Scientia Horticulturae 281 (2021) 110001

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Short communication

Variation of amino acids in *Prunus persica* cultivars leaves with regard to leaf age

Samia Dabbou [a,b,*], Samira Maatallah [c,d], Andrea Antonelli [e], Giuseppe Montevecchi [e]

[a] Unit of Bioactive and Natural Substances and Biotechnology UR17ES49, Dentistry Faculty, University of Monastir, Avicenne Street, 5019, Monastir, Tunisia
[b] Dentistry Faculty, University of Monastir, Avicenne Street, 5019, Monastir, Tunisia
[c] Institution of Research and Higher Education Agriculture (IRESA), Regional Center for Agricultural Research, Sidi Bouzid, 9100, Tunisia
[d] Non-Conventional Water Valuation Research Laboratory (LR VENC), INRGREF, Hedi EL Karray Street, El Menzah IV, 1004 Tunis, Tunisia
[e] Department of Life Sciences (Agro-Food Science Area), BIOGEST - SITEIA Interdepartmental Centre, University of Modena and Reggio Emilia, Piazzale Europa 1, 42124, Reggio Emilia, Italy

ARTICLE INFO

Keywords:
Leaf age
Peach
Amino acid source
Leaf valorization

ABSTRACT

The central role of amino acids in cellular and plant physiology is of current interest. Information related to the implication of amino acids during leaf age is still scarce. This study examined changes in the profile of amino acids extracted from leaves of five peach cultivars ('Early Maycrest', 'Sweet Cap', 'O'Henry', 'Flordastar', and 'Rubirich') grown in the region of Sidi Bouzid, central-western Tunisia with respect to leaf age.

Seventeen amino acids, aspartic acid (Asp), serine (Ser), glycine (Gly), proline (Pro), tyrosine (Tyr), valine (Val), methionine (Met), cysteine (Cys), tryptophan (Trp), leucine (Leu), phenylalanine (Phe), isoleucine (Ile), lysine (Lys), glutamic acid (Glu), arginine (Arg), alanine (Ala), and histidine (His) were quantified. Significant variations were observed for Asp, Glu, Ser, Gly, Ala + Arg, Pro, Val, Ile + Trp, and Lys in relation to the leaf age. On average, young leaves (106.48–142.18 mg g^{-1} DW) contained higher amounts of most amino acids than mature leaves (97.33–110.27 mg g^{-1} DW). Approximately identical contents of amino acids were found in the different cultivars.

In conclusion, *P. persica* leaves are a very rich source of amino acids (about 10% DW), and therefore deserves further investigation for their potential application in the nutraceutical, food, feed, and pharmaceutical industries.

1. Introduction

The climate, genetics, cultural practices affect drastically the composition of free amino acids in the leaves. During his life, the plant goes through different stages that can be correlated with changes in various components of the leaf, and with the development of new leaves. The developing leaves constitute the major sink for nitrogen transported from roots.

In higher plants, amino acids constitute the key compounds of the nitrogen (N) metabolism and have different roles (Ohyama et al., 2017). Indeed, they are known as constituents of proteins and nucleic acids and can act as precursors for anabolism, and, as signaling molecules or as a response to a changing environment (Batista-Silva et al., 2019; Häusler et al., 2014). The latter function is well documented in the medical/mammalian field, and evidence for similar functions is also emerging for the plant system (Häusler et al., 2014) especially in cellular and plant physiology (El-Yazal and Rady, 2014; Bagh et al., 2004). Furthermore, amino acids regulate ion transport, leaf stomatal opening, and redox homeostasis, thus helping the plants to cope with the harmful effects of the osmotic stress (Rai, 2002). Previous researches showed the close link between the biochemistry of amino acids and carbohydrate metabolism, as well as secondary metabolism (Ohyama et al., 2017).

Quantities of free amino acids are on average 100-to-1000-folds lower than the corresponding quantities of protein-bound amino acids. At the same time, the amino acid content varies dynamically and substantially responds to either environment and/or developmental stages (Hildebrandt et al., 2015). Indeed, their regulation, fluxes, and transport through the plant is tightly linked to plant adaptation to carbon and

* Corresponding author at: Unit of Bioactive and Natural Substances and Biotechnology UR17ES49, Dentistry Faculty, University of Monastir, Avicenne Street, 5019, Monastir, Tunisia.
E-mail addresses: dabbou_samia@yahoo.fr, samiadabbou@gmail.com (S. Dabbou).

https://doi.org/10.1016/j.scienta.2021.110001
Received 21 September 2020; Received in revised form 20 January 2021; Accepted 25 January 2021
Available online 10 February 2021

nitrogen status and development (Atanasova, 2008; Kohl et al., 2015; Pratelli and Pilot, 2014).

Leaves represent an important source of metabolites that could be positively applied to the agricultural, food, and nutraceutical industries (Bouhajeb et al., 2020). Studies on peach trees mainly focused on fruits composition and sensory characteristics (Dabbou et al., 2016; Montevecchi et al., 2016, 2013), as well as on innovative agricultural practices (Guizani et al., 2019), while a gap is still present in the valorization of peach leaves (Gomez et al., 2020; Maatallah et al., 2020).

In this context, this study aimed at establishing changes in the composition of amino acids synthesized during the young and mature phases of peach leaves from five cultivars in order to implement a strategy to valorize these deciduous leaves.

2. Material and methods

2.1. Plant material

Peach leaves (young and mature) were collected from five different cultivars of *Prunus persica* L. [Batsch], viz. Sweet Cap, Early Maycrest, O'Henry, Flordastar, and Rubirich, grafted on the Germen wild rootstock during the two summer seasons 2013–2014. The five cultivars studied had different vigors and shapes. Rubirich and Flordastar had high vigor, while, O'Henry and Early May Crest were characterized by medium vigor, and finally Sweet Cap had the lowest vigor. As for the shape, Rubirich, O'Henry and Early Maycrest had an open-center (vase) shape, while Sweet cap and Flordastar were characterized by a modified central leader shape. All cultivars were eight years old at the start of the experiment.

The orchard was arranged at a spacing of 4 m × 6 m, under a network drop-by-drop with two pipes per row (4 L h^{-1}) and similar fertilization of nitric acid, magnesium, and potassium, were selected at the experimental orchard located in the Regional Centre of Agricultural Research Farm in the region of Sidi Bouzid, Central-West Tunisia (35°2′0″N, 9°30′0″E; at 313 m a.s.l.). The region is characterized by a semi-arid climate, a mean annual rainfall of 251.8 mm (concentrated mainly from autumn to spring), a mean annual temperature varying from 12.5 °C to 25.3 °C, average evapotranspiration (ETc) of 1634.9 mm and silt-clay-loam soil texture. The leaves were picked from the branches of the previous year. Seven-month-old mature leaves were taken from the central part of the branches, while young leaves which were only a few days old were collected from the extremity of the same branches. All leaves were collected by hand immediately after fruit harvest, gently washed with tap water, lyophilized, and stored at −20 °C until analysis. Three replicated samples were used for each peach cultivar.

2.2. Chemicals

Pure reference compounds: alanine (Ala), arginine (Arg), aspartic acid (Asp), cysteine (Cys), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), and valine (Val); acetic acid, acetonitrile, borate acid, diethyl 2-(ethoxymethylidene)propanedioate (DEEMM), hydrochloric acid (HCl), methanol, and sodium acetate were purchased from Fluka Sigma–Aldrich (Milan, Italy).

Deionized HPLC-grade water was obtained by an Elix Essential^{3UV} purification system (Merck Millipore, Milan, Italy).

2.3. Analytical methods

2.3.1. Hydrolysis of amino acids present in proteins

The determination of the amino acid composition was carried out according to a standard method described by Peace and Gilani (2005). Each sample (0.5 g, exactly weighed) was introduced into a test tube (carefully cleaned prior analysis) with 6-M HCl. Each tube was flushed with nitrogen before sealing, then vortexed and, finally, placed into a Thermoblock well at 110 °C for 24 h. Afterward, the tube was centrifuged at 2066 *g* for 6 min. The supernatant was collected and the pH was adjusted to 9 using NaOH solution and pH-9 borate buffer.

2.3.2. Pre-column derivatization of amino acids with DEEMM

The derivatization method described by Gómez-Alonso et al. (2007) was used after minor modifications to convert primary and secondary amino acids into aminoenones. Briefly, the derivatization was carried out using 200 μL of the sample, 750 μL of borate buffer, 300 μL of methanol, and finally 30 μL of pure DEEMM. The reaction occurred in a test tube tightly closed at 70 °C for 2 h.

2.3.3. Amino acid determination via HPLC

The amino acid determination was performed with HPLC (PU4180, Jasco Europe Srl, Cremella, LC) equipped with a UV/vis detector (UV4070, Jasco) set at 280 nm (λ_{max}). By means of an auto-sampling system, 20 μL of each sample were injected into the RP-C_{18} column (Poroshell, 120 SB-C18, Agilent) with a mobile phase flow of 0.6 mL min^{-1}. The mobile phase was constituted by phase A, 25 mM acetate buffer pH = 5.34; phase B, 80:20 mixture of acetonitrile and methanol. The elution was carried out according to the following gradient: 0% B (0.0 min), 4% B (7.0 min), 6% B (18.0 min), 8% B (23.0 min), 8% B (25.0 min), 15% B (28.0 min), 23% B (45.0 min), 45% B (50.0 min), 60% B (55.0 min), 80% B (57.0 min), 80% B (64.0 min), 0% B (66.0 min), and 0% B (70.0 min).

Samples extracted from different sample batches were analyzed and injected in triplicate. Peak identification included the comparison of peak retention times to those obtained with chemical standards. Quantification was performed via external standard calibration. The chromatograms were acquired and processed by ChromNAV software v2 (Jasco).

2.4. Statistical analysis

Three repetitions were performed for each sample. The data were expressed as mean values (± standard deviations) and assessed by the analysis of the variance (two-way ANOVA, using 'cultivar' and 'leaf age' as factors) elaborated through Statistica v8.0 software (Stat 180 Soft Inc., Tulsa, USA). When a significant effect (at least $p \leq 0.05$) was showed, comparative analyses were carried out using the post-hoc Duncan multiple comparison tests to distinguish between the mean values of the five cultivars of *P. persica* and two leaf ages have been included in Table 1, while the *p* values referred to cultivar, leaf age, and interaction cultivar × leaf age have been reported in Table 2. The comparison between the two leaf ages within each cultivar was evaluated using Student's *t*-test. Statistically significant differences between groups were defined as $p \leq 0.05$. Correlation matrix analysis and principal component analysis (PCA) were also applied to the whole data set.

3. Results and discussion

The HPLC analysis of peach leaves from cultivars grown in Tunisia allowed to determine 17 amino acids (Table 1). However, two peak coelutions were present in the chromatogram. In particular, the couples of amino acids alanine-arginine and isoleucine-tryptophan were not resolved, so these amino acids were determined as sums. Furthermore, asparagine and glutamine were hydrolyzed in the extraction process and converted into their respective acids.

The individual amino acid contents varied from 0.4 to 26.5 mg g^{-1} DW. These large differences reflect the various functional roles that individual amino acid plays in the protein structure and overall plant metabolism (Hildebrandt et al., 2015). Among the amino acids detected, eight of them are essential for humans (valine, methionine, tryptophan, leucine, isoleucine, phenylalanine, histidine, and lysine), while some others are considered "conditionally essential", meaning their synthesis

Table 1
Amino acids contents (mg g^{-1} DW) in young and mature leaves from five *Prunus persica* cultivars.

	Sweet Cap			Early Maycrest			O'Henry			Flordastar			Rubirich		
Amino acids	Young leaves	Mature leaves		Young leaves	Mature leaves		Young leaves	Mature leaves		Young leaves	Mature leaves		Young leaves	Mature leaves	
Asp	0.63 ± 0.10	0.67 ± 0.16		0.42 ± 0.11	0.70 ± 0.19		0.48 ± 0.13	0.90 ± 0.16*		0.46 ± 0.10	0.84 ± 0.14*		0.52 ± 0.11	0.89 ± 0.25	
Glu	9.94 ± 2.86*	4.67 ± 0.68	ab	6.25 ± 1.67	4.23 ± 0.88	b	9.61 ± 2.63	9.98 ± 2.50	a	11.89 ± 3.51	8.65 ± 2.05	a	10.43 ± 2.59	9.23 ± 2.28	a
Ser	10.98 ± 1.94	10.14 ± 0.73		12.15 ± 1.04*	9.81 ± 0.67		9.16 ± 1.99	9.38 ± 1.79		11.54 ± 2.13	8.36 ± 2.01		13.07 ± 1.74	9.84 ± 0.55	
His	10.32 ± 2.66	9.80 ± 0.65		9.48 ± 2.69	9.71 ± 0.76		8.59 ± 1.64	7.82 ± 1.71		10.97 ± 1.14	7.99 ± 2.25		11.49 ± 1.45	9.40 ± 1.27	
Gly	5.89 ± 0.97	5.46 ± 0.43		5.52 ± 1.14	5.56 ± 0.59		5.12 ± 0.75	3.75 ± 0.45		6.08 ± 0.50	4.47 ± 1.24		6.68 ± 1.05	4.54 ± 1.03	
Ala + Arg	22.14 ± 4.44	19.83 ± 1.98		25.13 ± 4.87	19.98 ± 1.59		18.97 ± 3.05	15.75 ± 2.79		23.73 ± 1.78*	16.91 ± 3.77		26.54 ± 3.57*	18.69 ± 1.91	
Pro	11.60 ± 2.72	10.33 ± 1.70		11.82 ± 1.22	10.65 ± 0.49		9.60 ± 2.10	7.89 ± 1.34		12.14 ± 0.32*	9.54 ± 1.41		13.43 ± 1.99*	9.34 ± 0.87	
Tyr	6.01 ± 1.56	5.75 ± 0.71		6.17 ± 0.61	5.88 ± 0.37		4.68 ± 0.62	4.89 ± 0.53		6.03 ± 0.71	5.15 ± 1.20		6.64 ± 1.70	5.61 ± 0.51	
Val	6.34 ± 0.89	6.41 ± 1.13	ab	7.17 ± 0.90	7.24 ± 1.34	a	5.70 ± 1.35	4.84 ± 0.52	b	7.12 ± 0.51*	5.49 ± 0.63	ab	7.79 ± 1.47	5.29 ± 0.60	a
Met	2.03 ± 0.36	2.09 ± 0.28		2.00 ± 0.14	2.12 ± 0.10		1.94 ± 0.49	1.94 ± 0.36		1.83 ± 0.36	2.08 ± 0.33		2.49 ± 0.54	2.19 ± 0.57	
Cys	5.85 ± 1.30	5.53 ± 0.20		5.57 ± 0.99	5.41 ± 0.56		5.10 ± 1.39	4.46 ± 1.32		5.81 ± 0.87	4.82 ± 1.01		6.61 ± 1.43	5.06 ± 1.03	
Ile + Trp	6.30 ± 1.66	5.90 ± 0.61		6.57 ± 0.27*	5.87 ± 0.25		5.14 ± 1.09	4.83 ± 0.72		6.50 ± 0.49	5.18 ± 0.91		7.09 ± 1.54	5.40 ± 0.04	
Leu	13.31 ± 3.96	12.58 ± 1.09		13.34 ± 2.04	12.33 ± 1.73		11.23 ± 2.32	9.81 ± 1.70		13.51 ± 1.78	10.66 ± 1.96		15.07 ± 3.69	11.74 ± 1.90	
Phe	5.55 ± 0.69	5.02 ± 1.32		5.13 ± 0.50	4.89 ± 0.97		4.61 ± 1.15	5.63 ± 0.72		4.39 ± 1.14	5.34 ± 1.57		5.08 ± 1.51	6.51 ± 1.45	
Lys	7.98 ± 1.39	7.51 ± 0.65		8.46 ± 1.29	7.29 ± 0.85		6.54 ± 1.93	5.45 ± 0.96		8.13 ± 0.90	6.04 ± 0.95		9.26 ± 1.73	6.48 ± 0.83	
Total	*124.85 ± 24.63*	*110.12 ± 8.52*		*125.19 ± 13.56*	*110.27 ± 8.43*		*106.48 ± 17.15*	*97.33 ± 10.87*		*130.13 ± 3.15**	*101.55 ± 14.95*		*142.18 ± 20.90*	*106.93 ± 11.65*	

Duncan test was reported as non-capitalized letters ("a" > "b") only for significant average values among cultivars. All other average values were not significant. "*" indicates significant differences between the young and mature leaves of every single cultivar, and it is located close to the highest value of each pair.

Table 2
Two-way ANOVA *p* values of related to cultivars, leaf age, and interaction cultivar × leaf age.

Factor	Cultivar *p* value	Leaf age *p* value	Cultivar × Leaf age *p* value
Asp	0.1555	**0.0001**	**0.0286**
Glu	**0.0210**	**0.0361**	0.4410
Ser	0.2339	**0.0171**	0.3592
His	0.2754	0.1650	0.6031
Gly	0.0721	**0.0133**	0.3126
Ala + Arg	0.0662	**0.0017**	0.7915
Pro	0.0703	**0.0019**	0.8235
Tyr	0.2378	0.2659	0.8476
Val	**0.0269**	**0.0262**	0.1770
Met	0.6245	0.9100	0.8046
Cys	0.5252	0.1708	0.8777
Ile + Trp	0.2469	**0.0266**	0.7818
Leu	0.3166	0.0979	0.8749
Phe	0.8598	0.7192	0.3410
Lys	0.0691	**0.0138**	0.7272
Total	*0.1254*	***0.0057***	*0.7040*

Significant *p*-values are indicated in bold.

can be lacking in particular conditions (Manta-Vogli et al., 2020). These results are in agreement with the findings of Navruzova et al. (2016) for peach leaves, harvested in the Tajikistan and Ukraine.

Apart from essential amino acids, from Table 1, it was determined that peach leaves accumulated other amino acids such as glutamic acid, serine, glycine, alanine, arginine, proline, tyrosine, and cysteine, in concentrations higher than 5.0 mg g^{-1} DW and 3.5 mg g^{-1} DW in young and mature leaves, respectively. Peach leaves harvested in Ukraine accumulated glutamic acid, leucine, proline mainly, whereas cysteine was in trace concentrations (Navruzova et al., 2016).

Significant differences were found among the cultivars only in the case of glutamic acid and valine (Table 1). As regards glutamic acid, the highest concentrations were found in O'Henry, Flordastar, and Rubirich cultivars, while for valine Early Maycrest and Rubirich showed the highest concentrations.

The young leaves (106.48–142.18 mg g^{-1} DW) of the peach trees contained a higher concentration of total amino acids than the mature leaves (97.33–110.27 mg g^{-1} DW). Although it is not possible to make a wide bilbiographical comparison with botanical species close to *P. persica*, there are some studies that compare the amount of amino acids in young leaves and mature ones. No significant differences were found in the total amino acid content in *Sporobolus stapfianus* leaves (Martinelli et al., 2007), while young mustard leaves are richer in amino acids than mature leaves (Kim et al., 2014), cosistently with the results described in the present study.

Specifically, young leaves contained higher amounts of Ser (in Early Maycrest), Glu (in Sweet Cap), Ala + Arg (in Flordastar and Rubirich), Pro (in Flordastar and Rubirich), Val (in Flordastar), and Ile + Trp (in Early Maycrest) than mature leaves, while levels of Asp were higher in mature leaves (in O'Henry and Flordastar). Only Flordastar showed statistically significant differences in the two types of leaves though there were differences in amino acid summations (Table 1).

The two-way ANOVA applied to the data matrix, using cultivar and leaf age as factors, showed significant differences regarding leaf age (Table 2). In particular, these significant differences were found for Asp, Glu, Ser, Gly, Ala + Arg, Pro, Val, Ile + Trp, Lys, as well as for the total amount of amino acids. In all cases, the young leaves showed a concentration of amino acids higher than the mature leaves, while the only case in which the concentration of amino acids was at the highest in the

mature leaves concerned Asp. However, the concentrations of Asp were rather low and, consequently, the analytical error was more relevant.

The study of linear correlations showed interesting results (Table 3). In particular, Asp and Glu had very different behaviors than all the other amino acids. Aspartic acid had significantly negative correlations with most amino acids except Phe (r = 0.53, $p \leq 0.01$), while Glu did not show any significant linear correlation.

Most of the amino acids, i.e. Ser, His, Gly, Ala + Arg, Pro, Tyr, Val, Cys, Ile + Trp, Leu, and Lys showed highly significant positive correlations among them (in almost all cases $p \leq 0.001$), thus indicating their simultaneous presence in the samples. Conversely, Met and Phe represented two exceptions. The former showed significant positive correlations with some amino acids but of less magnitude ($p \leq 0.05$ and $p \leq 0.01$). However, the correlation between Met and Cys, the only other sulfur-containing amino acid, was highly significant (r = 0.60, $p \leq 0.001$). On the other hand, Phe displayed a significant positive correlation only with Met ($p \leq 0.01$), apart from the previously mentioned correlation with Asp.

Principal component analysis (PCA) was carried out on the autoscaled values to explore the data set and to gather information about the relationship among variables and the overall distribution of samples on score plots. The first three principal components (PCs), all with eigenvalues higher than 1.0, explained 87.35% of the total variance. All factors with eigenvalues lower than 1.0 were discarded, according to Kaiser's criterion (Kaiser, 1958).

The loading plot of PC1 (66.11% of the total variance) versus PC2 (13.62% of the total variance) showed that most of the amino acids (including Ser, His, Gly, Ala + Arg, Pro, Tyr, Val, Cys, Ile + Trp, Leu, and Lys) weighed on PC1 with a negative sign, while Met, Phe, and Asp weighed on PC2 with a positive value (Fig. 1a). The score plot of PC1 versus PC2 did not show high data repeatability between replicates, thus reflecting the fact that biological samples have different concentrations of metabolites (Fig. 1b). However, the separation between the young leaf samples in the left part of the score plot and the mature leaf samples in the right part was partially evident.

PC3 (7.63% of the total variance) showed only the relevant weight of Glu with a negative value (Fig. 2a). In the score plot of PC1 against PC3, the separation between the samples of young leaves on the left side and the mature ones on the right side of the graph emerged again (Fig. 2b).

All the statistical treatments applied to the dataset resulted in a coherent framework.

For most amino acids, the concentration in young leaves was higher than that in mature leaves. Indeed, the foliar development and the cellular enzymatic machinery necessary for photosynthesis are put into action (Gomez et al., 2020).

Only some amino acids, such as Asp and Glu (which accounted also for asparagine and glutamine), Met, and Phe deviated from this pattern. However, the differences between young and mature leaves were not so striking. Finally, the differences between the leaves' ages were far more evident than those observed among the cultivars.

4. Conclusion

The present study demonstrated that young and mature peach leaves accumulated amino acids in different ways. The leaf age heavily affected the synthesis of some amino acids. Ser, Gly, Ala + Arg, and Pro contents decreased when the leaves reached maturity. No significant variation for Tyr, Met, Cys, and Phe contents were observed throughout ripening.

The caducity of peach tree leaves means that they can be simply recovered by placing nets underneath the trees. Although the difference in amino acid concentrations in young and mature leaves is statistically evident, both types of leaves contain a discreet amount of amino acids which makes them a promising and easily available source of nitrogen. The satisfactory results obtained through this research lay the foundations for subsequent investigations, such as the evaluation of the amino acids' concentration near the falling of the leaves when the organ

Table 3
Significant correlation matrix of main parameters data set.

	ASP	GLU	SER	HYS	GLY	ALA + ARG	PRO	TYR	VAL	MET	CYS	ILE + TRP	LEU	PHE	LYS	Total
ASP																
GLU	−0.53**															
SER	−0.50**		0.61***													
HYS	−0.67***		0.69***	0.91***												
GLY	−0.69***		0.76***	0.82***	0.90***											
ALA + ARG	−0.62***		0.70***	0.83***	0.89***	0.92***										
PRO			0.69***	0.76***	0.81***	0.85***	0.81***									
TYR	−0.58**		0.57**	0.74***	0.82***	0.82***	0.82***	0.77***								
VAL								0.53**	0.38*							
MET	−0.41*		0.55**	0.75***	0.81***	0.81***	0.75***	0.87***	0.75***	0.60***						
CYS	−0.49**		0.73***	0.80***	0.84***	0.90***	0.92***	0.91***	0.83***	0.39*	0.79***					
ILE + TRP	−0.48*		0.71***	0.77***	0.86***	0.88***	0.81***	0.94***	0.78***	0.52**	0.94***	0.86***				
LEU	0.53**									0.50**						
PHE	−0.65***		0.77***	0.83***	0.93***	0.95***	0.91***	0.86***	0.83***		0.85***	0.91***	0.90***			
LYS	−0.56**		0.77***	0.88***	0.92***	0.96***	0.95***	0.89***	0.84***		0.84***	0.95***	0.90***		0.94***	
Total																

"*", "**", "***" indicates $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, respectively.

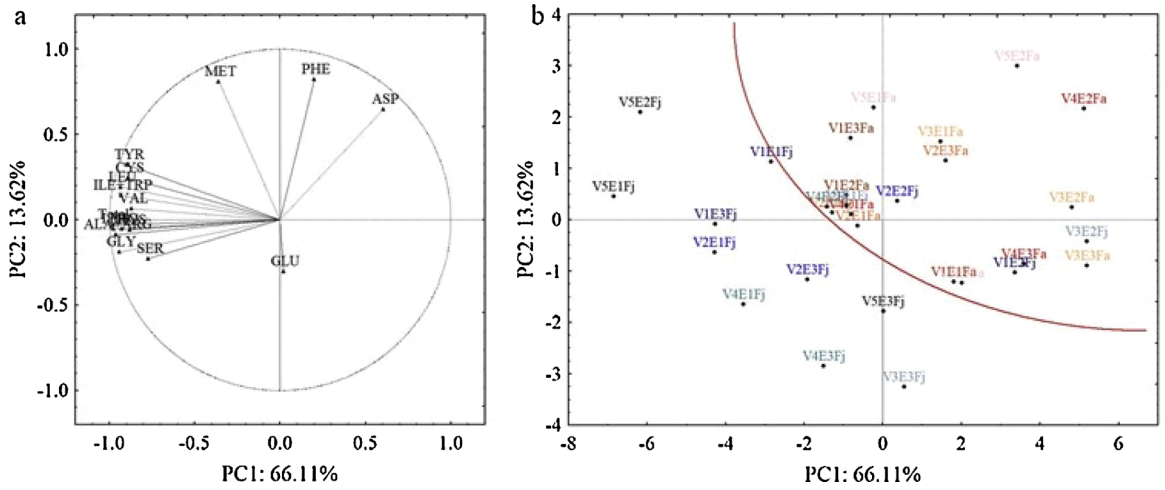

Fig. 1. (a) PC1 vs PC2 loading plots of the individual and total sum of amino acids determined in the sample set. (b) PC1 vs PC2 score plots of the samples.

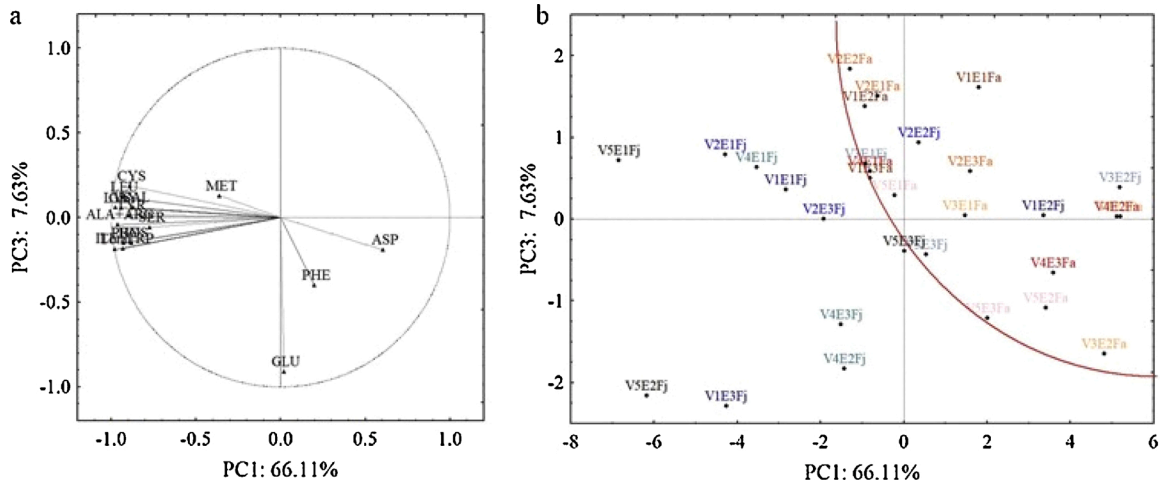

Fig. 2. (a) PC1 vs PC3 loading plots of the individual and total sum of amino acids determined in the sample set. (b) PC1 vs PC3 score plots of the samples. V1 = Sweet Cap; V2 = Early Maycrest; V3 = O'Henry; V4 = Flordastar; V5 = Rubirich. E1-E2-E3 = replications 1, 2, and 3. Fj = young leaves; Fa = mature leaves.

gradually loses its functions.

CRediT authorship contribution statement

Samia Dabbou: Conceptualization, Methodology, Writing - review & editing. **Samira Maatallah:** Resources, Visualization. **Andrea Antonelli:** Visualization, Funding acquisition. **Giuseppe Montevecchi:** Methodology, Formal analysis, Writing - review & editing.

Declaration of Competing Interest

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

References

Atanasova, E., 2008. Effect of nitrogen sources on the nitrogenous forms and accumulation of amino acid in head cabbage. Plant Soil Environ. 54, 66–71. https://doi.org/10.17221/438-pse.

Batista-Silva, W., Heinemann, B., Rugen, N., Nunes-Nesi, A., Araújo, W.L., Braun, H.P., Hildebrandt, T.M., 2019. The role of amino acid metabolism during abiotic stress release. Plant Cell Environ. 42, 1630–1644. https://doi.org/10.1111/pce.13518.

Bouhajeb, R., Selmi, S., Nakbi, A., Jlassi, I., Montevecchi, G., Flamini, G., Zarrad, I., Dabbou, S., 2020. Chemical composition analysis, antioxidant, and antibacterial activities of eggplant leaves. Chem. Biodivers. 17 https://doi.org/10.1002/cbdv.202000405.

Dabbou, S., Lussiana, C., Maatallah, S., Gasco, L., Hajlaoui, H., Flamini, G., 2016. Changes in biochemical compounds in flesh and peel from *Prunus persica* fruits grown in Tunisia during two maturation stages. Plant Physiol. Biochem. 100, 1–11. https://doi.org/10.1016/j.plaphy.2015.12.015.

Gomez, L., Vercambre, G., Jordan, M.O., 2020. Spatial-temporal management of nitrogen and carbon on the peach tree (*Prunus persicae* L. Batsch.). Sci. Hortic. (Amsterdam). 273, 109613. https://doi.org/10.1016/j.scienta.2020.109613.

Gómez-Alonso, S., Hermosín-Gutiérrez, I., García-Romero, E., 2007. Simultaneous HPLC analysis of biogenic amines, amino acids, and ammonium ion as aminoenone derivatives in wine and beer samples. J. Agric. Food Chem. 55, 608–613. https://doi.org/10.1021/jf062820m.

Guizani, M., Dabbou, S., Maatallah, S., Montevecchi, G., 2019. Physiological responses and fruit quality of four peach cultivars under sustained and cyclic deficit irrigation in center-west of Tunisia. Agric. Water Manag. 217, 81–97. https://doi.org/10.1016/j.agwat.2019.02.021.

Häusler, R.E., Ludewig, F., Krueger, S., 2014. Amino acids - A life between metabolism and signaling. Plant Sci. 229, 225–237. https://doi.org/10.1016/j.plantsci.2014.09.011.

Hildebrandt, T.M., Nunes Nesi, A., Araújo, W.L., Braun, H.P., 2015. Amino acid catabolism in pants. Mol. Plant 8, 1563–1579. https://doi.org/10.1016/j.molp.2015.09.005.

Kaiser, H.F., 1958. The varimax criterion for analytic rotation in factor analysis. Psychometrika 23, 187–200. https://doi.org/10.1007/BF02289233.

Kim, Y.B., Uddin, R., Lee, M.K., Kim, S.J., Kim, H.H., Chung, E., Lee, J.H., Park, S.U., 2014. Accumulation of free amino acids in different organs of green and red mustard cultivars. Asian J. Chem. 26, 396–398. https://doi.org/10.14233/ajchem.2014.15417.

Maatallah, S., Dabbou, S., Castagna, A., Guizani, M., Hajlaoui, H., Ranieri, A.M., Flamini, G., 2020. Prunus persica by-products: a source of minerals, phenols and

volatile compounds. Sci. Hortic. (Amsterdam). 261, 109016 https://doi.org/10.1016/j.scienta.2019.109016.

Manta-Vogli, P.D., Schulpis, K.H., Loukas, Y.L., Dotsikas, Y., 2020. Birth weight related essential, non-essential and conditionally essential amino acid blood concentrations in 12,000 breastfed full-term infants perinatally. Scand. J. Clin. Lab. Invest. 80, 571–579. https://doi.org/10.1080/00365513.2020.1818280.

Martinelli, T., Whittaker, A., Bochicchio, A., Vazzana, C., Suzuki, A., Masclaux-Daubresse, C., 2007. Amino acid pattern and glutamate metabolism during dehydration stress in the "resurrection" plant *Sporobolus stapfianus*: A comparison between desiccation-sensitive and desiccation-tolerant leaves. J. Exp. Bot. 58, 3037–3046. https://doi.org/10.1093/jxb/erm161.

Montevecchi, G., Antonelli, A., Masino, F., Vasile Simone, G., Gabriella Mellano, M., 2013. Fruit sensory characterization of four Pescabivona, white-fleshed peach [*Prunus persica* (L.) Batsch], landraces and correlation with physical and chemical parameters. Fruits 68, 195–207. https://doi.org/10.1051/fruits/2013067.

Montevecchi, G.G., Simone, M.M., Bignami, G.V., Antonelli, C., A, 2016. Physico-chemical and sensory characterization of Pescabivona P.G.I., A sicilian white flesh peach cultivar [*Prunus persica* (L.) Batsch]: A case study. In: Hansen, J. (Ed.), Apricots and Peaches: Nutritional Properties, Post-Harvest Management and Potential Health Benefits. Nova science Publishers, Hauppauge, N.Y., U.S.A, pp. 93–124.

Navruzova, H.F., Lenchyk, L.V., Kyslychenko, V.S., Sharyfov, K.S., Diakonova, Y.V., 2016. Comparative study of amino acids and mineral composition of *Prunus persica* leaves, harvested in Tajikistan and Ukraine. ФарМацевтичний часопис 1, 30–33. https://doi.org/10.11603/2312-0967.2016.1.6045.

Ohyama, T., Ohtake, N., Sueyoshi, K., Ono, Y., Tsutsumi, K., Ueno, M., Tanabata, S., Sato, T., Takahashi, Y., 2017. Amino acid metabolism and transport in soybean plants. Amino Acid - New Insights and Roles in Plant and Animal, pp. 171–196. https://doi.org/10.5772/intechopen.68992.

Peace, R.W., Gilani, G.S., 2005. Chromatographic determination of amino acids in foods. J. AOAC Int. 88, 877–887. https://doi.org/10.1093/jaoac/88.3.877.

Pratelli, R., Pilot, G., 2014. Regulation of amino acid metabolic enzymes and transporters in plants. J. Exp. Bot. 65, 5535–5556. https://doi.org/10.1093/jxb/eru320.

Rai, V.K., 2002. Role of amino acids in plant responses to stresses. Biol. Plant. 45, 481–487. https://doi.org/10.1023/A:1022308229759.