Ecological differentiation of the halophyte Suaeda salsa under different habitat conditions

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Abstract

Suaeda salsa serves as an excellent species for environmental remediation in saline-alkali areas. However, its ecological differentiation in different habitats is not clear. Therefore, plant samples of *S. salsa* from two main habitats in northern China, including populations of saltmarshes and inland were collected, and their differences in external morphology, gene and chemical composition were comprehensively compared. The results showed 1) the basic composition of various organs of *S. salsa* plants in two habitats was consistent, but there were certain differences in height, color, size, etc. 2) 102 *psbA-trnH* sequences of *S. salsa* under different habitats belonged to two distinct haplotypes, with a high level of intraspecific genetic variation. The NJ phylogenetic tree analysis revealed the *psbA-trnH* sequence successfully distinguished 105 samples of *S. salsa* from two different habitats. 3) A total of 699 differential metabolites were detected in the two different habitats of *S. salsa*. The results showed that *S. salsa* populations were morphologically, genetically and chemically differentiated in different habitats, but there was no substantial difference in the characteristics of reproductive organs. Based on the above analysis, we concluded that *S. salsa* produced different ecotypes at morphological, genetic and chemical levels to adapt to different habitats.

Introduction

Coastal wetlands represent the most productive, biodiverse, and ecologically valuable ecosystems on Earth[1]. However, these coastal wetland ecosystems have experienced severe degradation. Currently, the comprehensive protection of coastal wetlands has become a globally urgent and feasible endeavor. The biological improvement measures centered on the cultivation of local native halophytes have become an important choice for the improvement of heavy saline alkali land[2]. *Suaeda salsa* (L.) Pall. stands as a representative flora of coastal wetlands. It widely distributed on coastal saline-alkali soils in Europe and Asia. *S. salsa* belonging to the *Suaeda* genus of the Chenopodiaceae family, is an annual herbaceous plant predominantly found in China, particularly in the eastern coastal areas, North China, and the northwest regions[3]. *S. salsa*, with its ability to effectively reduce soil salinity, is characterized as a salt-tolerant and drought-resistant true halophyte[4,5]. This plant has the capacity to considerably decrease the surface salt content of the soil, increase organic matter content, enhance the levels of nitrogen, phosphorus, potassium in the soil, and reduce the concentration of the heavy metal cadmium[6]. Wherefore, *S. salsa* can also be used in the restoration of salinized or contaminated saline land[7].

Ecotype refers to different groups of individuals of the same species, living in different ecological environments and artificial cultivation conditions for a long time, divergent adaptation, and natural and artificial selection to form different genotype groups with different ecological, morphological, and physiological characteristics[8–11]. Ecotype is a taxonomic unit below species, which is the result of the divergent adaptation of the same species to different environments. Ecotypic differentiation is fundamental to species evolution[12]. The study of different ecotypes of *S. salsa* holds significant ecological and scientific importance. Investigating the ecotypes of *S. salsa* provides critical insights into plant evolution, ecological adaptation, and ecosystem functionality. It offers guidance for a deeper comprehension of the ecological adaptability differences within this species. This research can unveil their survival strategies in saline-alkali environments, contributing to the development of more effective conservation strategies. Moreover, it is conducive to the deliberate improvement, efficient management, and comprehensive utilization of *S. salsa* resources, which can aid in finding more effective approaches to reclaim saline-alkali lands. At present, the research on *S. salsa* currently focuses on the effects of environmental factors on seed germination, growth and development of *S. salsa*[13–17], the response of *S. salsa* plants to salt stress, and the molecular mechanisms of salt resistance[18–23]. A series of *S. salsa* genes related to salt tolerance have been cloned and their functions tested[7,24,25]. While there has been no research on the ecological differentiation of *S. salsa*. In addition, the taxonomic classification of *S. salsa* in this region has also been a subject of debate. Xing suggested that the *S. salsa* population in Dongying saltmarshes might not be an ecotype of *S. salsa*, but rather a new species termed "*Suaeda kenliensis*" [26]. However, this taxon has not been documented in the Flora of China.

The Yellow River Delta in China falls under the category of coastal wetland ecosystems, with over 50% of its land being affected by varying degrees of saline-alkali soil[27]. In this region, the ecosystem degradation poses a considerable threat to vegetation habitats and various habitats for rare and endangered wildlife species. *S. salsa* is a pioneer species in the coastal saline alkali wetlands of the Yellow River Delta, widely distributed in the region. Therefore, it is of great ecological significance to conduct in-depth research on the ecological differentiation and to use *S. salsa* for the ecological restoration of severely degraded saline alkali wetlands. The objective of this study is to investigate plant materials of the *S. salsa* under two different habitats of inland and saltmarshes, within four sampling areas of the Yellow River Delta in China, as well as samples downloaded by NCBI. First, we compared the morphological differences of samples from various sampling points. Then, we employed DNA barcoding technology to analyze sample sequences and assess their differences. Simultaneously, utilizing comprehensive targeted metabolomics analysis, we examined the differential metabolites in the leaves of *S. salsa* populations from inland and saltmarshes habitats. Based on the results from these three approaches, a study was conducted on the ecological differentiation of *S. salsa* in different habitats to reveal the characteristics and laws of the plant's adaptive growth to the environment, and to provide scientific basis for understanding the intra species differentiation mechanism and classification of *S. salsa*. It will also be of great significance for the functional restoration and governance of the ecosystem in the region.

Materials and Methods

Plant Materials

The sampling sites for the morphological observation experiments were located in the saltmarshes zones of Dongying (DT), Binzhou (BT), Qingdao (QT) and the inland areas of Dongying (DL) in Shandong Province. Plants (either cultivated or wild), including the collection of plant material, complies with relevant institutional, national, and international guidelines and legislation. It is worth noting that the DT and DL populations were situated in close proximity to each other. At each site, three random 1 m*1 m quadrats were selected, and the *S. salsa* plants within these quadrats were chosen as experimental subjects. The inland populations characterized by high soil salinity, low topography, periodic inundation, low plant density, and typically appearing as monospecific populations. In contrast, the populations from the saltmarshes thrive in flat terrain with lower soil salinity, absence of water inundation, primarily relying on seawater, featuring higher plant density and co-occurring with common reed species. Additionally, at the first three sampling points, ten plants in the flowering stage were randomly selected as experimental subjects, with each plant providing ten flowers for anatomical analysis. Various parts of the flowers, including the corolla, anthers, filaments, pistils, and bracts, were dissected and observed using a Carl Zeiss microscope (Axiocam 105 color). Measurements of flower diameter and anther length were recorded for each flower.

Genetic diversity was assessed using experimental materials sourced from four distinct populations of *S. salsa*. A total of 93 samples were collected, comprising 28 samples from inland in Dongying (DL), 35 samples from saltmarshes in Dongying (DT), 10 samples from Qingdao saltmarshes (QT), and 20 samples from Binzhou saltmarshes (BT) (Table 1). In addition, we retrieved all nine available *psbA-trnH* sequences of *S. salsa* from GenBank. Subsequently, we performed BLAST comparisons for the different *psbA-trnH* haplotypes of *S. salsa* and downloaded the top 10 most similar sequences from GenBank (retained only one sequence for those with identical matches). Multiple sequence alignment analysis was conducted on these sequences. Genetic distances were calculated using the Kimura-2-parameter (K2P) model for between *S. salsa* and its closely related species.

Metabolomics analysis materials were prepared by randomly selecting 15 leaf samples from 28 *S. salsa* inland samples from (DL) and 35 samples from saltmarshes habitat (DT) in Dongying. These samples were thoroughly mixed within their respective groups and divided into three portions, resulting in a total of six leaf samples, denoted as DT-1, DT-2, DT-3; DL-1, DL-2, DL-3. Extensive targeted LC-MS/MS metabolite profiling was conducted on the above materials.

Table 1
Sampling information of *Suaeda salsa*

Sampling site	Initialism	Longitude	Latitude	No. of samples
Inland, Dongying	DL	118°51'41.47"	37°35'05.96"	28
Saltmarshes, Dongying	DT	118°56'38.64"	37°34'28.50"	35
Saltmarshes, Binzhou	BT	120°06'22.39"	36°06'30.50"	10
Saltmarshes, Qingdao	QT	118°10'01.77"	38°08'20.12"	20

Experiment process.

The total DNA of *S. salsa* leaves was extracted using the modified CTAB method[28]. The *psbA-trnH* primer sequence and PCR reaction procedure were performed according to Gao[29]. The PCR products were sent to Sangon Bioengineering (Shanghai) Co., LTD for bidirectional sequencing and assembly.

Leaf samples were subjected to vacuum freeze-drying. The samples were ground to a fine powder. Subsequently, 50 mg of powder was accurately weighed and combined with 1200 µL of 70% methanol aqueous internal standard extract, pre-cooled to -20°C. The mixture was vortexed for 30 seconds every 30 minutes, with a total of six vortex cycles. After centrifugation (12000 r/min for 3 minutes), the supernatant was collected and filtered through a microporous membrane (0.22 µm) for use in UPLC(ExionLC™ AD)-MS/MS□Applied Biosystems 4500 QTRAP□analysis. Phase A comprised ultrapure water with 0.1% formic acid, and phase B was acetonitrile with 0.1% formic acid.

Data processing and analysis

The obtained *psbA-trnH* sequencing results from bidirectional sequencing and splicing was manually curated against the *psbA-trnH* sequences of closely related species in the NCBI database. The lengths, GC content, and sequence features of the *psbA-trnH* sequences were statistically analyzed using MEGA 5.0 and DNAMAN software. The Kimura 2-parameter (K2P) genetic distances between samples were calculated, and a phylogenetic tree was constructed based on the Neighbor-Joining method with a Bootstrap value of 1000, using MEGA 5.0 software. Metabolites were identified by comparing fragment patterns, retention times, and m/z values with a database created by MaiWei (Wuhan) Biotechnology Co., Ltd., and public databases[30]. The raw mass spectrometry data were processed in Analyst 1.6.3, and principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed on the identified metabolites. By establishing an orthogonal partial least squares OPLS-DA model graph and combining orthogonal signal correction (OSC) with PLS-DA method, irrelevant differences are removed to screen for differential variables and improve the reliability of experimental results[31]. Metabolites with a variable importance in projection (VIP) score of VIP ≥ 1, a fold change ≥ 2, or a fold change ≤ 0.5 within the specified interval were defined as significant changed metabolites (SCMs). Further analysis of these SCMs was conducted using the KEGG Pathways (www.genome.jp/kegg) metabolic pathway database[32].

Results

Morphological differentiation of *S. salsa* under different habitat

The shape, color and size of *S. salsa* in saltmarshes and inland habitats were considerably different. Specifically, the saltmarshes population of *S. salsa* can be categorized into treelike (Fig. 1, a, b) and single-branched (Fig. 1, c) forms. The plants from saltmarshes were comparatively shorter, exhibited numerous branching, spreading in various directions, with branches primarily developing in the middle-lower regions of the main stem. They possessed robust root systems with well-developed lateral and fine roots. In contrast, single-branched plants in inland population exhibited fewer branches,

grew vertically upwards, and branch from the middle part of the main stem. Their root systems were relatively shorter, and they had fewer lateral roots. Plants growing on saltmarshes soils were generally taller, exhibited a greater number of branches, with branching occurring approximately 3 to 5 cm above the ground, growing vertically upwards, and possessing shorter root systems with underdeveloped lateral roots (Fig. 1, d, e, f). In terms of plant coloration, saltmarshes plants were almost red. Conversely, inland plants predominantly exhibited green coloration. Regarding leaf morphology, saltmarshes plants had thick, fleshy leaves without distinct edges, with round and well-fleshed tips. In contrast, inland plants had shorter leaves with pronounced edges on the tips and sides, displaying less fleshy characteristics (Table 2).

In terms of fruit structure, the growth interval of *S. salsa* fruit in saltmarshes ranged from 0.3 to 0.5 cm (Fig. 1, g), occurring in clusters of 3 to 5 fruits. The central fruit in each cluster ad the largest volume, with a gradual decrease in volume for the fruits located on both sides. The seeds exhibited dimorphism, with predominantly brown disc-shaped seeds. On the other hand, the fruit growth of *S. salsa* in inland habitats was more compact, with a growth spacing not exceeding 0.3 cm (Fig. 1, h). The size differences among seeds in each cluster were less pronounced, and the seeds primarily exhibited black, biconvex-mirror-shaped seeds. Regarding the floral morphology, there was no significant difference in the size of *S. salsa* flowers between two saltmarsh populations($P > 0.05$). They had the larger diameter of approximately 2.36 ± 0.064 mm. However, the flowers of the inland population had a significantly smaller diameter of approximately 1.80 ± 0.091 mm compared to two saltmarsh populations ($P < 0.01$). Among three populations, the single-grained anther length of the inland population was significantly shorter ($P < 0.01$), measuring approximately 0.794 mm. While the anther lengths of the saltmarshes populations were 0.964 and 1.102 mm, respectively. The color of the floral parts exhibited variation among different populations. In the saltmarshes populations, the floral parts of *S. salsa* were predominantly purple-red, the styles, stigmas, and ovaries displayed alternating shades of transparent and pale purple. Whereas the floral parts of the inland populations were greenish-blue, the saltmarshes population's styles, stigmas, and ovaries exhibited alternating shades of transparent and light green (Table 3, Fig. 2).

Genetic differentiation of *S. salsa* under different habitat

The PCR amplification results of the *psbA-trnH* gene of *S. salsa* samples revealed a 100% efficiency in amplification and successful sequencing among all 93 samples. A distinct and sharp band was observed at the target position for all amplified samples, indicating the robustness and reliability.

In the analysis of genetic distances among different populations of *S. salsa* based on the *psbA-trnH* sequence (Table 4), it was observed that the genetic distances within the four populations (DL, DT, BT, and QT), were all found to be 0. The genetic distances between the saltmarshes and inland populations ranged from 0.000 to 0.027, with an average value of 0.027. When considering all four populations together, the genetic distances ranged from 0.000 to 0.027, with an average value of 0.016.Notably, the genetic distance between the nine *S. salsa* sequences downloaded from NCBI and the inland (DL) population was 0.000, while the genetic distances between these sequences and the saltmarshes populations (DT, BT, and QT) were 0.027. This observation suggested that populations within the same habitat exhibited relatively high genetic stability, while there were certain genetic variations among populations of *S. salsa* in different habitats.

The *psbA-trnH* sequence lengths of different *S. salsa* populations remained within the range of 394bp to 399bp. The GC content for the inland group was 28.75%, and the saltmarshes groups shared a common GC content of 28.57%. Through a comparative analysis of *psbA-trnH* sequences from four different populations of *S. salsa*, it was determined that the land population from Dongying (DL) and sequences downloaded from NCBI (SSA) belonged to one haplotype, while the saltmarshes samples from three different sampling points (DT, BT, and QT), represented a separate haplotype. The similarity between these two haplotypes was found to be 96.06%, with a difference of 14 bp bases (Table 5).

The genetic distances among different species of *S. salsa* ranged from 0.000 to 0.591, with an average value of 0.320. The minimum interspecific genetic distance between inland population (DL) of *S. salsa* and *S. australis* was 0.002 to 0.029, with an average value of 0.027. The minimum interspecific genetic distance between saltmarshes populations (DT, BT, QT)

of *S. salsa* and *S. australis* and *Suaeda japonica* was 0.000. The interspecific genetic distances between *S. salsa* and *Suaeda heteroptera* and *Suaeda maritima* were also close. The interspecific genetic distance from *Suaeda glauca* and *Suaeda corniculata* was relatively far (Table 6). In general, the interspecific genetic distance was greater than the intraspecific genetic distance. However, the interspecific genetic distance between *S. salsa* and some related species was 0, which was smaller than the intraspecific genetic distance.

Table 2
Differences of *Suaeda salsa* plants in different populations

Characteristics	Inland	Saltmarshes, Dongying			Saltmarshes, Binzhou (single branch)	Saltmarshes, Qingdao (single branch)
	Dongying	Tree like (red)	Tree like (green)	Single branch		
Plant color	Green /red	Overall bright red, slightly greenish	Green	Overall red, slightly green	Overall red, slightly green	Overall red, slightly green
Plant height(cm)	8 ~ 55	20 ~ 35	23 ~ 35	12 ~ 21	11 ~ 19	13 ~ 27
No. of branches	4 ~ 7	4 ~ 9	4 ~ 9	1 ~ 5	3 ~ 5	3 ~ 6
Leaf shape	Narrow, long, sharp top	Narrow, long, sharp top	Round and without edges, with a high degree of fleshy texture	Round and without edges, with a high degree of fleshy texture	Round and without edges, with a high degree of fleshy texture	Round and without edges, with a high degree of fleshy texture
Branching	The main stem is short, above the ground, with lignification at 1-5cm.	The main stem is stout, lignified and hard in texture.	The main stem is stout, lignified and hard in texture.	The main stem is slender, lignified, and the branches have high toughness.	The main stem is slender, lignified, and the branches have high toughness.	The main stem is slender, lignified, and the branches have high toughness.
Root system	Taproot short and thin, side curved, with few lateral and capillary roots.	The taproot is strong, with developed lateral and capillary roots.	The taproot is strong, with developed lateral and capillary roots.	The taproot is thin, with few lateral and capillary roots.	The taproot is thin, with few lateral and capillary roots.	The taproot is thin, with few lateral and capillary roots.
Seed type	Black seeds dominate, with few brown seeds	Brown seeds dominate, with a few black seeds	Brown seeds dominate, with a few black seeds	Brown seeds dominate, with a few black seeds	Brown seeds dominate, with a few black seeds	Brown seeds dominate, with a few black seeds
Growth site	Within saline alkali land above sea level; No flooding	In the high saltmarshes, short daily flooding time	In the high saltmarshes, short daily flooding time	In the low saltmarshes, long daily flooding time	In the high saltmarshes, short daily flooding time	In the saltmarshes near the coast ,short daily flooding time
Population distribution	Dense and patchy	With a certain interval	Large intervals, scattered	Dense and patchy	Large intervals, scattered	Densely distributed in patches

Table 3
Comparison of flower structure of *Suaeda salsa*

Characteristic	Dongying inland (DL)	Dongying coastal salt marshes(DT)	Qingdao coastal salt marshes(QT)
Perianth size(mm)	1.80 ± 0.091[b]	2.28 ± 0.094[a]	2.36 ± 0.064[a]
Perianth color	Yellow-green	Purple	Purple
Number of petals (pieces)	5	5	5
Number of anthers (grains)	5	5	5
Anther length (mm)	0.794 ± 0.025[b]	0.964 ± 0.037[a]	1.102 ± 0.027[a]
Anther color	Yellow	Yellow	Yellow
Style color	Yellow-green	Purple	Purple

Table 4
Genetic distances based on *psbA-trnH* sequences in different populations of *Suaeda salsa*

Population	Genetic distance	
	Max ~ min	Average
DL + DT + BT + QT	0.000 ~ 0.027	0.016
DL + DT/BT/QT	0.027	0.027
DT + BT	0	0
DT + QT	0	0
BT + QT	0	0
DL	0	0
DT	0	0
BT	0	0
QT	0	0
DL + SSA	0	0
DT/BT/DT + SSA	0.027	0.027

Table 5
Differences in *psbA-trnH* sequences of different haplotypes of *Suaeda salsa*

Population	Habitat	Haplotype	47	99	100	111	219	227–231	253	276	319	320	328
DL/SSA	Inland	H1	A	T	G	C	C	-	C	A	G	A	G
DT/BT/QT	Saltmarshes	H2	T	A	A	T	A	GGTT	A	C	T	C	T

Table 6
Genetic distances among samples of *Suaeda salsa* and its related species based on *psbA-trnH* sequence

Related species		GenBank No.	Genetic distance (Max ~ Min/average)	
			Inland(DL)H1	Saltmarshes(DT、BT、QT)H2
Suaeda australis	SAU	MF064571	0.027 ~ 0.029/0.028	0 ~ 0.003/0.001
Suaeda heteroptera	SHE	MH845120	0.034	0.000 ~ 0.027/0.013
Suaeda japonica	SJA	MT873731	0.035	0
Suaeda maritima	SMA	MT873756 MT873752	0.034	0.007
Suaeda glauca	SGL	JN047269 JN047268	0.490 ~ 0.494/0.492	0.498 ~ 0.502/0.500
Suaeda corniculata	SCO	JN047264 JN047265	0.189 ~ 0.567/0.559	0.565 ~ 0.575/0.570

Chemical differentiation of *S. salsa* under different habitat

The results revealed a distinct separation between the two groups of samples in the two-dimensional plot (Fig. 4a), with PC1 contributing 82.72% and PC2 contributing 4.91% to the variance. The OPLS-DA model (Fig. 4b) clearly demonstrated a considerable differentiation between the inland (DL) and saltmarshes (DT) samples from different habitats. The high R^2 and Q^2 values within the OPLS-DA model, both exceeding 0.5, with $Q^2 > 0.9$, indicated a well-constructed OPLS-DA model for the two groups of saltbush leaf samples, ensuring predictive reliability. Cluster analysis results (Fig. 5) further supported the presence of substantial metabolic differences between inland (DL) and saltmarshes (DT) samples, with higher contents of specific metabolites in region 'a' for DT and in region 'b' for DL.

A total of 699 differential metabolites accounted for 56.14% of the total metabolite constituents (1245), were identified between the two populations, consisting of 389 upregulated and 310 downregulated metabolites. These metabolites primarily belonged to various chemical classes, including 199 Amino acids and derivatives, 137 Flavonoids, 84 Lipids etc. (Fig. 6). It indicated a considerable disparity in the metabolite profiles between populations under different habitats.

There were 74 significantly altered metabolites primarily distributed across 20 metabolic pathways. Notably, the differentially regulated metabolites exhibited pronounced enrichment in secondary metabolite biosynthesis, flavonoid biosynthesis, flavone and flavonol biosynthesis (Fig. 7). Secondary metabolites played a pivotal role in enhancing plant self-protection, competitive survival, and their interaction with the environment, closely associated with stress resistance. From these findings, we hypothesize that *S. salsa* plants from saltmarshes inhabiting exhibits greater resilience and better adaptation to environmental stress compared to the plants in inland habitat.

Discussion

The sampling locations for this study included two distinct habitats of *S. salsa*: inland zones and saltmarshes areas. Taking flowers as an example,there were no structural differences observed of flowers from different habitats. However, variations were evident in flower size, color, and the duration of flowering. Specifically, populations of *S. salsa* in saltmarshes areas exhibited a notable increase in the thickness of their floral structures. Moreover, both the floral structures and anther diameters of saltmarshes plants were significantly greater when compared to those in inland habitat($P < 0.01$). Research has indicated that factors such as high salinity and trace elements in the soil environment

stimulate plant development, resulting in a fleshy appearance of the floral structures in *S. salsa*[33]. Subsequently, during the fruiting phase, these floral structures transform into fruit peel, accompanied by a considerable increase in inner wall thickness. The flower color of *S. salsa* populations in saltmarshes appears purple, flowering period of populations in the saltmarshes is more prolonged. While the predominant flower color of populations in inland is yellow-green, and the flowering period of the *S. salsa* community in inland is short. Previous study has indicated that regular precipitation effectively reduces seawater salinity and soil salt content, directly impacting the color changes in plants[34]. It is speculated that comprehensive stress stimuli in saltmarshes environments lead to the accumulation of anthocyanins and carotenoids in plants while concurrently inhibiting the accumulation of chlorophyll a and b, reducing the removal of reactive oxygen species (ROS). This ensures the growth, development, and completion of the life cycle of *S. salsa* under abiotic stress[35]. It is also hypothesized that the higher Na+/K + ratio in the saltmarshes environment effectively promotes floral bud differentiation and increases the number of flowering plants[36]. In conclusion, in different habitats, the various organs of *S. salsa* can better adapt to the development and growth pattern of plants by changing their own morphology and growth mode at different stages. In angiosperms, the height, thickness and color of the plant, the depth and thickness of the root system, and the shape and size of the leaves all vary greatly due to environmental changes. Flowers and fruits, as reproductive organs of plants, have relatively stable morphology and structure in the process of parental inheritance, and are often used as an important basis for plant morphological classification[37]. The morphological differences between *S. salsa* plants in the inland and saltmarshes were mainly reflected in plant type, size and color, while there was little difference in flower and fruit morphology and structure.

In the early stages of species identification, morphological differences serve as the primary basis for categorizing plant populations; however, it is susceptible to environmental influences, which may introduce biases and impose substantial limitations on identification outcomes. DNA barcoding technology emerges as a pivotal tool for species identification and genetic diversity analysis, offering a swift and accurate means to identify species. Consequently, it has found widespread application in the identification of plant populations[38,39]. Genetic and chemical classification methods were also employed to complement the research findings. Among the various barcode sequences used in plant identification, the *psbA-trnH* barcode, characterized by its rapid evolutionary rate, provides more comprehensive phylogenetic information[40]. This sequence is particularly well-suited for studying species with closely related geographical populations[41]. In previous study, comparisons were made among six commonly used DNA barcode loci of 15 species within the Chenopodiaceae family. Regarding the identification method of the best close match analysis, it was found that the *psbA-trnH* and *matK* loci exhibited the highest identification efficiency, both exceeding 93%. Although the *matK* barcode sequence was also attempted in the preliminary stages of our research, its identification efficiency was inferior to that of *psbA-trnH*, and consequently, it was not further analyzed. Yao et al[42]. analyzed inland and coastal salt marsh samples of *Chenopodium album* and *Chenopodium glucum* belonging to the same family as *S.salsa.* The results indicated that coastal samples exhibited increased levels of intraspecific genetic variation. A relatively stable level of intraspecific variation could be achieved when the sample size was increased to a certain extent (13 to 15 samples).In this analysis, the results indicated the 28 *S. salsa* samples from inland habitat (DL) and 9 sequences from NCBI (SSA) belonged to a single haplotype, while 65 samples collected from three different saltmarshes zones were categorized under a different haplotype. Despite the capacity of *S. salsa* to promote ovule development within the pistil through self-pollination, the analysis revealed a considerable level of genetic polymorphism within populations inhabiting different environments. In some cases, the genetic divergence within populations exceeded that between species, suggesting that environmental factors might drive the accumulation of high genetic diversity in *S. salsa*. This differentiation in genetic makeup among populations inhabiting diverse environments was believed to aid in adaptation to their respective ecological niches. A phylogenetic tree constructed effectively distinguished between inland and saltmarshes populations (Fig. 3). However, utilizing only the *psbA-trnH* sequence was insufficient for complete differentiation between *S. salsa* and its four closely related species (*S. hermaphrodita, S. sinica, S. australis, and S. japonica*). Therefore, additional barcode sequences or chloroplast genomic analysis may be necessary to achieve a more comprehensive distinction. Xing's research uncovered that *S. salsa*

populations in the Yellow River markedly differed from the typical growth patterns of *S. salsa*. Instead, they were more similar to *S. australis*[27]. In this research, both inland and saltmarshes *S. salsa* populations were genetically closest to *S. australis*. These results were consistent with those obtained by Xing. Furthermore, the analysis has revealed that two distinct morphotypes of *S. salsa*, namely the single-stem and treelike forms, along with plants of different colors (red, green, yellow), shared identical barcode sequences with a 100% similarity. This suggests that the plant morphology and coloration represent different ecotypes developed by *S. salsa* as adaptations to their respective environments. And the plants markedly differed from the typical growth patterns of *S. salsa* should still be regarded as same species.

In this study, a total of 10 classes of 1245 metabolites were identified by UPLC-MS/MS metabolomics analysis in the metabolites of two *S. salsa* populations, and 699 differentially expressed metabolites were identified, accounting for 56.14% of the total metabolites. In the cluster analysis, the land populations of *S. salsa* were clustered into different branches, indicating significant differences of chemical composition between them. In this study, it was found that the colors of plants of *S. salsa* in different habitats were different. The color of plants is mainly influenced by anthocyanins, which belong to the flavonoids of phenolic compounds. It is suggested that plant color change might be related to 137 flavonoids which are significantly different among *S. salsa* populations in different habitats. KEGG metabolic pathway analysis showed that the differential metabolites between the saltmarshes and land populations were significantly enriched in flavone and flavonol biosynthesis, which were mainly involved in plant secondary metabolism in response to stress. The contents of flavonoids such as quercetin and kaempferol were significantly higher in plants in saltmarshes habitat. The results showed that the contents of quercetin and kaempferol in the treatment group and the control group increased significantly after the plants were exposed to salt stress, low temperature stress, and mechanical damage. Therefore, it is speculated that distinct environmental pressures have driven plant evolution. Under the stress of seawater and low temperature, *S. salsa* accumulated more diverse array of secondary metabolites to resist the stress and adapt to survival. Apigenin-7-O-glucoside (Cosmosiin) is also a labeled metabolite adapted to the high-salt environment, and its content in saltmarshes populations was significantly higher than that in inland population. In addition, kaempferol-3-O-glucose rhamnoside negatively regulated auxin transport, leading to plant short stature. The results showed that its content in saltmarshes populations was also significantly higher than that in inland, which was consistent with the observation that the plants in the saltmarshes were relatively short. From this, we can also know that in the long process of evolution, plants have continuously evolved more targeted material accumulation to cope with the pressure brought by the external environment.

Conclusion

Based on the above analysis of traditional morphological characteristics classification, DNA barcoding identification results of *psbA-trnH* sequence and metabolomics of *S. salsa* in land and saltmarshes habitat, it was found that although there were some differences in barcoding sequence, chemical composition and external morphological characteristics between the two populations in different habitats. However, there was no substantial difference in the characteristics of reproductive organs between the two groups. Overall, we concluded that both *S. salsa* in saltmarshes and in inland are different ecotypes of the same species, which is equivalent to the concept of a taxonomic variety or variant. Different habitats drive plant evolution, resulting in relatively stable morphological, genetic, and metabolomic differentiation. The formation of different ecotypes is conducive to the rapid emergence of plant differentiation, which also promotes the multi-dimensional adaptation of *S. salsa* to the coastal environment. Understanding the different ecotypes of *S. salsa* improves our understanding of these halophytes and contributes to solving important problems such as saline-alkali land restoration, resource utilization and ecological protection.

Declarations

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Author contributions

Z.W., L.X., T.G. and X.D. wrote the main manuscript text; J.P., Q.W., D.Z., H.Y., X.Z.prepared figures and tables. All authors reviewed the manuscript. These authors contributed equally: Z.W., J.P., T.G.

Competing interests

The authors declare no competing interests.

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Data availability

The datasets used and/or analyzed in this study are available from the corresponding author upon reasonable request.

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Figures

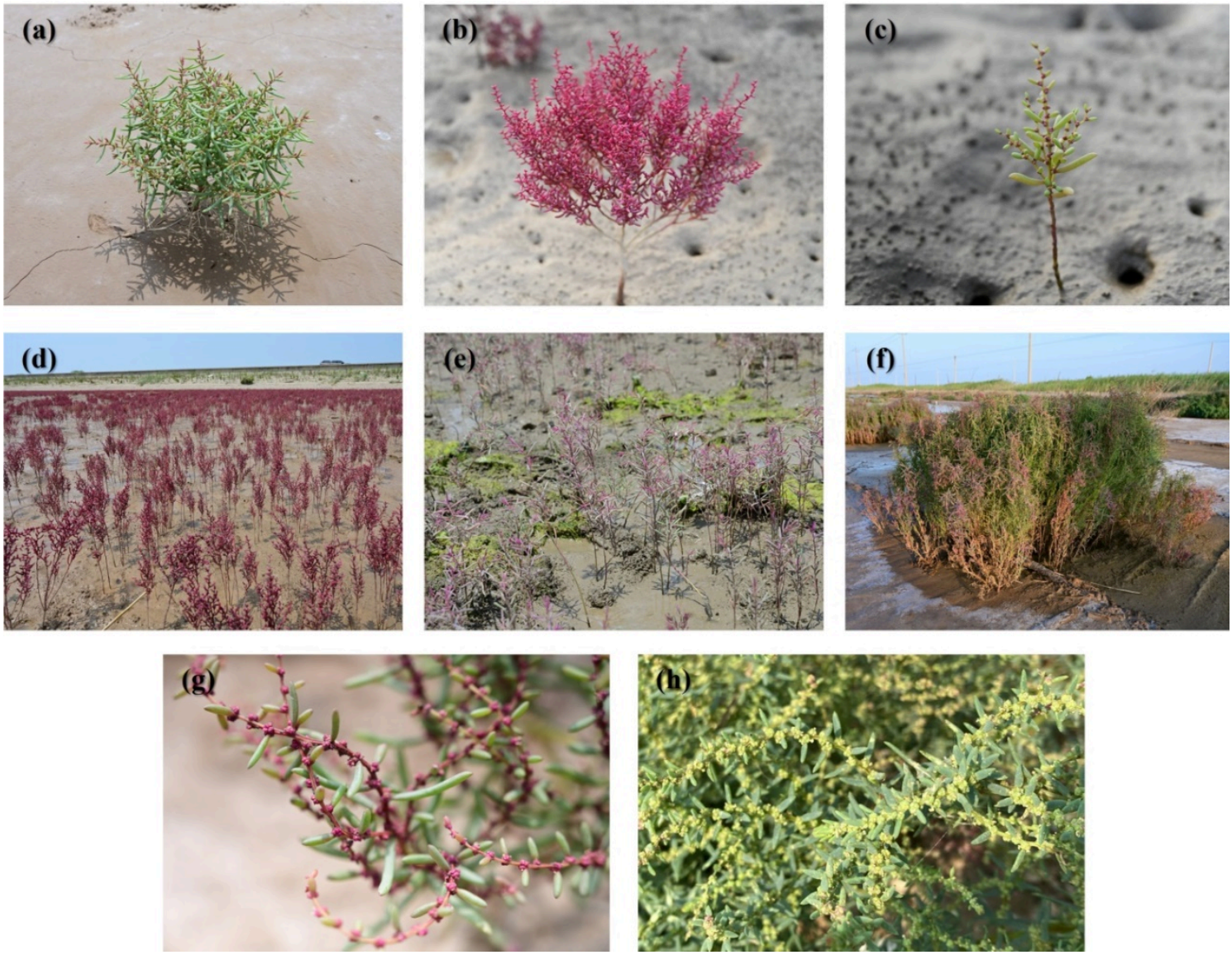

Figure 1

Morphological differences of *Suaeda salsa* in different populations

Notes: (a) Green treeliform *S. salsa*in saltmarshes of Dongying City; (b) The red tree-like *S. salsa* in saltmarshes of Dongying City; (c) Single branch *S. salina* in saltmarshes of Dongying City; (d) *S. salsa* in saltmarshes of Binzhou City; (e) *S. salsa* in inland of Dongying City; (f) *S. salsa* in saltmarshes of Qingdao City; (g) Leaves, flowers and fruits of *S. salsa* in saltmarshes of Dongying City; (h) Leaves, flowers and fruits of *S. salina* in inland, Dongying City

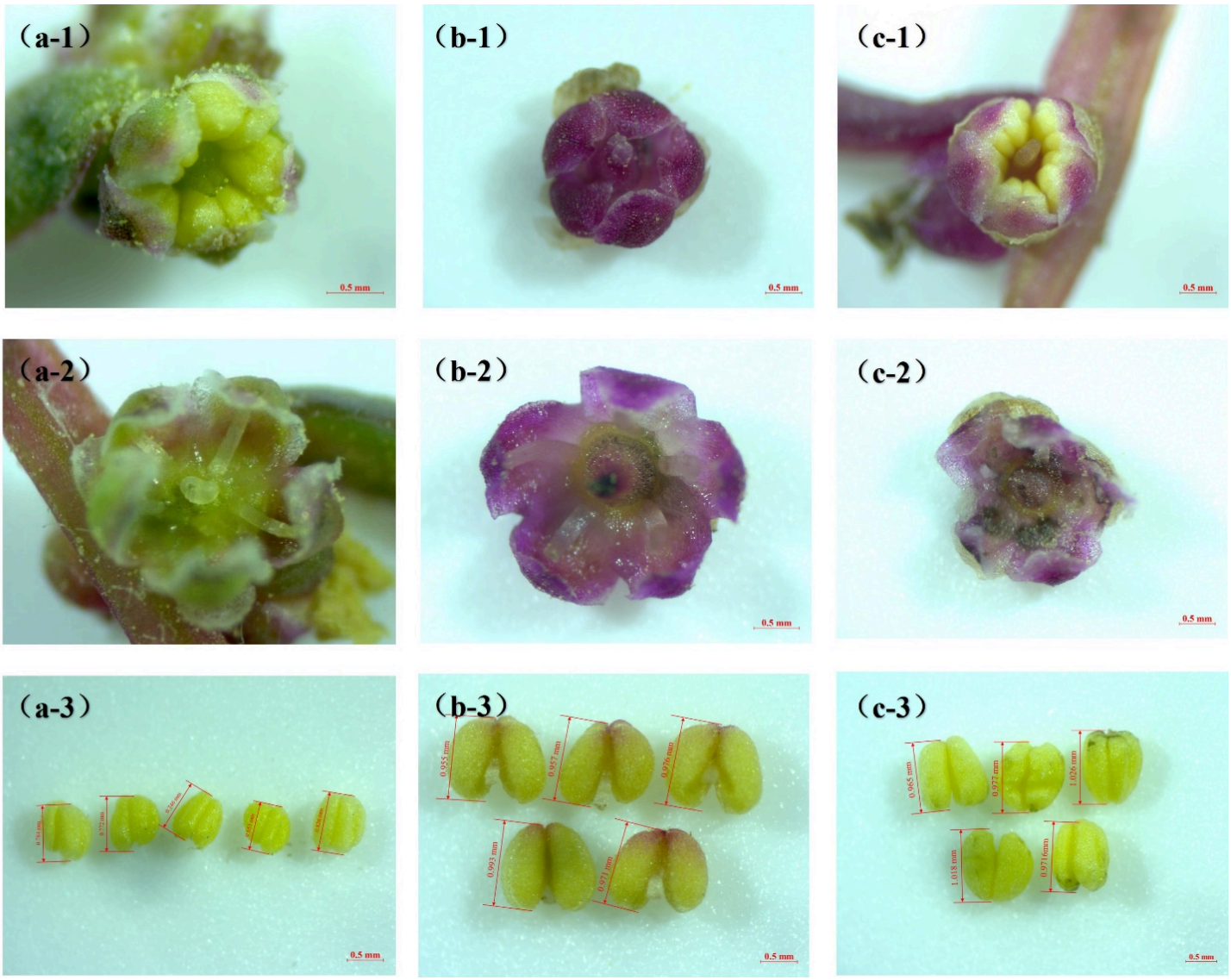

Figure 2

Comparison of flower structure of *Suaeda salsa* under different habitats

Note: (a) The land population in Dongying City; (b) The saltmarshes population in Dongying City; (c) The saltmarshes population in Qingdao City (1) the overall shape and structure of flowers; (2) perianth structure; (3) anther diameter

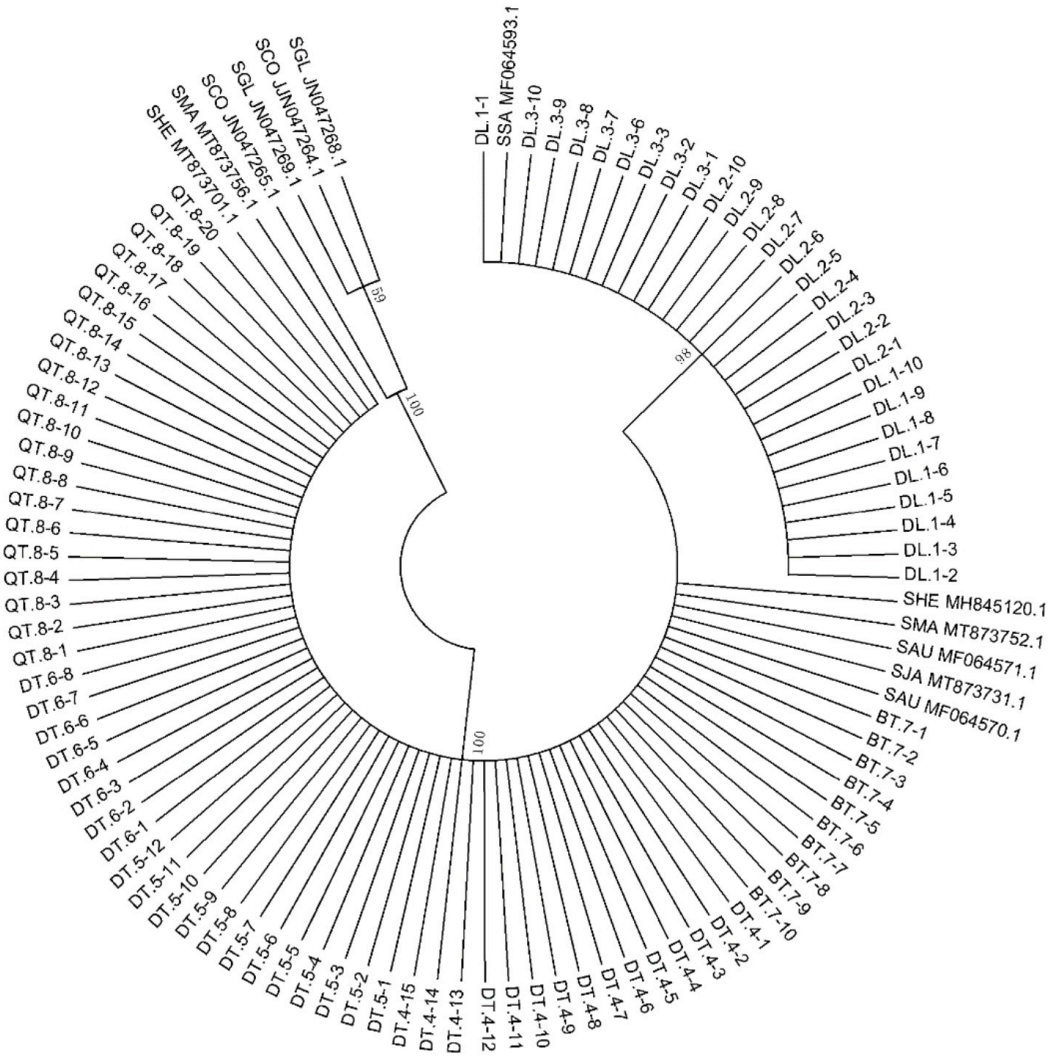

Figure 3

NJ tree of *Suaeda salsa* and its related species *samples based on psbA-trnH sequence*

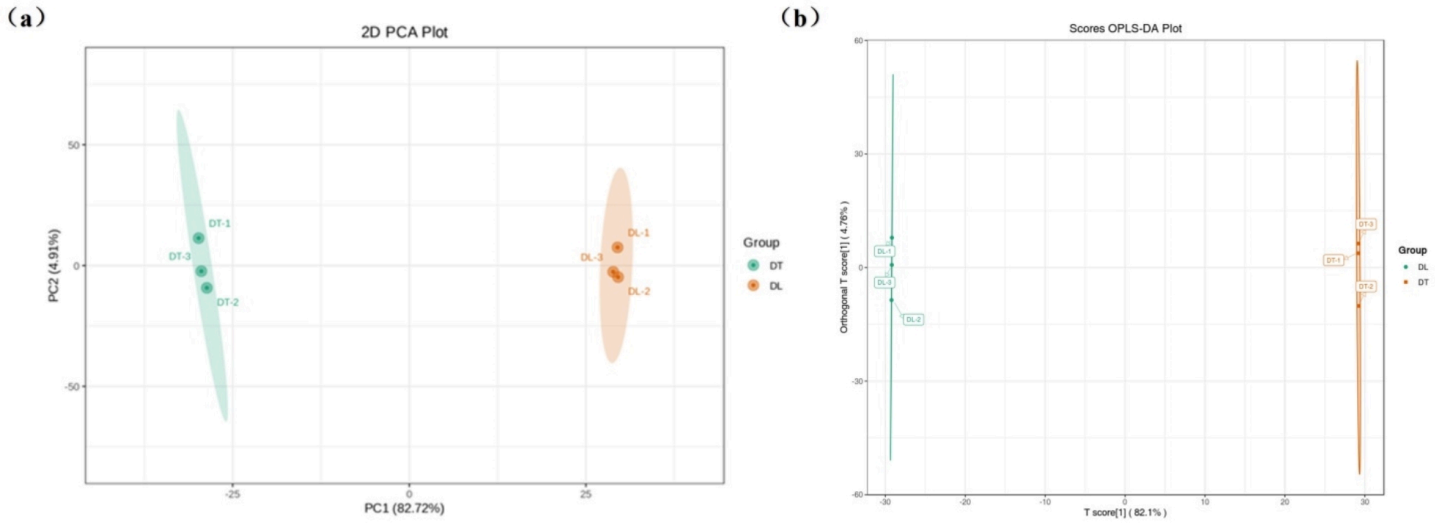

Figure 4

Multivariate Statistical Analysis Results of Leaf Samples of *Suaeda salsa* under Different Habitats Note：(a)PCA score chart; (b) OPLS-DA score chart

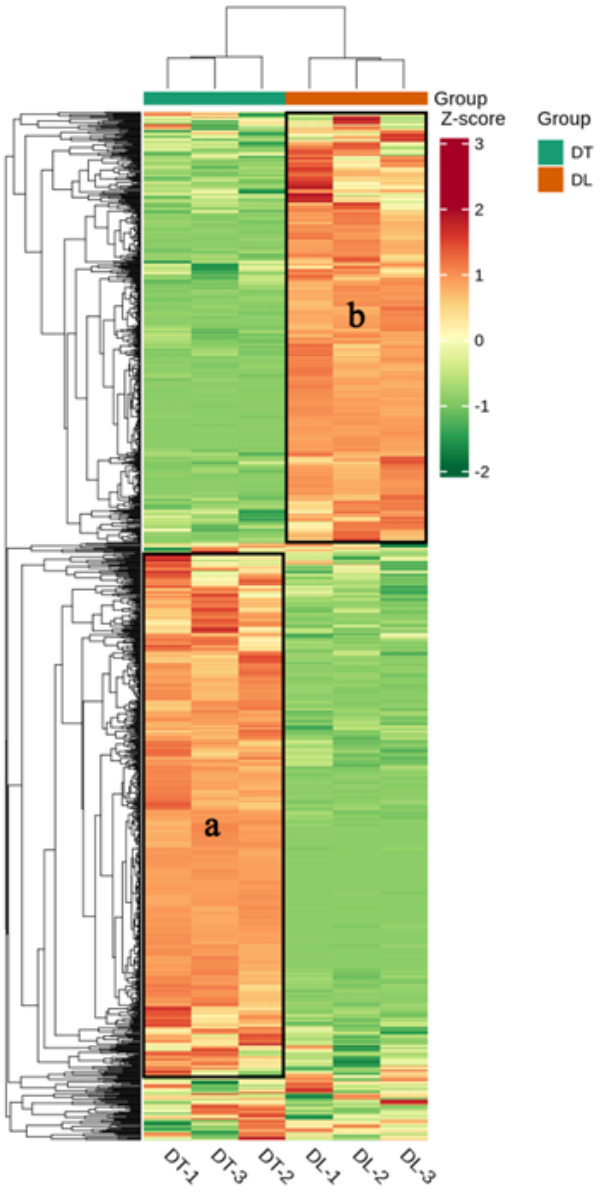

Figure 5

Cluster heat map of metabolites of *Suaeda salsa*

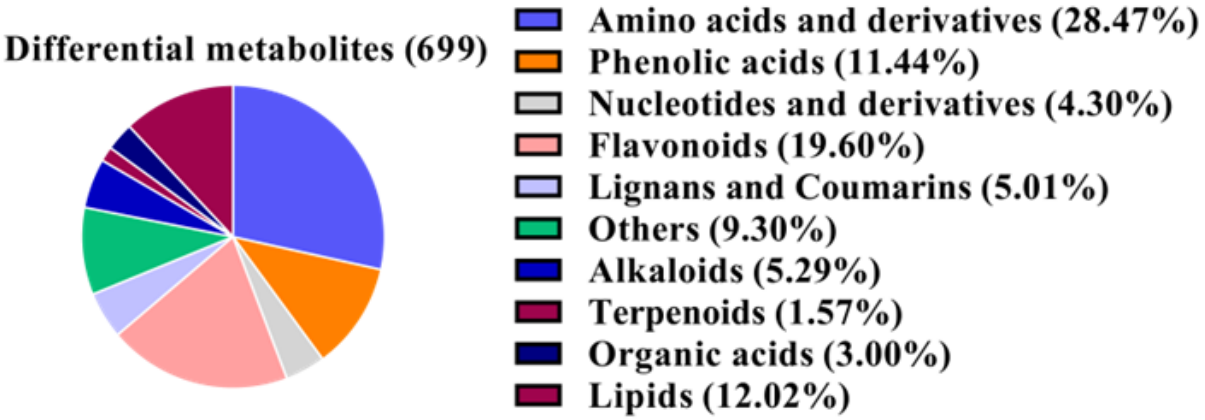

Figure 6

Differential metabolites in leaves of *Suaeda salsa* under different habitats

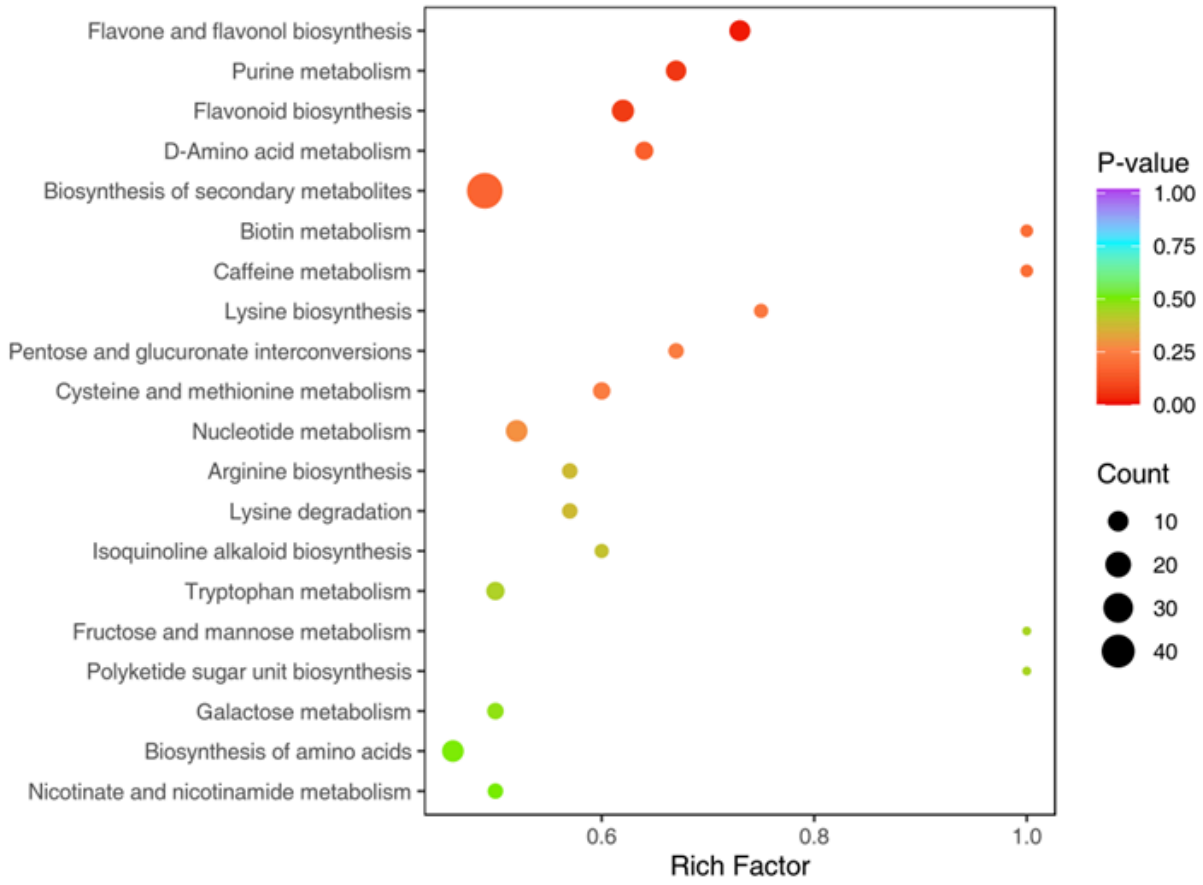

Figure 7

Significant changed metabolites KEGG enrichment map