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Research Article

Salinity generates varying chemical and biochemical responses in *Physalis ixocarpa* (Solanaceae) during different times of exposure

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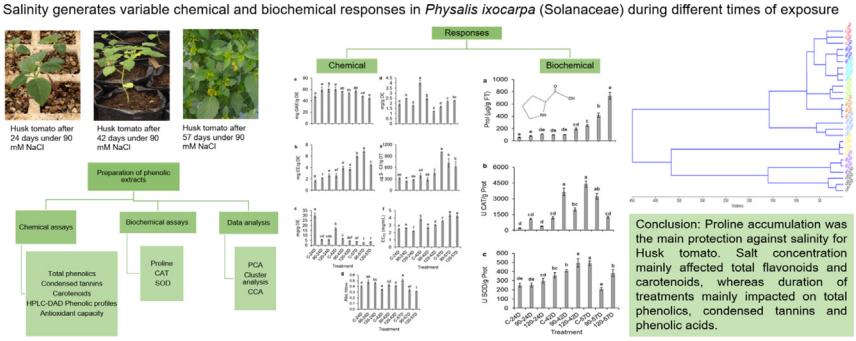
GRAPHICAL ABSTRACT



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ABSTRACT

Background: Diverse plants respond differently to similar saline conditions. The aim of the current study was to determine the variation in the foliar contents of phenolic compounds, carotenoids, and proline, and the variation of the activities of catalase (CAT) and superoxide dismutase (SOD) of the edible and medicinal *Physalis ixocarpa* throughout three different times of exposure (24, 42, and 57 d) to three salinity levels (0, 90, and 120 mM NaCl). The specific effects of salt concentration and time of exposure were also assessed.

Results: Proline increase was the only clearly salt-related response, evidencing its significant protective role in salinized *P. ixocarpa* under either short, medium, or chronic exposure. One phenolic acid, which increased up to 26.26 times its concentration (compared to control, under high salinity at the longest treatment) out of the eight compounds forming the phenolic profile of the species, and CAT and SOD, under 90 and 120 mM NaCl, respectively, and short and medium exposure, also made important contributions. Salt concentration mainly affected total phenolics, tannins, phenolic acids (PA), proline, and SOD, whereas exposure time mainly affected flavonoids, carotenoids, and CAT.

Conclusions: The participation of the different protection mechanisms of *P. ixocarpa* against salinity is dynamic and complementary, and it is differentially modulated by the salt concentration and the time

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of exposure. Proline is the main mechanism for the species. The accurate chronic registration of the responses is needed to determine its adaptation potential to salt stress. The results have agronomic and food quality implications.

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1. Introduction

Salinity cause inhibition of plant growth and development by diminishing the external water potential, generating ionic imbalance, ionic toxicity, and oxidative damage [1], which may lead to plant death.

Plants have developed adaptive chemical and biochemical mechanisms to reduce oxidative damage resulting from abiotic stress. The chemical mechanisms include the biosynthesis of secondary metabolites, such as antioxidant phenolic compounds and carotenoids [2,3]. The biochemical mechanisms include the accumulation of osmolytes such as proline [3], and the participation of antioxidative enzymes such as catalase (CAT) and superoxide dismutase (SOD) [4].

Most studies on the responses of plants to salinity have been carried out in a single stage of development [2,3,5,6] and have focused on short-term responses [2,3]. An important insight from these studies is that different plant species of comparable age respond differently to similar saline treatments. In the current study, we hypothesize that salt level and the duration of exposure differentially modulate the chemical and biochemical responses of plants, and we consider that the accurate knowledge of the particular defense strategies deployed by economically important plants throughout long exposures to different salinity levels would contribute to reveal their adaptive potential for cultivating in salinized soils and could support the development of improvement and crop expansion programs.

Physalis ixocarpa Brot. ex Hornem (husk tomato) belongs to Solanaceae. It is an important edible and medicinal plant in Mesoamerica [7]. Husk tomato is native to Mexico and is cultivated mainly in the tropical and subtropical regions of its territory, although, currently, it is cultivated in other countries [8]. In Mexico, due to the increase in its demand, it seeks to expand the areas of cultivation [9]. Arid and semiarid regions cover more than a half of the territory of this country [10]; nevertheless, for different reasons, these regions are being particularly affected by salinity, which is one of the major abiotic stress limiting plant productivity [11]. In this context, the assessment of the responses of *P. ixocarpa* to salinity becomes a relevant issue. Despite this issue and its importance as an edible and medicinal plant, the responses of *P. ixocarpa* to salt stress have been less explored [12,13]. The aim of the current study was to determine the variation in the foliar accumulation of phenolic compounds, terpenoids, and proline, and of the activities of SOD and CAT of salt-stressed *P. ixocarpa* through short, medium, and chronic exposure, and to determine whether the salt concentration and the exposure time differently affect the chemical and biochemical responses of the species.

2. Materials and methods

2.1. Plant material and saline treatments

Seeds of *P. ixocarpa* var. Rendidora were germinated (May 2018) in a greenhouse in Durango, Mexico, in peat moss–agrolite (8:2) [9]. The study was conducted under repeated measures designs with one factor and three replicas. The factor levels were 0, 90, and 120 mM NaCl [13], corresponding to 0.96, 10.35, and 14.04 dS/m electrical conductivity, respectively. The repeated measures were 24, 42, and 57 d (these times represent growth stages of the species). Experimental lots were formed by 15 plants. Each saline treatment (Table 1) had three experimental lots (replicas). Salt solutions were applied eight days after germination (when 100% germination was reached). The respective saline solutions were provided in a manner such that the respective electrical conductivity was maintained. Electrical conductivity was checked daily. Minimum and maximum temperatures varied between 14.9°C and 17.7°C, and 33.5°C and 46.7°C, respectively, all over the study. The daily photoperiod was 13 h.

2.2. Phenolic extracts

Leaves from the 15 plants of each experimental lot of each treatment (three lots for each saline treatment, representing three replicas) were collected, combined, dried at 40°C, ground, and independently analyzed. Extracts were prepared from 3 g of sample, according to Vasavilbazo-Saucedo et al. [14].

2.3. Total phenolics (TP)

TP was determined according to Noreen et al. [15], from a gallic acid standard curve (Abs_{765} = 11.2460 [gallic acid] – 0.0180, r = 0.9998). Contents were expressed as milligrams equivalents of gallic acid per gram of dry extract (mg GAE/g DE).

2.4. Condensed tannins (TCT)

TCT was estimated according to Julkunen-Tiitto [16], from an epicatechin standard curve (Abs_{500} = 2.1932 [epicatechin] + 0.015 0, r = 0.9949), and expressed as milligrams equivalents of epicatechin per gram of dry extract (mg EE/g DE).

2.5. Carotenoids (CR)

Extraction and quantification of carotenoids were carried out according to Conesa et al. [17] by using a standard β-carotene

Table 1
Saline treatments to which *Physalis ixocarpa* was submitted.

Treatment	Saline condition (mM NaCl)	Exposure time (Days)
C-24D	0	24
90-24D	90	24
120-24D	120	24
C-42D	0	42
90-42D	90	42
120-42D	120	42
C-57D	0	57
90-57D	90	57
120-57D	120	57

Control plants received only nutrient solution (potassium nitrate, 0.50 g/L; calcium nitrate, 0.70 g/L; magnesium nitrate, 0.20 g/L; mono ammonium phosphate, 1.00 g/L; potassium sulfate 0.50 g/L; and micronutrients 0.20 g/L).

curve (Abs_{450} = 0.6472 [β-carotene] – 0.0036, r = 0.9999). Contents were expressed as micrograms equivalents of β-carotene per gram of dry tissue (μg β-CE/g DT).

2.6. Phenolic profiles

The phenolic profiles were determined in a Perkin Elmer Flexar HPLC-DAD system, using the gradient method previously described [18], with a Perkin Elmer Brownlee Analytical C18 column (4.6 × 250 mm, 5 μm). Chromatograms were registered at 260 and 340 nm. UV spectra of the resolved peaks were registered between 200 and 400 nm, using a Perkin Elmer Flexar diode array-detector (DAD). The injection volume was 100 μL and the flow rate was 0.8 mL/min. Structural information was obtained by interpreting the UV spectra according to Campos and Markham [18], and by comparing the retention time (RT) and λ_{max} of the resolved compounds in the chromatograms with those of the standards. Concentrations were estimated by area measurements, using a standard curve of rutin (slope: 6564.40, intercept: 127.33, r = 0.9965) for flavonols and a standard curve of ferulic acid (slope: 39870.00, intercept: 6372.9, r = 0.9985) for phenolic acids (PA) and their derivatives. Concentrations were expressed as micrograms per gram of dry extract (μg/g DE). The total contents of flavonoids (TF) were estimated by adding the individual flavonoid concentrations of one sample, whereas the total contents of PA by adding the individual PA concentrations of one sample. Concentrations were reported as milligrams per gram of dry tissue (mg/g DE).

2.7. Antioxidant capacity

The DPPH**.** (1,1-diphenyl-2-picrilhidrazil) scavenging activity, determined according to Medina-Medrano et al. [7], was expressed as the efficient concentration at 50% (EC_{50}, mg/mL). The iron-reducing power (IRP) was evaluated according to Chavan et al. [19], registering the sample absorbance at 700 nm.

2.8. Proline

Proline content was determined according to Sarker and Oba [4], from a proline standard curve (Abs_{520} = 1.4946 [*L*-proline] + 0.0230, r = 0.9939) and expressed as milligrams per gram of fresh tissue (mg/g FT).

2.9. CAT and SOD activities

Enzyme extracts and the activities of CAT and SOD were according to Sarker and Oba [4]. The protein content in the enzyme extracts was estimated according to Mæhre et al. [20], from a standard curve of bovine serum albumin. CAT and SOD activities were expressed as units per gram of protein (U CAT/g Prot) and units per milligram of protein (U SOD/mg Prot), respectively.

2.10. Data analysis

Data were submitted to repeated measures analysis and means separated by Duncan's multiple range test ($p < 0.05$), using RStudio. Principal component analyses (PCA) was used to determine the contribution of the chemical and biochemical response to differentiation of plants from different treatments. The chemical and biochemical relations between plants from different treatments were separately determined by cluster analyses (paired group UPGMA and Euclidean similarity index). The effects of salt concentration and time of exposure on the chemical and biochemical responses were assessed using canonical correspondence analyses (CCA). Cluster analyses, PCA, and CCA were obtained using Past4.07b.

3. Results and discussion

3.1. Effect of treatments on chemical responses

Plants under any treatment had a good survival, suggesting that *P. ixocarpa* has an excellent adaptation potential to salinity.

Significant differences in the accumulation of the types of phenolic compounds analyzed were found (Fig. 1a-1d). The results revealed that the leaves of unstressed *P. ixocarpa* are a good source of TP and TF when compared with the leaves of other Solanaceae, such as *Solanum lycopersicum*, for which 7.34 mg/g extract of total phenolics and 18.51 mg/g extract of flavonoids were reported [2]. A salt-dependent increase of TP and TCT was observed only after 24 d of exposure, suggesting that these compounds represent an initial strategy of *P. ixocarpa* to cope with saline stress, whereas, only after d 57, a salt-dependent increase of PA was detected, suggesting their participation as protectors after chronic saline exposure. No salt-dependent increase but decreases were observed for TF after any time of exposure, revealing that flavonoids play no important role as protectors against salinity in husk tomato. The reduction in TF was also reported for *S. lycopersicum* [2], although in that study only one NaCl concentration, not progressive increases, was evaluated. Comparing our results with those found for other plant species, a great diversity of responses in the accumulation of TF and PA have been informed [5,6,21,22,23]; even, a minor significance of flavonoids as protectors against salt-induced oxidative damage has been reported [24]. This diversity of responses could be a consequence of the different experimental conditions, in which the studies were carried out. However, it can also indicate that diverse genetically controlled responses against saline stress have emerged among different plant species, even in related species. The species-specific responses found for species of the genus *Brassica* (Brassicaceae) [25] support this proposal.

Carotenoids are other secondary metabolites reported as protectors against salinity stress [26]. In plants, carotenoids play essential roles in photosynthesis and photoprotection [27]; however, according to the data shown in Fig. 1e, their involvement as protectors against salinity stress was little relevant in *P. ixocarpa*. A reduced role for these compounds was also found for other Solanaceae [2,3], suggesting a probable common response of carotenoids under salt stress in the family. Nevertheless, other species responded differently [21,26].

Our results suggest that the accumulation of phenolic compounds and carotenoids of a single species vary under short, medium, and long salt treatments. Thus, the accurate registration of the chemical changes taking place in chronically salinized plants is necessary for a better understanding of the accumulation processes and roles of secondary metabolites, which can represent useful markers to support the development of conservation, improvement, and crop expansion programs.

The DPPH**.** scavenging activity and the IRP of husk tomato leaves were not clearly dependent on phenolic contents and salt concentration (Fig. 1f-1g). Contrary, the increase of DPPH**.** scavenging activity as the increase of phenolic contents and abiotic stress has been found for other species [4]. However, not only the concentration of phenolic compounds but also the composition is important in determining the antioxidant activity in plant tissues [28].

The HPLC-DAD analysis revealed eight main phenolic compounds. Fig. 2a-2i shows the chromatograms registered for each sample. Fig. 3 displays the UV spectra of each compound resolved in the chromatograms, its RT, and λ_{max}. Compounds **1**–**4** were PA, **5** and **6** were flavonols. Compound **5** was proposed as rutin. **6** was a kaempferol-3-*O*-glycoside. **7** and **8** were *p*-coumaric acid derivatives.

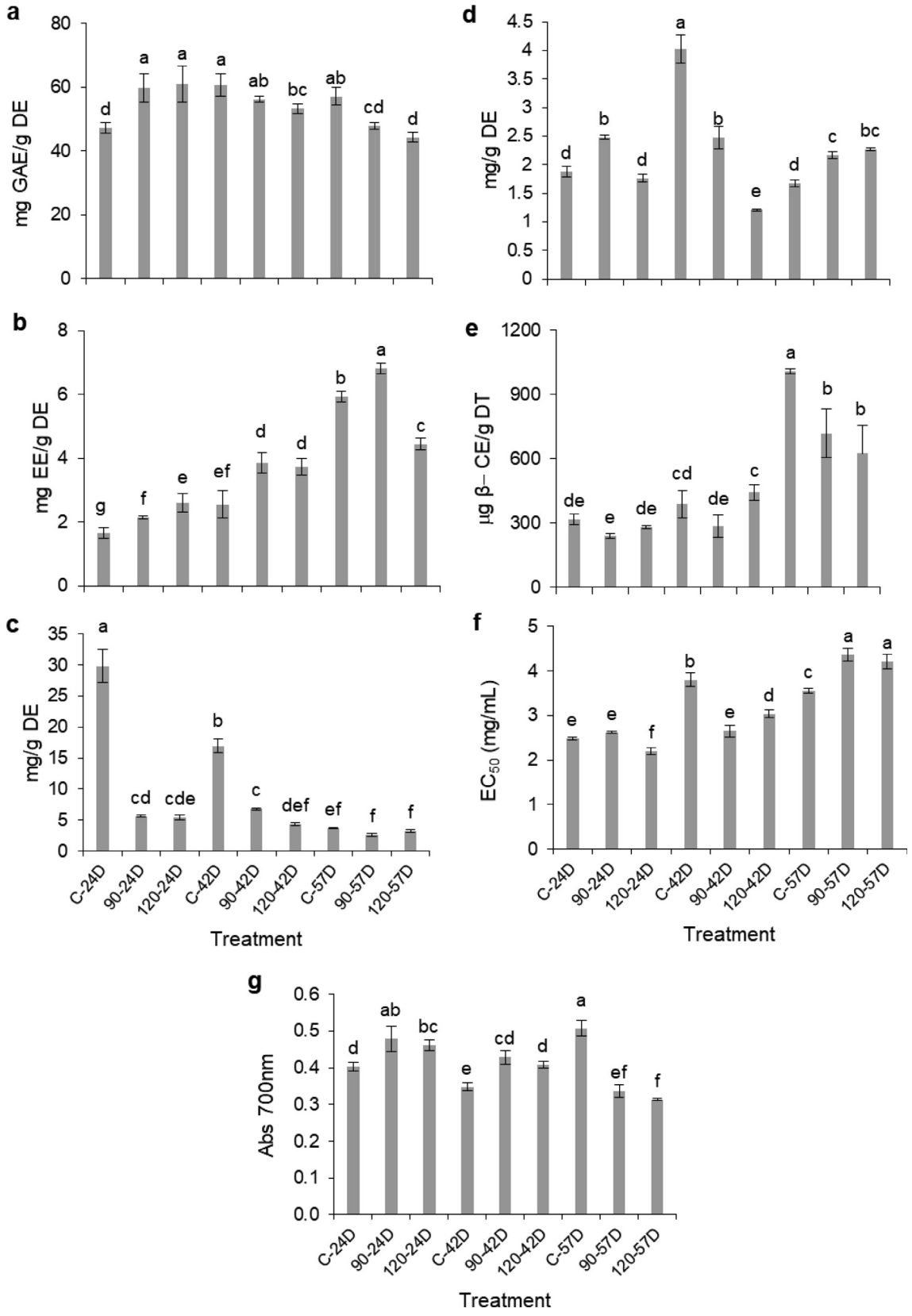

Fig. 1. Effect of saline treatments on the content of total phenolics (a), condensed tannins (b), flavonoids (c), PA (d), carotenoids (e), DPPH**.** scavenging capacity (f), and IRP (g) of *Physalis ixocarpa*. DE: Dry extracts, DT: Dry tissue.

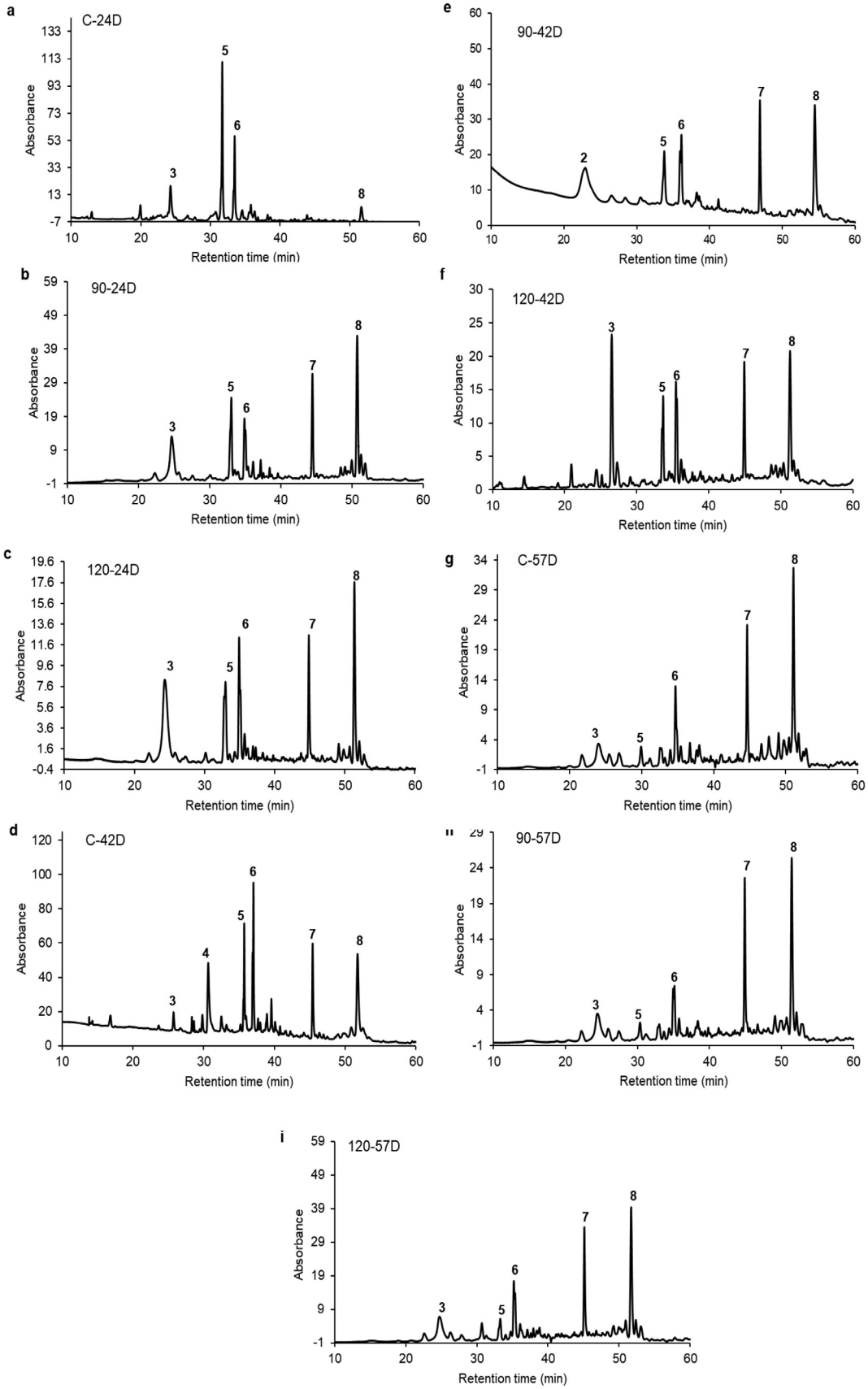

Fig. 2. HPLC-chromatograms of the foliar extracts of *Physalis ixocarpa* exposed to different saline treatments. Saline treatments according to Table 1. Numbers in bold represent compounds according to Table 2.

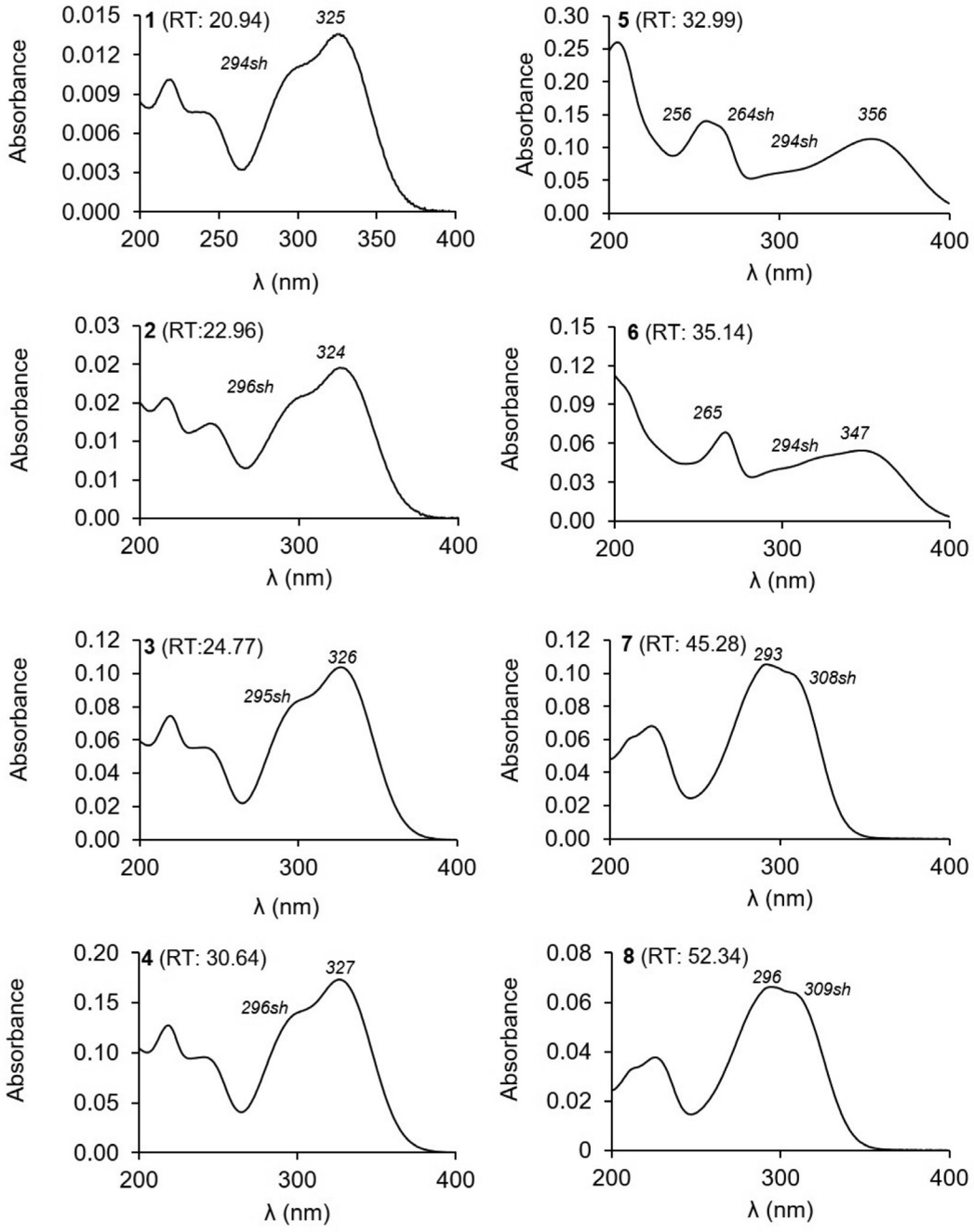

Fig. 3. UV spectra of the major foliar phenolics of *Physalis ixocarpa*. RT: Retention time (min). Numbers in bold represent compounds according to Table 2.

The foliar phenolic profile of untreated mature *P. ixocarpa* (Fig. 2g) was different from those reported for other species (also in the mature stage) of the genus, such as *P. patula*, *P. solanaceae*, *P. subulata*, *P. angulata*, and *P. hederifolia* var. *hederifolia* [7], supporting the proposal made by several authors that phenolic profiles have a species-specific trend [7,29].

Quantitative age-dependent differences were observed in the foliar phenolic profiles of untreated *P. ixocarpa* (Table 2), in accordance with the quantitative age-dependent variation found for *Physalis angulata* [30]. The high concentrations of **5** and **6** in plants from C-24D were notable.

In addition to the changes in the concentration of the individual foliar phenolic compounds of *P. ixocarpa* due to the natural demand during its growing process, changes caused by treatments were found (Table 2). Only under the longest treatments (57 d), **3** significantly increased in a salt-dependent manner by 17.19 and 26.26 times more under the moderate and high salinity, respectively, than the respective control, suggesting that this phenolic acid plays an important protective role in husk tomato against salinity after chronic exposure. The accumulation of some PA under a determined saline condition but not in others (Table 2) is in agreement with other reports [11,25], suggesting a dynamic participation against salt stress for some single phenolic compounds, according to defined combinations of salt concentration and time of exposure.

No clear protector role was found for the two flavonols **5** and **6** neither the two *p*-coumaric acid derivatives **7** and **8** (Table 2), in disagreement with that reported for other plants [11,31], including

Table 2
Concentrations (μg/g DE) of the individual compounds of *Physalis ixocarpa* leaves under different salt treatments.

Compound	Type of compound	Treatment									
		C-24D	90-24D	120-24D	C-42D	90-42D	120-42D	C-57D	90-57D	120-57D
1	Phenolic acid	NF	NF	NF	NF	NF	70.24 ± 6.76	NF	NF	NF
2	Phenolic acid	NF	NF	NF	NF	743.91 ± 111.22	NF	NF	NF	NF
3	Phenolic acid	1275.75 ± 46.71a	681.05 ± 14.93b	728.21 ± 37.09b	287.27 ± 34.26a	NF	124.05 ± 6.76b	14.45 ± 2.45c	248.40 ± 17.20b	379.48 ± 24.47a
4	Phenolic acid	NF	NF	NF	1551.99 ± 139.21	NF	NF	NF	NF	NF
5	Quercetin-3-O-[rhamnosyl(1-6) glucoside] (Rutin)	19498.72 ± 1538.41a	4340.24 ± 139.47b	2925.71 ± 320.65b	7853.40 ± 448.45a	2975.95 ± 208.83b	2220.53 ± 246.19c	925.88 ± 20.81b	894.29 ± 97.80b	1219.99 ± 106.17a
6	Kaempferol-3-O-glycoside	10343.16 ± 1380.20a	1320.91 ± 155.36b	2496.19 ± 142.06b	9114.48 ± 732.58a	3847.75 ± 231.32b	2121.69 ± 206.54c	2793.59 ± 134.77a	1722.49 ± 87.06c	1985.48 ± 116.08b
7	*p*-coumaric acid	NF	635.83 ± 31.70a	368.15 ± 20.01b	1182.28 ± 115.67a	654.97 ± 51.42b	401.40 ± 17.41c	584.13 ± 50.38b	744.45 ± 65.00a	728.93 ± 17.59a
8	*p*-coumaric acid	600.15 ± 48.28b	1159.25 ± 15.81a	668.20 ± 54.57b	999.53 ± 82.72a	1075.85 ± 143.39a	613.49 ± 17.55b	1074.61 ± 59.29b	1171.79 ± 22.26a	1161.29 ± 36.29ab

The values represent the mean and standard deviation for three independent samples. Different letters in the same line mean significant differences ($p < 0.05$). DE: Dry extract; NF: Not found.

tomato [32]. The variation of the individual compounds concentrations suggests that for a single plant species, variations in salt concentrations and exposure time can stimulate the accumulation of different flavonoids and PA, which could be useful to improve the selective accumulation of bio-products in plants.

3.2. Effect of treatments on the biochemical responses

The variations in proline, CAT, and SOD are shown in Fig. 4a-4c. The clear salt-dependent increase of proline content was observed in every exposure time, mainly after 57 d (Fig. 4a), suggesting that this amino acid is a key biochemical mechanism of *P. ixocarpa* against moderate and high saline stress, either under short, medium, or chronic exposure. This prominent role of proline in salt-stressed husk tomato can derive from its multiple roles in cells, such as an osmolyte, which protects plants against the osmotic unbalance generated by saline stress [33,34]; as a protector against oxidative damage, due to its capability to scavenge hydroxyl radical and quench singlet oxygen; and as a stabilizer of macromolecules and membrane structure [33]. The importance of proline in plant defense against salt has also been revealed for other species of Solanaceae [2,3], which could suggest a common strategy of proline accumulation against salinity in the family.

CAT played a major protective role after 24 and 42 d of exposure to 90 mM NaCl, its activity increased by about 5 and 3 times, respectively, compared to the respective controls (Fig. 4b), suggesting a hydrogen peroxide detoxification promotion in *P. ixocarpa* under these saline conditions, as CAT catalyzes that detoxification in cells [4]. SOD played a protective role in *P. ixocarpa* after the same times of exposure (24 and 42 d) but under the strong salt concentration (Fig. 4c), as its activity increased by 1.20 and 1.38 times, respectively, compared to the respective controls, suggesting a promotion of dismutation of superoxide radicals to H_2O_2 and O_2 in the leaves of *P. ixocarpa*, as this enzyme catalyzes this reaction in cells [4]. The results suggest a complementary role for these two enzymes under increased salt concentration for *P. ixocarpa* after short and medium time of exposure. Important protective roles of CAT and SOD were also found for the Solanaceae *S. lycopersicum* [2] and *L. ruthenicum* [3], although for these species it is difficult to assign a complementary role because the studies were carried out at a single time of exposure for the first species and at only one salt level and one time of exposure for the second one. Different results have been reported for other plants, for which neither CAT nor SOD responded to increases of salt concentration [22].

Regarding the chemical responses, the variation of biochemical responses may be the consequence of a diversity of genetically controlled responses against saline stress that have emerged in different plant species, with a single species being able to display a wide, complementary, and complex range of biochemical responses throughout chronic exposure to different salt concentrations.

3.3. PCA and cluster analysis based on the chemical and biochemical responses

PC1 and PC2 of the chemical responses-based PCA accounted for 99.94% of the variance; PC1 was correlated with CR, TCT, and the antiradical activity, whereas PC2 was correlated with TF (Fig. 5a). PC1 and PC2 of the biochemical responses-based PCA accounted for 99.74% of the variance; PC1 was correlated with CAT and PC2 was correlated with proline (Fig. 6a). All these responses sustained the discrimination between *P. ixocarpa* from different saline treatments.

The similarity between the chemical and biochemical responses of *P. ixocarpa* from different saline treatments was

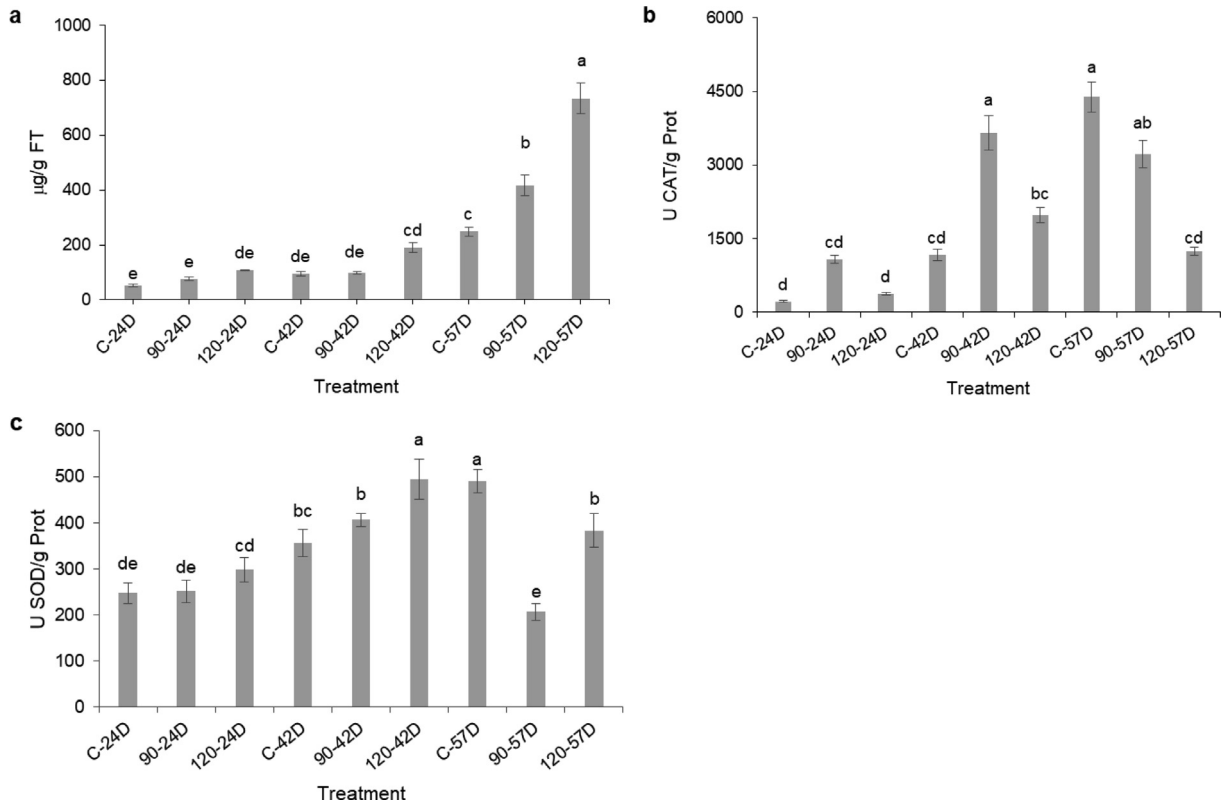

Fig. 4. Effect of saline treatments on proline content (a), CAT activity (b), and SAT (c) of *Physalis ixocarpa*.

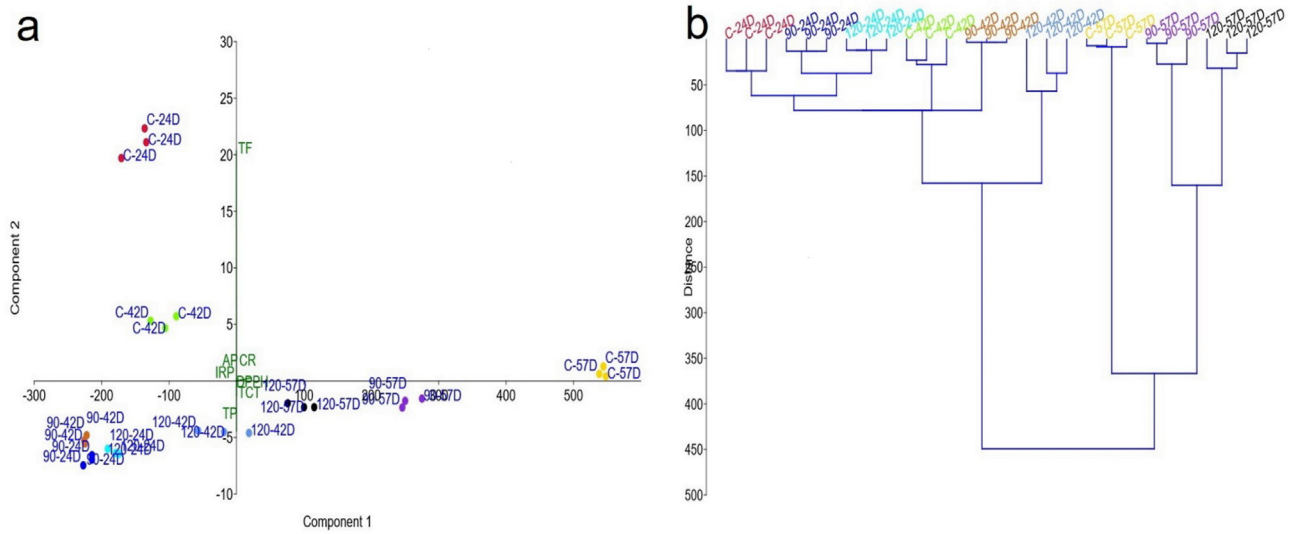

Fig. 5. Results of the PCA (a) and cluster analysis (b) based on the chemical responses of *Physalis ixocarpa* exposed to different saline treatments. Saline treatments according to Table 1. Each treatment is represented by three replicas.

assessed by two cluster analyses (Figs. 5b and 6b). Both cluster analyses revealed that at early growth stages the chemical and biochemical responses of *P. ixocarpa* to different treatments were less variable than at advance stages, as plants from the treatments 90-24D and 120-24D formed a single group with the highest similarity, whereas plants from the longer treatments formed a more heterogeneous group. However, both cluster analyses corroborated the potential of the chemical and biochemical responses to discriminate between husk tomato cultivated under different salt treatments, as plants from each treatment was grouped in a single clade. These results verify that the chemical and biochemical responses of the husk tomato represent a valuable fingerprinting with agronomic and food quality control implications.

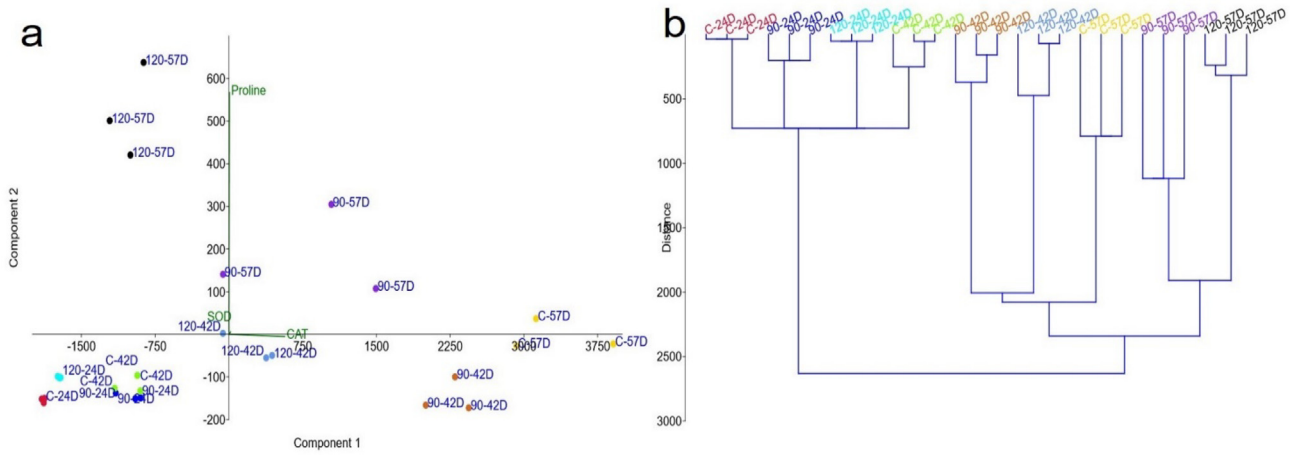

Fig. 6. Results of the PCA (a) and cluster analysis (b) based on the contents of proline and the activities of CAT and SOD of *Physalis ixocarpa* exposed to different saline treatments. Saline treatments according to Table 1. Each treatment is represented by three replicas.

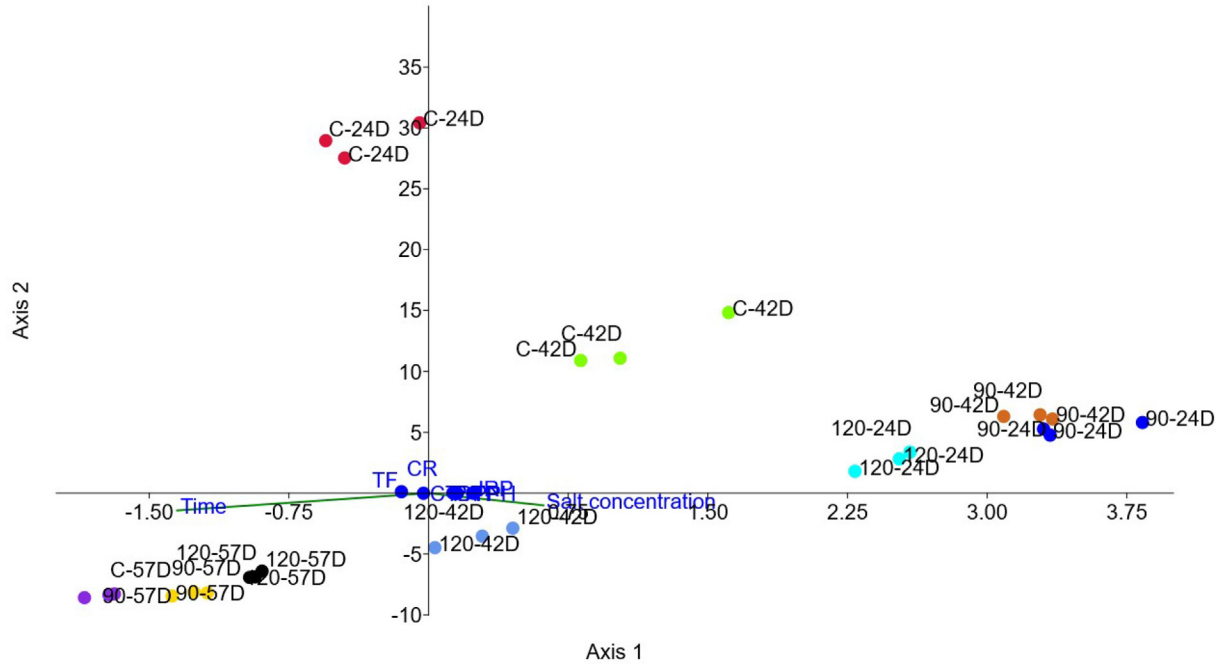

Fig. 7. CCA showing the influence of salt concentration and time of exposure on the chemical responses of *Physalis ixocarpa* exposed to different saline treatments. Saline treatments according to Table 1. Each treatment is represented by three replicas.

3.4. Relation of the chemical and biochemical responses and salt concentration and time of exposure

The effect of salt concentration and time of exposure on the chemical responses evaluated for salt-stressed *P. ixocarpa* were assessed by a CCA (Fig. 7). CCA1 explained 96.23% of total variance (1,000 permutations, $P = 0.001$). The results indicate that the time of exposure mainly affected TF and CR, whereas salt concentration had a greater impact on TP, TCT, PA, and the antioxidant properties.

The effect of salt concentration and time of exposure on the biochemical responses evaluated of salt-stressed *P. ixocarpa* were assessed by other CCA (Fig. 8). CCA1 explained 97.73% of total variance (1,000 permutations, $P = 0.001$). The time of exposure mainly influenced CAT activity, whereas salt concentration mainly influenced proline content and SOD activity.

The current results reveal that for *P. ixocarpa* some chemical and biochemical responses were mainly triggered by salt concentration, whereas others mainly responded to exposure time. As chemical changes determine variations in the organoleptic and functional properties of plants [35], the accurate registration of these changes can be useful as control quality tools.

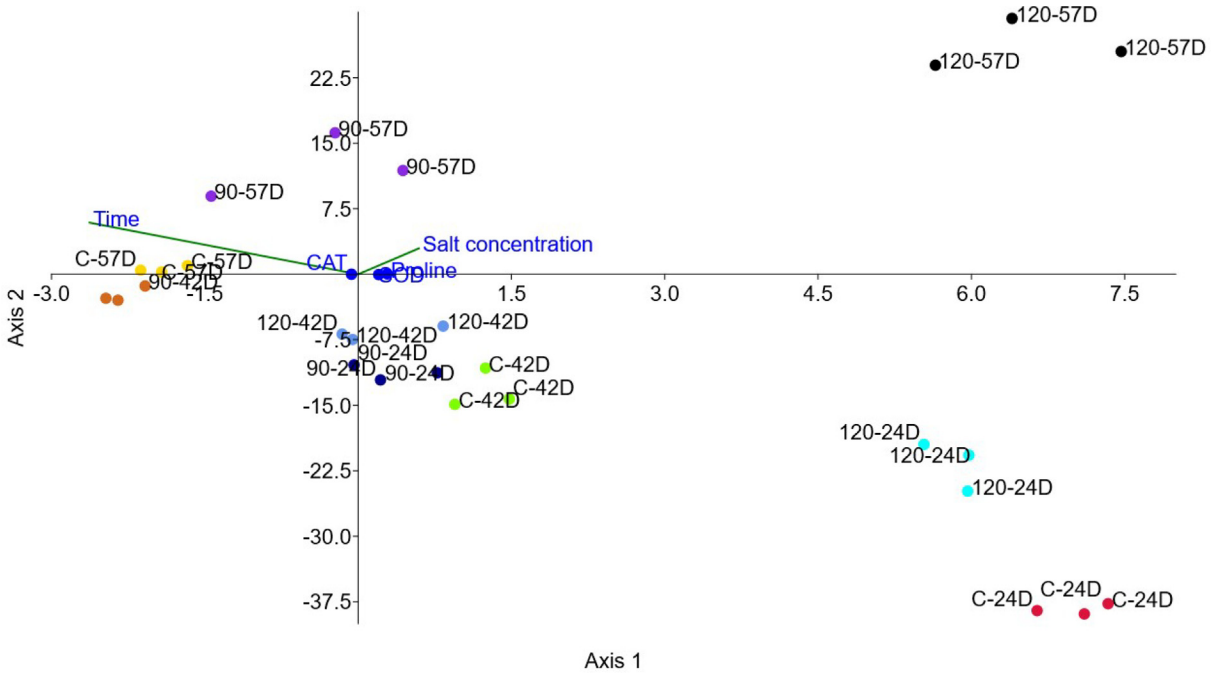

Fig. 8. CCA showing the influence of salt concentration and time of exposure on the biochemical responses of *Physalis ixocarpa* exposed to different treatments. Saline treatments according to Table 1. Each treatment is represented by three replicas.

4. Conclusions

Physalis ixocarpa has developed complex defense systems to cope with salt stress. Proline was revealed as the main defense mechanism; however, under particular saline conditions, proline can synchronize with the increase in compound **3** and CAT and SOD activities. The panorama revealed here for *P. ixocarpa*, suggests that the participation of the different mechanisms is dynamic and complementary, and it is differentially modulated by intensity of salinity and duration of the treatments. Since the responses can vary as the time of exposure increases, it is necessary to register not only short-time responses but long-time responses to determine the adaptation potential of economically important plants to salinity. The collected data have agronomic and food quality implications.

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Conflict of interest

The authors declare no conflict of interest.

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