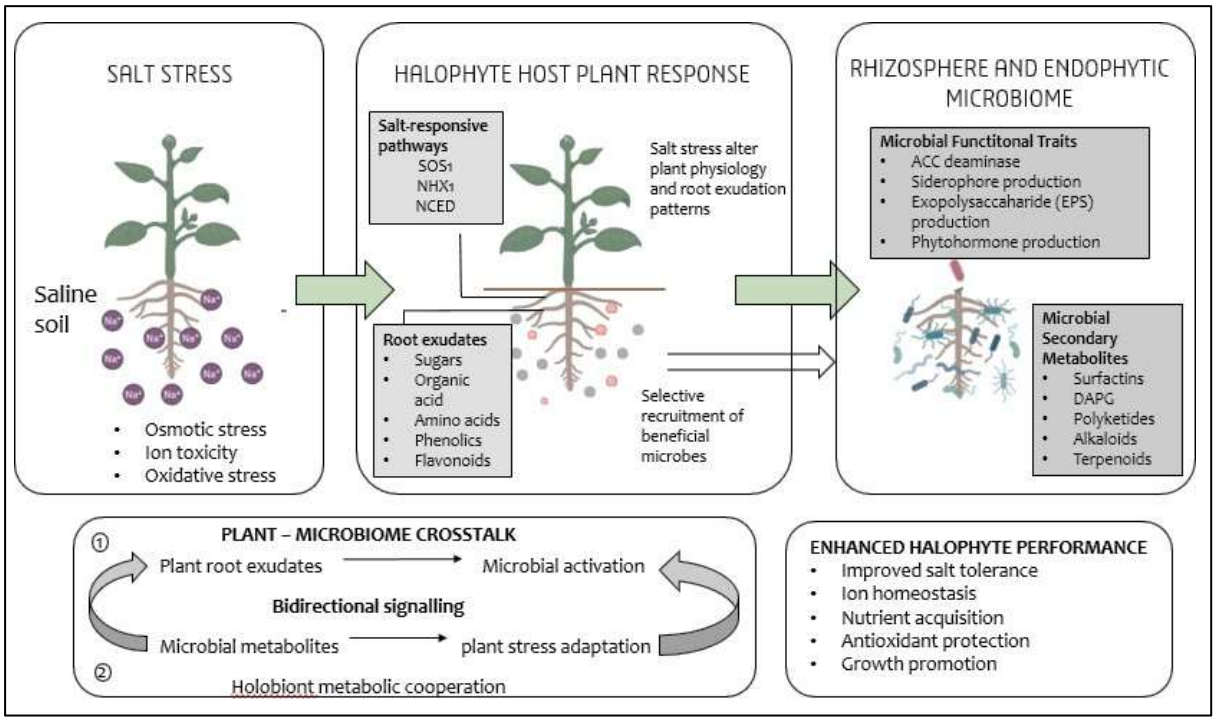

Fig. 2 Synergistic plant–microbiome interactions in halophyte under salt stress, highlighting root exudate–mediated recruitment, microbial metabolite production and coordinated stress adaptation