Metabolic Differences Between *Suaeda Salsa* and *Puccinellia Tenuiflora* Under Saline-alkali Stress

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Research Article

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Abstract

Background: Salinization of soil is an urgent problem that restricts agroforestry production and environment protection. Substantial accumulation of metal ion or high alkaline alters plant metabolites and may even cause plant death. In order to explore the differences in the response strategies between *Suaeda salsa* (*S. salsa*) and *Puccinellia tenuiflora* (*P. tenuiflora*), two main constructive species that survive in saline-alkali soil, their metabolic differences were characterized.

Result: Metabolomics was conducted to study the role of metabolic differences between *S. salsa* and *P. tenuiflora* under saline-alkali stress. A total of 68 significantly different metabolites were identified by GC-MS, including 9 sugars, 13 amino acids, 8 alcohols, and 34 acids. A more detailed analysis indicated that *P. tenuiflora* utilizes sugars more effectively and may be salt-alkali tolerant via sugar consumption while *S. salsa* mainly utilizes amino acids, alcohols, and acids to resist salt-alkali stress. Measurement of phenolic compounds showed that more C6C3C6-compounds were accumulated in *P. tenuiflora* while more C6C1-compounds, phenolic compounds that can be used to defense stress as signaling molecules, were accumulated in *S. salsa.*

Conclusion: Our observations suggest that *S. salsa* resists the toxicity of saline-alkali stress using aboveground organs and *P. tenuiflora* eliminates the poison of saline-alkali via roots. *S. salsa* has a stronger ability of habitat transformation and can provide better habitat for other plants.

Background

Soil salinization is a serious environmental problem that largely restricts the production of agroforestry [1]. More than 20% of irrigated soils are affected by saline-alkali stress worldwide and the situation is continuously deteriorating [2]. The area of salinized land is growing at a rate of 1.5 million ha per year. The increase of soluble salt in soil causes a hypertonic condition and hinders water absorption by roots. The substantial accumulation of metal ion in the cytoplasm destroys ionic equilibrium [3, 4] The change of pH leads to acid-base imbalance and damages plant cell membrane structure [5]. These factors seriously impair land utilization. Anthropogenic disturbance and intervention further aggravate soil erosion, exacerbate land desertification, and destructs aquifer resources. Salt-alkali affected soils cause the imbalance between plants and the external environment, decrease plant photosynthetic rate, and disrupt the normal metabolism of plants [6–8]. Saline-alkali stress reduces osmotic potential, causes ion imbalance, inhibits plant growth, and even leads to plant death [9, 10]. Salinization-induced lack of water and arid climates further affect the resistance to alkalinity and aggravate soil erosion [11].

Hulun Buir Grassland is a famous natural pasture located in Northeast China. However, it is reported that degradation, desertification, and salinization affect 46 million hectares in the Hulun Buir Grassland, accounting for 62.68% of total area [12]. The increase of salinized land results in decreased production and quality of herbage. Salinization-induced damage causes vegetation degradation, induces more

severe grassland salinization, and causes low-yield of pastures. Therefore, it is in urgent need of solving soil salinization of the Hulun Buir Grassland.

There are many saline-alkali grassland communities in the Hulun Buir Grassland. These saline-alkali resistant natural plants improve the properties of soil and provide better live conditions for the other plants. They are not only beneficial for ecological restoration, but also the ideal materials for studying salt-alkali stress. *Suaeda salsa* (*S. salsa*) and *Puccinellia tenuiflora* (*P. tenuiflora*) are two very important saline-alkali tolerant plants and community-building species in the Hulun Buir Grassland. *S. salsa* is recognized as the first-line warrior to defend salt-alkali stress [13, 14]. It exhibits high salt tolerance during germination, growth, and reproduction [15]. *P. tenuiflora* generally grows in degraded grasslands or salinized soils [16, 17]. It has strong salt-alkali resistance and is known as “pioneer in the saline-alkali grass”. It is found that *P. tenuiflora* can growth well in the soil of a pH value higher than 10 and a salt content greater than 5% [18]. Considering that *S. salsa* and *P. tenuiflora* can effectively improve the surrounding environment, it is important to explore the response mechanisms of *S. salsa* and *P. tenuiflora* under saline-alkali stress. In the current study, we collected *S. salsa* and *P. tenuiflora* from their communities and determined their metabolic changes, aiming to reveal the differences of their saline-alkali responses. Our study may help to seek a way to improve vegetation restoration, increase crop yield, and encourage the sustainable development of agriculture.

Result

Overview of S. salsa and P. tenuiflora communities

P. tenuiflora community shows more species diversity and land cover than *S. salsa* community (Fig. 1). Soils around *S. salsa* community present “leucophylline” and harden into a lump, indicating that *S. salsa* faces more serious saline-alkali stress. According to soil alkalization classification standard (SSC), both the rhizosphere soils of *S. salsa* and *P. tenuiflora* belong to salinization soils (Table 1). The alkalization of *S. salsa* rhizosphere soil is significantly higher than *P. tenuiflora* rhizosphere soil and is classified as severe salinization (pH > 9.0) (Table 1).

Table 1
The soil indicator around *S. salsa* and *P. tenuiflora.*

	pH	Na^+/K^+	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}
S1	9.74 ± 0.14**	32.96 ± 2.34**	0.018 ± 0.003	0.037 ± 0.009**	0.090 ± .014*	0.20 ± .021
S2	8.53 ± 0.25**	21.20 ± 2.62**	0.015 ± 0.001	0.005 ± 0.001**	0.037 ± 0.009*	0.09 ± .011

S1: the soil around *S. salsa*, S2: the soil around *P. tenuiflora*. *, $p < 0.05$; **, $p < 0.01$.

The responses of primary metabolites to saline-alkali stress

In order to explore the differences of saline-alkali resistance between *S. salsa* and *P. tenuiflora*, GC-MS was used to detect the amounts of metabolites related to the response of saline-alkali stress. *S. salsa* and *P. tenuiflora* were clearly separated by means of PC1 (22.9%) and PC2 (23.0%) of OPLS-DA, a supervised method which could classify observations into the group with largest predicted indicator variable (Fig. 2. a). A total of 68 significantly different metabolites between *S. salsa* and *P. tenuiflora* were obtained according to their variable importance in the projection (VIP, VIP > 1) and *p*-values ($p < 0.05$). These significantly different metabolites could be classified to 9 sugars, 13 amino acids, 8 alcohols, 34 acids, and 4 other compounds (Table S1). The calculation of principal component Q value showed that sugars were obviously accumulated in *P. tenuiflora* (Fig. 2b) while other primary metabolites, i.e., amino acids, alcohols, and acids were all significantly higher in *S. salsa* than *P. tenuiflora* (Fig. 2c-2e). The distributions of these primary metabolites were also different in different parts (root, stem, and leaf) of plants. For instance, the root of *P. tenuiflora* had the lowest Q value of sugars while the leaf of *P. tenuiflora* had the highest Q values of sugars (Fig. 2b). On the contrast, the root of *P. tenuiflora* had the highest Q values of amino acids, alcohols, and acids while the leaf of *P. tenuiflora* had the lowest Q values (Fig. 2c-2e).

The distributions of these primary metabolisms were further analyzed. The sugars showed that among 9 significantly different sugars, only two kinds of 6-carbon sugars, sorbose and fucose, were enriched in *S. salsa* (Fig. 3). Other 7 kinds of sugars were all highly accumulated in *P. tenuiflora*, including 6-carbon sugars tagatose, D-talose, fructose, and D-galactose, 12-carbon sugars sucrose and maltotriitol, and 18-carbon sugar melezitose (Fig. 3). These sugars not only provide energy in plants, but also play a key role in resisting saline-alkali stress.

Different from sugars, detailed analyses of differentially expressed amino acids showed that most kinds of amino acids were remarkably accumulated in *S. salsa* (Fig. 4). Only isoleucine, norlecucine, and aspartic acid were accumulated at higher levels in *P. tenutiflora*, with isoleucine largely accumulated in the leaf and norlecucine as well as aspartic acid largely accumulated in the root (Fig. 4). Other amino acids were discovered to be highly accumulated in *S. salsa*, especially in the aboveground part of *S. salsa* (Fig. 4). These amino acids play an important role on osmoregulation under saline-alkali stress.

Similar as amino acids, the majority of differentially expressed alcohols showed high abundances in *S. salsa* (Fig. 5). And these alcohols were mainly accumulated in the aboveground part of *S. salsa*. Two other alcohols, cuminic alcohol and xylitol, were found to be accumulated in *P. tenutiflora* (Fig. 5). Cuminic alcohol was enriched in the root of *P. tenutiflora* while xylitol was enriched in the leaf.

Acids accounted for a large proportion of significantly different metabolites (Table 2). Differentially expressed acids were grouped into phenolic compounds, organic acids, and volatile compounds, artificially. Detailed investigation of organ-specific expression of acids showed that acids were largely accumulated in the aboveground part of *S. salsa* and the root of *P. tenuiflora* (Table 2).

Table 2
Significantly different acids in root, stem, and left of *S. salsa* and *P. tenuiflora.*

Acids		*S. salsa*			*P. tenuiflora*		
		root	stem	Leaf	root	stem	Leaf
phenolic compounds	gallic acid	27.73 ± 3.0[a]	54.35 ± 5.97[a]	76.9 ± 12.26[a]	22.58 ± 7.05[a]	8.26 ± 2.85[b]	10.92 ± 2.74[b]
	protocatechuic acid	5.2 ± 1.72 [a]	4.86 ± 1.98 [a]	8.41 ± 2.23 [a]	5.12 ± 1.16 [a]	0.95 ± 0.3 [b]	0.77 ± 0.34 [b]
	catechol	0.419 ± 0.26 [a]	1.87 ± 0.82 [a]	1.42 ± 0.91 [a]	0 [b]	0.29 ± 0.09 [b]	0.24 ± 0.01 [b]
	epigallocatechin	1.30 ± 0.32 [a]	2.34 ± 0.44 [a]	4.92 ± 1.08 [a]	1.01 ± 0.19 [a]	0.22 ± 0.04 [b]	0 [b]
	vanillic acid	2.01 ± 0.69 [a]	4.47 ± 1.70 [a]	7.11 ± 1.57 [a]	1.73 ± 0.35 [a]	0.48 ± 0.12 [b]	0.48 ± 0.09 [b]
	vinylphenol	5.88 ± 1.46 [a]	7.69 ± 1.29 [a]	7.55 ± 0.73 [a]	6.15 ± 1.66 [a]	1.9 ± 0.93 [b]	1.77 ± 0.66 [b]
	guaiacol	0.96 ± 0.43 [a]	1.78 ± 0.43 [a]	2.23 ± 0.62 [a]	1.03 ± 0.34 [a]	0.24 ± 0.05 [b]	0.22 ± 0.04 [b]
organic acids	citraconic acid	8.21 ± 1.22 [a]	10.85 ± 2.79 [a]	7.97 ± 0.7 [a]	4.49 ± 1.8 [b]	1.43 ± 0.81 [b]	1 ± 0.48 [b]
	malonic acid	34.97 ± 10.72 [a]	43.78 ± 16.69 [a]	75.82 ± 22.5 [a]	30.84 ± 8.08 [a]	15.04 ± 5.2 [b]	15.68 ± 2.08 [b]
	succinic acid	44.36 ± 12.5 [a]	144.7 ± 17.51 [a]	51.73 ± 17.3 [a]	47.51 ± 18.11 [a]	15.77 ± 2.66 [b]	14.59 ± 4 [b]
	tartaric acid	1.02 ± 0.37 [a]	2.43 ± 1.26 [a]	1.75 ± 0.58 [a]	0.98 ± 0.35 [a]	0 [b]	0.33 ± 0.12 [b]
	itaconic acid	20.76 ± 4.65 [a]	39.92 ± 8.93 [a]	28.12 ± 8.73 [a]	19.04 ± 6.52 [a]	3.38 ± 1.42 [b]	4.44 ± 1.63 [b]
	pelargonic acid	0.52 ± 0.17 [a]	0.88 ± 0.16 [a]	1.91 ± 0.82 [a]	0.91 ± 0.23 [a]	0.31 ± 0.1 [a]	0.19 ± 0.08 [b]
	glycolic acid	17.41 ± 6.24 [a]	40.57 ± 11.34 [a]	55.77 ± 8.35 [a]	19.86 ± 7.74 [a]	6.31 ± 2.07 [b]	10.11 ± 2.02 [b]

The relative contents of acids are summarized from 6 biological replicates and presented as the mean ± standard error of 6 biological replicates. Different letters indicate significant differences among treatments ($p < 0.05$).

Acids		*S. salsa*			*P. tenuiflora*		
		root	stem	Leaf	root	stem	Leaf
	3-methylglutaric acid	1.46 ± 0.59 [a]	0 [a]	0.9 ± 0.58 [a]	0 [b]	0 [a]	0 [b]
	aminooxyacetic acid	11.64 ± 4.1 [a]	13.44 ± 3.72 [a]	18.03 ± 6.91 [a]	8.44 ± 3.7 [a]	0.79 ± 0.36 [b]	0.63 ± 0.27 [b]
	oxalic acid	2.58 ± 0.97 [a]	3.63 ± 0.6 [a]	4.01 ± 0.85 [a]	3 ± 0.49 [a]	0.81 ± 0.25 [b]	1.15 ± 0.2 [b]
	L-gulonic acid	2. 5 ± 1.1 [b]	3.84 ± 1.15 [a]	2.01 ± 0.39 [b]	12.86 ± 5.38 [a]	10.44 ± 3.03 [a]	10.18 ± 2.14 [a]
	cumic acid	4.15 ± 1.26 [a]	6.76 ± 2.23 [a]	9.27 ± 2.82 [a]	5.43 ± 1.72 [a]	1.43 ± 0.58 [b]	0.63 ± 0.18 [b]
	palmitic acid	295.15 ± 86.1[a]	401.49 ± 42.39[a]	577.35 ± 140.8[a]	508.25 ± 214.83[a]	99.91 ± 31.2[b]	76.59 ± 18.34[b]
volatile compounds	methylfumarate	0.94 ± 0.29 [a]	1.83 ± 0.36 [a]	1.46 ± 0.24 [a]	0.71 ± 0.3 [a]	0.16 ± 0.06 [b]	0.16 ± 0.04 [b]
	hydroxybutyrate	0 [b]	0 [b]	0.32 ± 0.08 [a]	0.35 ± 0.08 [a]	0.16 ± 0.02 [a]	0.31 ± 0.12 [a]
	gluconic lactone	2.89 ± 0.86 [a]	2.46 ± 0.85 [a]	0.98 ± 0.19 [a]	0 [b]	0 [b]	0 [b]
	methyl hexadecanoate	0.33 ± 0.07 [a]	0.52 ± 0.19 [a]	0.92 ± 0.43 [a]	0 [b]	0 [b]	0.11 ± 0.01 [b]
	dioctyl phthalate	9.13 ± 3.25 [a]	13.3 ± 2.6 [a]	20.1 ± 6.35 [a]	8.92 ± 1.62 [a]	1.39 ± 0.5 [b]	1.24 ± 0.24 [b]
	methyl heptadecanoate	3.76 ± 0.59 [a]	8.3 ± 1.01 [a]	4.16 ± 0.52 [a]	1.87 ± 0.77 [b]	0.69 ± 0.28 [b]	0.44 ± 0.09 [b]
	nonanoic acid methyl ester	78.7 ± 17.98 [a]	105.39 ± 22.62[a]	149.24 ± 28.53[a]	113.66 ± 33.08 [a]	23.24 ± 6.98[b]	19.38 ± 4.56 [b]
	methyl octanoate	44.05 ± 6.29 [a]	58.75 ± 12.81 [a]	91.8 ± 16.65 [a]	62.31 ± 18.85 [a]	14.03 ± 3.21[b]	10.16 ± 3.31 [b]

The relative contents of acids are summarized from 6 biological replicates and presented as the mean ± standard error of 6 biological replicates. Different letters indicate significant differences among treatments ($p < 0.05$).

Acids		*S. salsa*			*P. tenuiflora*		
		root	stem	Leaf	root	stem	Leaf
	L-gulonolactone	0 [a]	0 [b]	0 [b]	0 [a]	1.61 ± 0.04 [a]	8.46 ± 3.61 [a]
	phenylacetic acid	0 [b]	0.64 ± 0.22 [a]	0.64 ± 0.2 [a]	0.33 ± 0.18 [a]	0 [b]	0 [b]
	hydroxymandelic acid	10.65 ± 2.1 [a]	8.9 ± 0.96 [a]	13.56 ± 3.56 [a]	11.85 ± 4.5 [a]	3.24 ± 0.83 [a]	2.1 ± 0.5 [b]
	5-hydroxyindole-2-carboxylic acid	0.73 ± 0.32 [a]	1.57 ± 0.88 [a]	1.89 ± 0.54 [a]	0 [a]	0.22 ± 0.04 [b]	0.36 ± 0.09 [b]
	5-hydroxyindole-3-acetic acid	1.41 ± 0.82 [a]	3.17 ± 0.68 [a]	2.39 ± 0.37 [a]	1.37 ± 0.39 [a]	0.27 ± 0.13 [b]	0 [b]

The relative contents of acids are summarized from 6 biological replicates and presented as the mean ± standard error of 6 biological replicates. Different letters indicate significant differences among treatments ($p < 0.05$).

The responses of phenolic compounds to saline-alkali stress

Phenolic compounds from phenylalanine metabolism are important for plant development and defense and play an essential role in saline-alkali stress. To explore the involvement of phenolic compounds in saline-alkali stress, HPLC-qTOF-MS was performed, and the accumulation of a total of 34 phenolic compounds were measured. 20 phenolic compounds were found to be accumulated in *S. salsa* and *P. tenuiflora*, and 8 phenolic compounds were identified to be significant differently expressed (VIP > 1 and $p < 0.05$) by OPLS-DA (Figure S1). These 8 significantly different phenolic compounds could be divided into 2 C6C1-compounds (protocatechuic acid and gallic acid), 2 C6C3-compounds (chlorogenic acid, p-hydroxycinnamic acid), and 4 C6C3C6-compounds (luteolin, quercetin, myricitrin, and petunidin), according to their carbon skeleton (Fig. 6). C6C1-compounds were found to be notably accumulated in *S. salsa*, especially the aboveground part of *S. salsa* while C6C3-compounds and C6C3C6-compounds were identified to be mainly enriched in *P. tenuiflora* (Fig. 6).

Discussion

Saline-alkali tolerant plants show excellent potentials for preventing soil salinization, improving the ecological environment, and providing live conditions for the other plants that have lower tolerance to saline-alkali stress [2]. Emerging studies have focused on biological responses of saline-alkali tolerant plant, aiming to decipher saline-alkali tolerant mechanisms. For example, *Chenopodium quinoa* Wild. has been used to investigate the genotype-dependent variability in salinity responses from morphological,

physiological, cellular, and molecular aspects [21]. However, these plant tolerance or defense studies are mainly conducted by indoor control [16, 22, 23]. The application of indoor control cannot fully reflect the real responses of plants to saline-alkali stress as plants survive from saline-alkali soil usually undergo a long-term adaptation and evolution with habitat. In the current study, we measured the expressions of metabolites in *S. salsa* and *P. tenuiflora*, which survive in saline-alkali soil, using GC-MS and LC-qTOF-MS and demonstrated diverse metabolites with varied intensities in *S. salsa* and *P. tenuiflora*.

Many sugars have been identified as regulatory components in the control of glycolytic flux in a variety of stress survival strategies [19]. They not only act as readily available energy source for plant growth under stress, but function as osmoprotectants to maintain osmotic balance and stabilize macromolecules [24]. Soluble sugars provide adaptive buffer for plants under saline-alkali stress and play an important role in regulating osmotic pressure [25, 26]. In our studies, many soluble sugars, including sorbose, fucose, and D-talose, are highly expressed. They have the ability to balance osmotic pressure and protect the biological structures of plants from desiccation damage [27, 28]. Notably, many metabolites in glycolysis/gluconeogenesis pathways were found to be significantly accumulated in *P. tenuiflora*, indicating that the production of downstream products through metabolic flux from these pathways is essential for salt-alkali tolerance. Therefore, it is likely that *P. tenuiflora* can regulate the central metabolism by effectively utilizing carbon, accumulating carbon assimilation production, and providing more material and energy to promote the tolerance against salt-alkali stress.

Nitrogen metabolism has been reported to be strongly interconnected with carbon metabolism [29]. Sufficient carbon skeleton source and energy supply are important for the assimilation of nitrogen and the synthesis of amino acids [30]. Plants respond to high salinity by limiting protein synthesis, promoting protein degradation, and changing amino acid compositions [31, 32]. Here, significant enrichment of amino acids was discovered in *S. salsa*. Therefore, we speculate that *S. salsa* uses different salt-alkali response strategy as *P. tenuiflora.* In *P. tenuiflora*, a large portion of carbon influx to sugars while only amino acids isoleucine, norleucine, and aspartatic acid were highly accumulated (Figure. 3a). Isoleucine and norleucine can improve salt resistance and maintain the metabolic and osmotic homeostasis under stress [24]. Aspartic acid can act as an immediate donor of amino groups for the synthesis of other amino acids [30]. Many other amino acids, including glutamine, proline, alanine, tyrosine, ornithine, and 3-hydroxynorvaline, were identified to be significantly accumulated in *S. salsa*. Glutamine has an elevated nitrogen-to-carbon ratio and can use limited carbon skeletons to response to environmental stresses [33, 34]. Proline is generally considered as an osmotic regulator and an active oxygen scavenger in response to high salinity [35]. Like molecular chaperone, proline can form a protective film [36]. Proline are mainly produced by glutamate synthesis pathway and ornithine synthesis pathway [36]. Activation of ornithine synthesis pathway also plays a vital role in improving plant salt tolerance. Noteworthy, aminobutyric acid, which is involved in various stress response and defense mechanisms, is also considerably accumulated in *S. salsa*. Aminobutyric acid can maintain carbon and nitrogen balance, protect plants from oxidative stress, and regulate the pH value of cytoplasmic. These differentially regulated amino acids help *S. salsa* to survive under saline-alkali stress.

Alcohols help to reserve available water in plants and thus are considered as essential osmotic regulators. In our current study, accumulated alcohols in the aboveground part of *S. salsa* were discovered (Fig. 5). These alcohols benefit the maintenance of osmotic pressure balance in cytoplasm and contribute to the regulation of water loss [37]. In addition, alcohols work as natural scavengers of salinity-induced reactive oxygen species and protect the biomolecules against oxidative damage [38].

The roles of soluble sugars, alcohols, and amino acids in resisting saline-alkali stress have been well acknowledged. Our current study revealed that a large proportion of differently expressed metabolizes were acids, implying the potential involvement of acids in plant protection. Acids can enhance plant stress resistance and stabilize intracellular pH [39]. We found that many acids were accumulated in the aboveground part of *S. salsa*. These acids may help to maintain ionic balance by neutralizing alkali and excess toxic ions. They can also affect the fluidity and hydrophobicity of cell membrane, which is crucial for cell membrane activity maintenance and saline-alkali stress defense [40]. Notably, some acids, such as nonanoic acid methyl ester, methyl hexadecanoate, and phenylacetic acid, have obvious flavors and are volatile. Secretion of these acids may affect the surrounding environment and influence soil composition (Table 2). Volatile substances are also communication factors that contribute to plant defense and reproduction [41]. Therefore, these volatile substances may improve soil properties via signal transmission and communication. The pioneer role of *S. salsa* may be partially attributed to the successful secretion of these allelopathic compounds under saline-alkali stress.

Our GC-MS results showed that gallic acid, vanillic acid, protocatechuic acid, and catechol, acids subordinated to phenolic compounds, were obviously accumulated. These compounds are secondary metabolites that originate from phenylalanine metabolism [20, 42, 43]. Gallic acid and protocatechuic acid are the precursors of tannin, which can affect plant thickness and reduce water evaporation [19]. Moreover, a larger number of other phenolic compounds were investigated. Bioactive phenolic compounds are important biofactories of plants under stress [44, 45]. These compounds are mainly divided into the benzoid acid derivatives with C6C1 carbon skeleton (C6C1-compounds), the hydroxycinnamic acid derivatives with C6C3 carbon skeleton (C6C3-compounds), and the flavonoids with C6C3C6 carbon skeleton (C6C3C6-compounds). Our results demonstrated the enrichment of C6C1-compounds in *S. salsa* as well as enrichment of C6C3- and C6C3C6-compounds in *P. tenuiflora* (Figure. 5). C6C1-compounds, usually induced by biotic elicitors, are signaling molecules that defense stress. C6C3C6-compounds are flavonoids that can directly enhance the chemical defense of plants and help plants adapting to their environments [46]. Significantly accumulation of phenolic compounds in *S. salsa* and *P. tenuiflora* may thus benefit their saline-alkali tolerance.

Conclusion

In short, our result showed that *S. salsa* resists the toxicity of saline-alkali stress using aboveground organs and *P. tenuiflora* eliminates the poison of saline-alkali via roots. *S. salsa* has stronger ability of habitat transformation and is more tolerant of saline-alkali than *P. tenuiflora*. The analyses of different metabolites of *S. salsa* and *P. tenuiflora* provide an important theoretical basis for understanding the

mechanisms of saline-alkali tolerance and may help to deepen the knowledge of plant metabolism regulation under stress.

Materials And Methods

Materials collected

S. salsa and *P. tenuiflora* were selected from *S. salsa* and *P. tenuiflora* communities of the open area of the Hulun Buir Grassland in China (115°31'00"-121°34'30", 47°20'00"-50°50'30"). Samples were collected from three different plots with similar transitional communities from *S. salsa* to *P. tenuiflora*. Distances between sample plots were greater than 500 kilometers. 3 plants were selected from each community with 6 repeats and stored in drikold. Soil samples derived from the habitats of *S. salsa* and *P. tenuiflora* were collected along the vertical length of 20 cm depth for characterizing salinization with 3–6 repeats.

The detected of soil salinity and alkalinity

Soil samples were dried at room temperature for 2 weeks, pulverized, and sieved through a 2 mm mesh sieve. Saturation paste extract was prepared for soil detection. Soil samples were digested with HF-$HClO_4$-HNO_3 method. Contents were determined by flame photometry (410, Corning, Halstead, England), colorimetry (Double beam spectrophotometer, UV-140-02, Shimadzu), and titration method (carbonates and bicarbonates, and chlorides). The pH of soil was measured with a glass electrode pH meter (pHM-2000, Eyela, Rikakikai Co., Tokyo, Japan) in the saturation paste titration.

GC-MS analysis

GC-MS was performed as previously described [19]. 60 mg samples were mixed with 360 μL cold methanol and 40 μL internal standards. Samples were homogenized (Tissuelyser-192, Shanghai, China), ultrasonicated for 30 min, mixed with 200 μL chloroform and 400 μL water, and centrifuged at 10,000 g for 10 min at 4℃. Finally, 400 μL supernatant was transferred to a glass sampling vial for vacuum-dry at room temperature. The residue was derivatized using a two-step procedure. First, 80 μL methoxyamine (15 mg Ml-1 in pyridine) was added to the vial, and kept at 37℃ for 90 min followed by 80 μL BSTFA (1 % TMCS) and 20 μL n-hexane at 70℃ for 60 min. After derivatization, 1 μL solution was injected into the Agilent 7890A-5975C GC-MS system (Agilent Corporation, USA) with a split ratio of 30 to 1. Separation was carried out on a non-polar DB-5 capillary column (30 m × 250 μm I.D., J&W Scientific, Folsom, CA) with high purity helium as the carrier gas at a constant flow rate of 1.0 mL/min. The temperatures of injection and ion source were set to 260℃ and 230℃, respectively. Electron impact ionization (-70 eV) at full scan mode (m/z 30 – 600) was used, with an acquisition rate of 20 spectrum/s in the MS setting. QC sample was prepared by mixing aliquots of tissues samples to be a pooled sample and analyzed using the same method with the analytic samples.

Acquired MS data were analyzed by Chroma TOF software (v 4.34, LECO, St Joseph, MI). Briefly, after alignment with Statistic Compare component, the CSV file was obtained with three-dimension data sets

including sample information, retention time, and peak intensities. The internal standard was used for data quality control (reproducibility). Internal standards and any known pseudo positive peaks, such as peaks caused by noise, column bleed, and BSTFA derivatization procedure, were removed from the data set. The data set was normalized using the sum intensity of the peaks in each sample.

Phenolic compound detection

Phenolic compound detection was performed as previously described [20]. After treatment with liquid nitrogen, 1.0 g pulverized sample was dissolved in 20 mL methanol for extraction and ultrasonicated at low-frequency for 40 min. The simple solution was centrifuged for 10 min at 8,000 rpm. The analysis was performed by Waters ACQUITY UPLC system (Waters, Japan) coupled to a quadrupole time-of-flight (qTOF) mass spectrometer (XEVO G2 QTOF, Waters). The chromatographic conditions were: A%: 0.05% formic acid-water; B%: 0.05% formic acid-acetonitrile; m/z: 120–1200; positive scan mode; chromatographic columns: ACQUIT UPLC-BEH C18 Column (1.7 mm, 2.1 mm, × 50 mm). Leu-Enkephalin was used as the internal standard.

Statistical analysis

Data sets obtained from GC-MS and LC-qTOF-MS were imported into SIMCA-P14.1 software package (Umetrics, Umeå, Sweden), separately. After mean centering and unit variance scaling, orthogonal partial least-squares discrimination analysis (OPLS-DA) was carried out to visualize metabolic alterations among experimental groups. Differentially expressed compounds were selected by comparing compounds in the treated group with the control group using the multivariate statistical method. Metabolites with both multivariate and univariate statistical significances (VIP > 1.0 and $p < 0.05$) were screened. Default 7-round cross-validation was applied with 1/7 of the samples being excluded from the mathematical model in each round to avoid overfitting.

Data were log2 transformed to improve normality and Min-Max Normalization was performed. Data were subjected to hierarchical clustering analysis by R software to study the variations of *S. salsa* and *P. tenuiflora*. Significantly different metabolites were screened by SIMICA14.1. The score of principal component Q was calculated using Statistical software SPSS version 21.0 software (Chicago, IL, USA). Heat maps, histograms, and pathway maps were drawn with R-3.2 language software, GraphPad Prism8, and Visor, respectively.

Abbreviations

Suaeda salsa: *S. salsa*; *Puccinellia tenuiflora*: *P. tenuiflora*; Discriminant analysis by orthogonal partial least square: OPLS-DA; soil alkalization classification standard: SSC.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare no competing financial interests.

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Author contributions

QC and XYL performed the experiments, analyzed and interpreted the data, prepared figures. QC and and ZHT wrote the manuscript. HSX and GYW performed part of the experiments and data analysis. XRG and ZJ revised the article critically. All authors have read and approved the manuscript.

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Not applicable

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Biomolecules, 10, 504-525.

Supplemental Figure

Supplemental Figure S1 is not available with this version.

Figures

Figure 1

The appearance of S. salsa community and P. tenuiflora community. (a) S. salsa community and S. salsa; (b) P. tenuiflora community and P. tenuiflora.

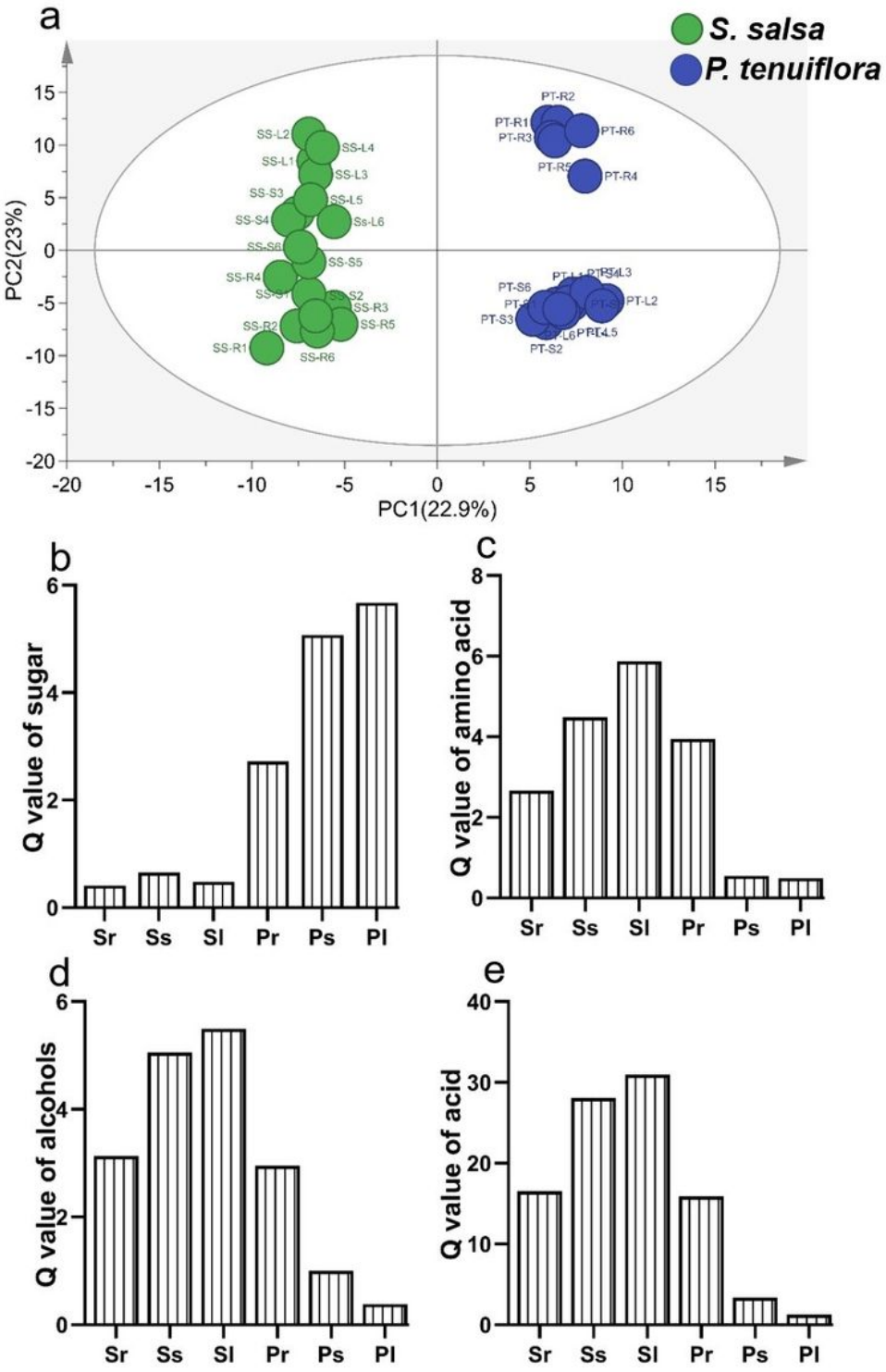

Figure 2

The OPLS-DA score plot of primary metabolites and the Q value of them significantly metabolites between S. salsa and P. tenuiflora. (a) The OPLS-DA score plot of primary metabolites; (b) Q value of sugar; (c) Q value of amino acid; (d) Q value of alcohol; (e) Q value of acids. Sr: the root of S. salsa. Ss: the stem of S. salsa. Sl: the leaf of S. salsa. Pr: the root of P. tenuiflora. Ps: the stem of P. tenuiflora. Pl: the leaf of P. tenuiflora.

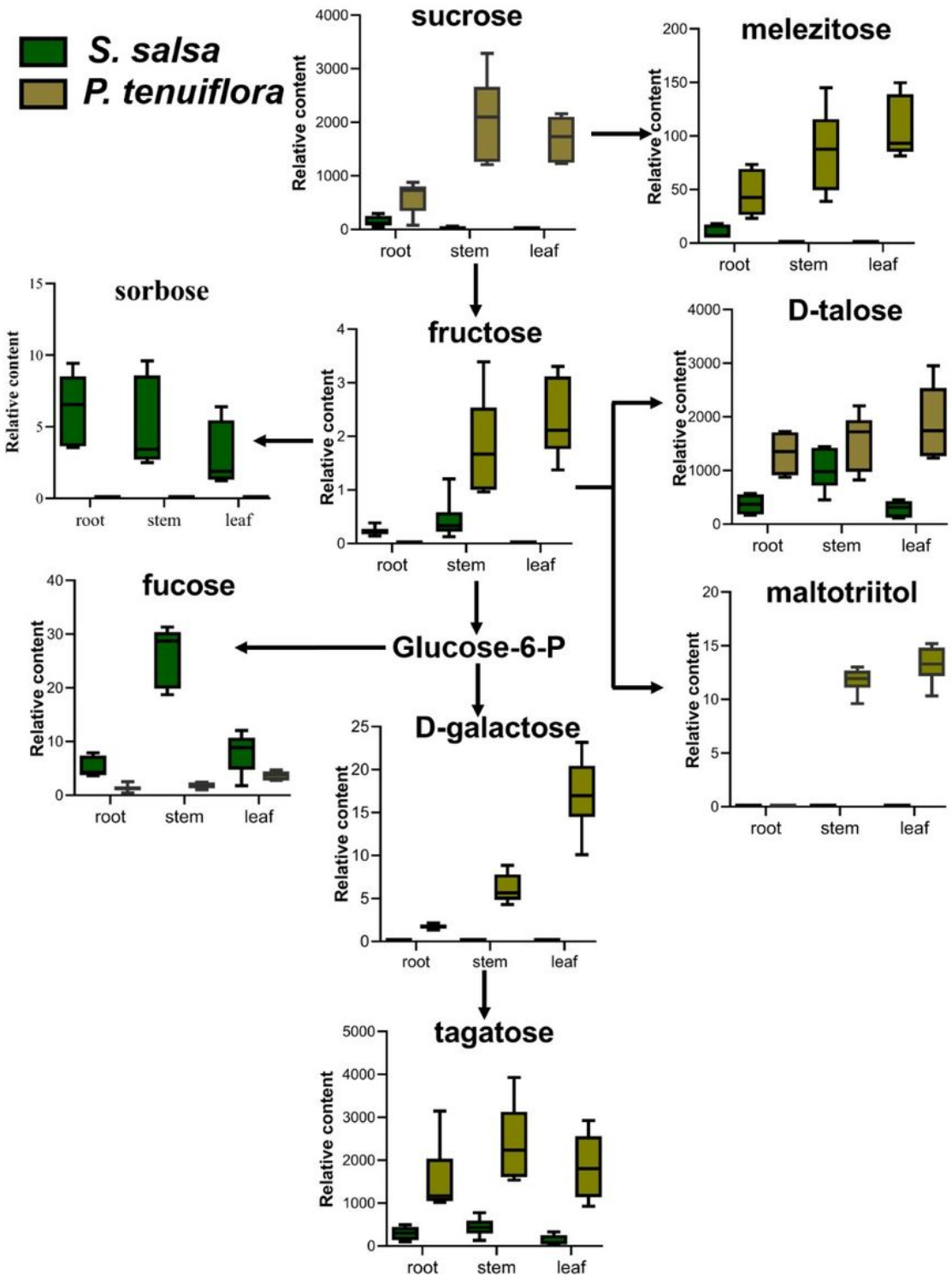

Figure 3

The metabolic network of significantly sugars between S. salsa and P. tenuiflora.

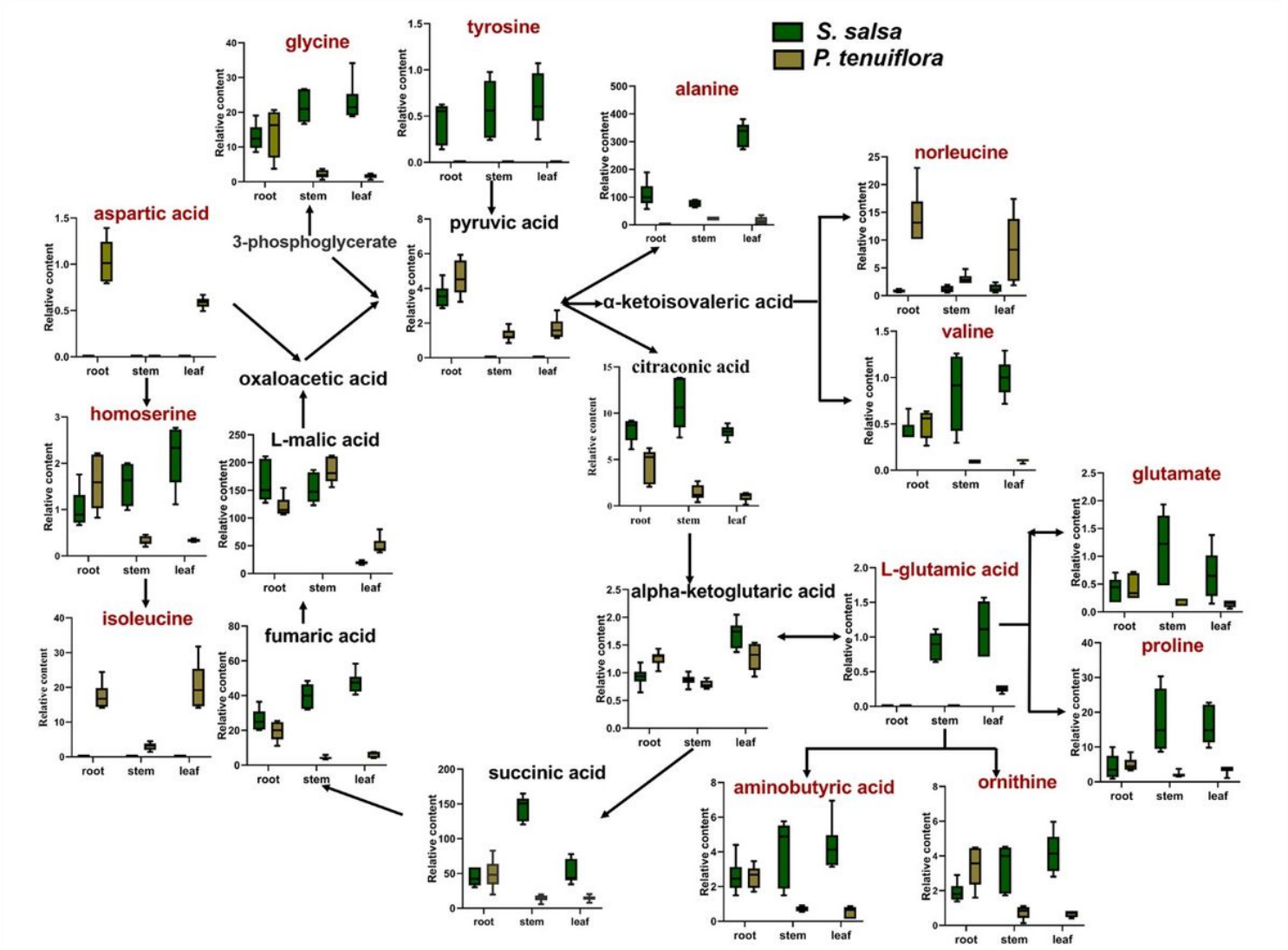

Figure 4

The metabolic network of amino acids between S. salsa and P. tenuiflora. The significantly different metabolites were marked red.

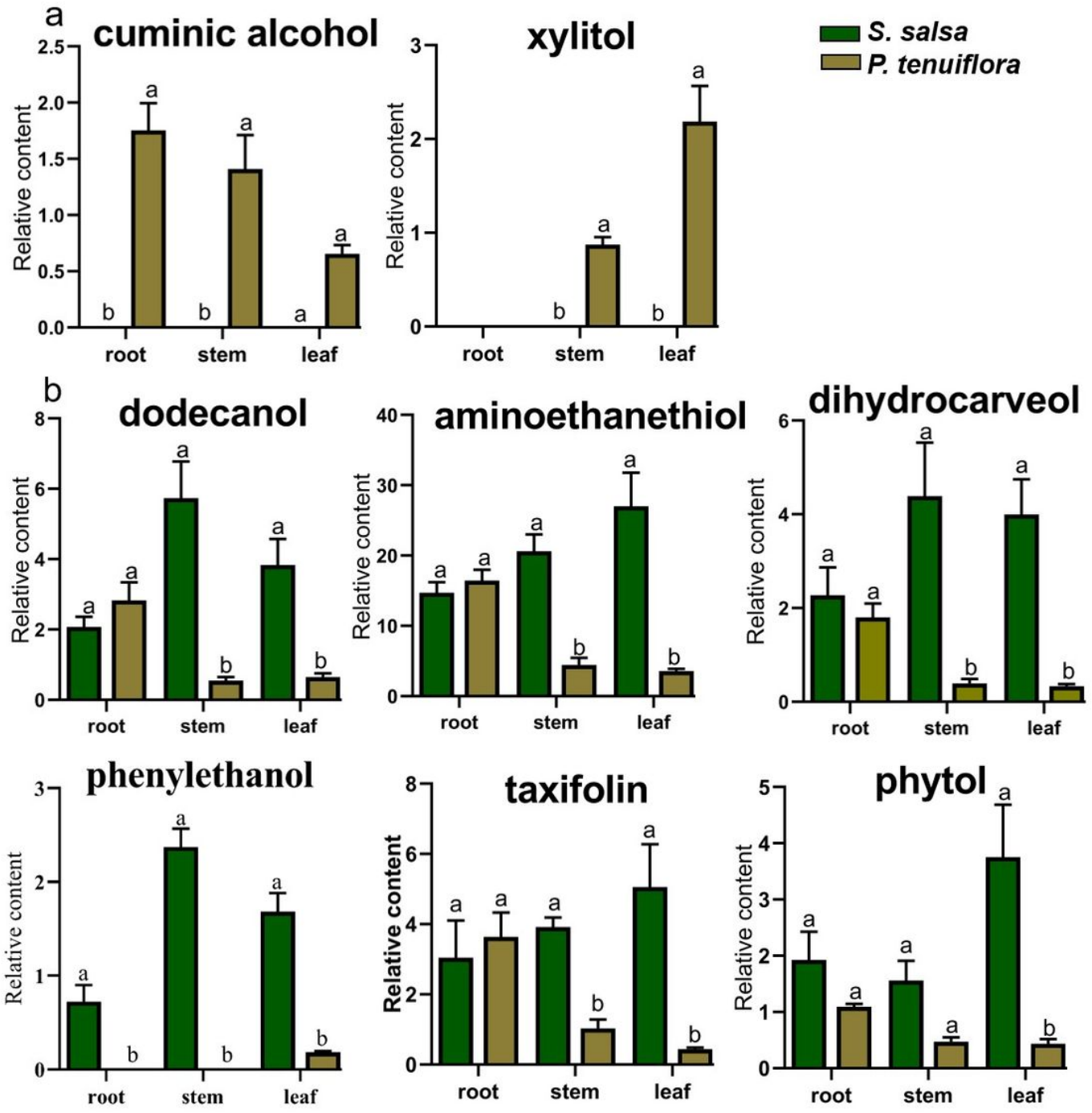

Figure 5

The significantly different alcohols between S. salsa and P. tenuiflora. (a) The significantly different alcohols mainly accumulated in P. tenuiflora; (b) The significantly different alcohols mainly accumulated in S. salsa. The relative contents of significantly different alcohols are summarized from 6 biological replicates and presented as the mean ± standard error of 6 biological replicates. Different letters indicate significant differences among treatments ($p<0.05$).

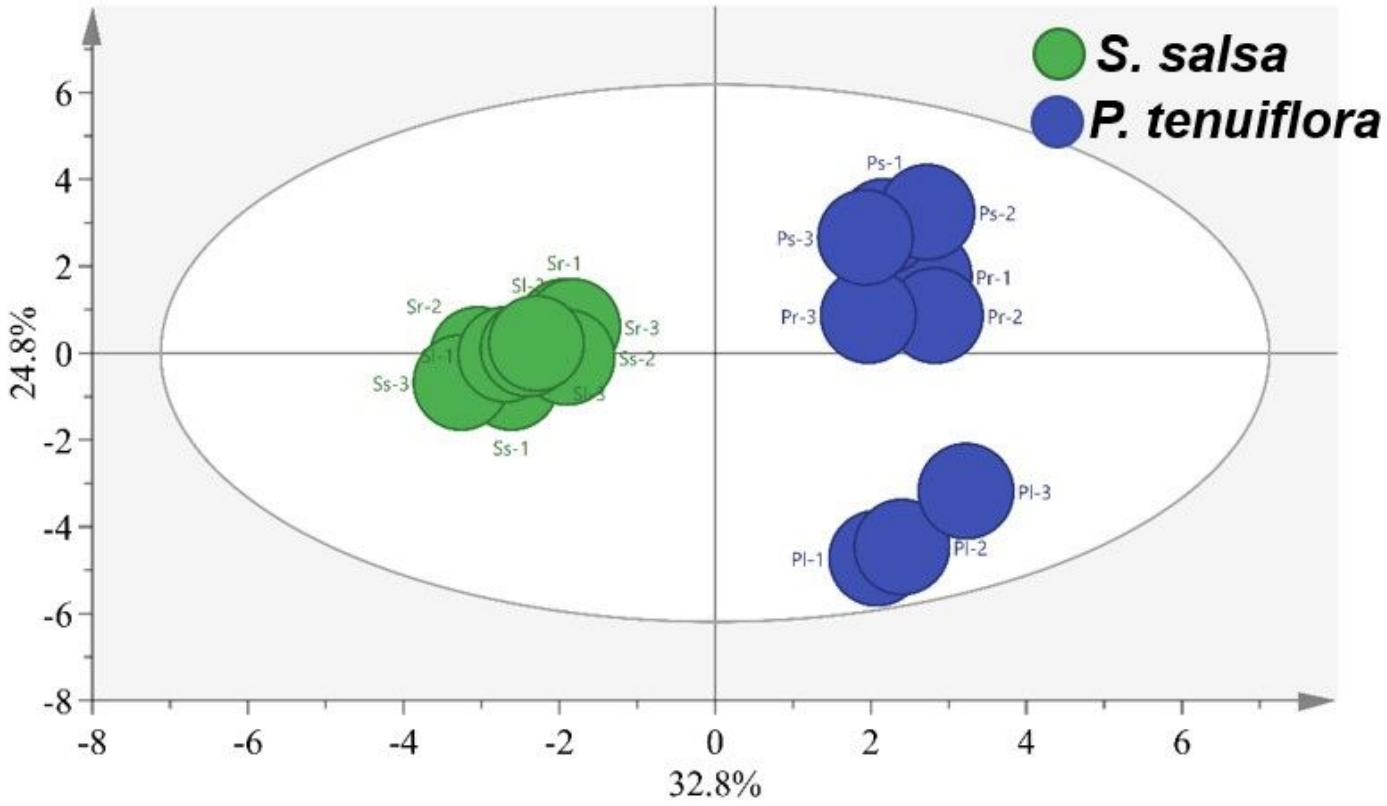

Figure 6

The OPLS-DA score plot of phenolic compounds.

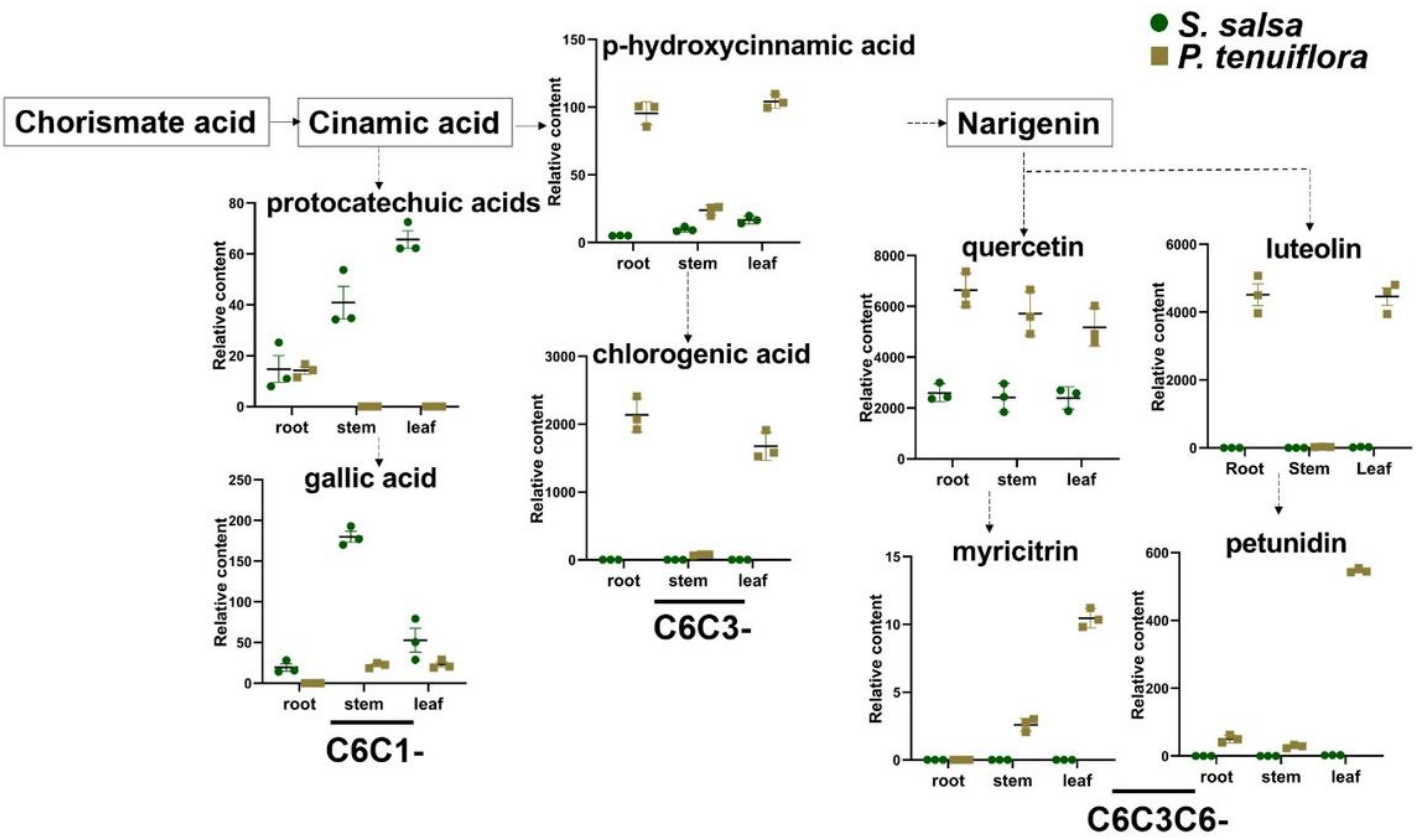

Figure 7

Visualization of the difference of significantly phenolic compounds on a biochemical pathway map.

Supplementary Files

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