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Prediction of Fruit Free Amino Acids by Foliar Nutrient Diagnosis in Longan (*Dimocarpus longan* Lour.)

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Abstract. **Free amino acid (FAA) profile is an important indicator of the quality of fruit and fruit product. Foliar nutrient diagnosis has been used for crop yield prediction for decades but not for fruit quality evaluation. Concentrations of 11 leaf nutrients including N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, and B at stages of terminal shoot maturation and fruit development and fruit FAA profile at harvest were examined in longan in South China. The relation between leaf nutrient and fruit FAA was then investigated by multiple stepwise regression analysis. Foliar N content was greatest among the nutrients among the detected elements at both stages. Twenty-nine FAAs were determined in longan flesh, with alanine (19.9%), γ-aminobutyric acid (17.5%), glutamic acid (15.2%), and asparagine (10.7%) as the main components. Flesh individual FAA, essential amino acid (AA), umami-, and sweet and bitter taste AA strongly depended on foliar nutrients. However, the relation between flesh FAAs and foliar nutrients varied with FAA species. Leaf N was the dominant indicator for most pulp FAAs at two growth stages, while other nutrients (e.g., B, Zn, P, K, Ca, Mg) also played versatile roles on flesh FAAs. This work provides a novel tool to predict fruit FAAs via foliar nutrient diagnosis, which supports the practicality of producing specific target fruit or improving fruit quality through regulation of fertilization strategies in fruit production.**

FAAs, a type of biologically active compounds present in food and beverages, are important for human nutrition and health (Efeyan et al., 2015; Nagao and Yamakado, 2016), and affect the quality of foods including taste, aroma, and color (Kocadağlı et al., 2013). Enhancement of essential amino acids (EAAs) in horticultural plants has recently been emphasized because vegetables are one of the main contributors of EAA in the human diet (Wang et al., 2017). Therefore, enhancement of the FAA profile is important to evaluate the quality of fruit and fruit product (Khan et al., 2018; Silva et al., 2004).

Nutrients regulate plant processes involved in the formation of yield and quality, such as pollination, flowering, tuber initiation, and storage processes in the sink organs (Engels et al., 2012). It has been recognized for decades that mineral nutrients are dispensable to AA synthesis and protein production (Harper and Paulsen, 1969a, 1969b). In higher plant, N is assimilated mainly in mature leaf mesophyll cells and is then used for leaf protein synthesis or exported as AAs or AA precursors to sink tissues via the phloem (Femandes and Rossiello, 1995). The rate of AA export from leaf to phloem is dependent on N nutritional status and AA species per se (Caputo and Barneix, 1997). For example, N is used for synthesis of leaf protein when the supply is low and exported to the phloem when supply is ample, then accumulated in the storage pool when supply is greater than plant demand. Further, glutamic acid (Glu), superior to aspartic acid (Asp), is easily exported to phloem in conditions of low N supply. However, although some sink-related elements, such as genetic attributes and environmental factors, affect the biosynthesis of storage proteins, protein synthesis in storage organs such as seed and fruit is largely dependent on the supply of AAs or AA precursors being transported into the sink from the source in plant (Johansson et al., 2013; Zahedi et al., 2004).

Foliar nutrient diagnosis has been developed and used in crops for decades, targeting to assess plant nutritional status and adjust nutrient management to promote crop productivity (Amundson and Koehler, 1987; Rubio-Covarrubias et al., 2009). Foliar diagnosis is performed in perennial fruit crops such as citrus (Raveh, 2013), litchi (Luo et al., 2019), and others. However, advance assessment of fruit quality by leaf nutrition diagnosis has not been fully studied to date. Field experiments show that AA accumulation in reproductive sink is strongly dependent on leaf N and AA levels (Barneix and Guitman, 1993; Blumenthal et al., 1990; Kano et al., 1999). Therefore, it may be possible to predict FAA composition in fruit by foliar nutrient diagnosis before fruit maturation in fruit crops.

Longan (*Dimocarpus longan* Lour.), a widely cultivated *Sapindaceae* fruit in the tropics, is highly appreciated for its succulent, sweet, and unique taste and health-related nutrients (Wall, 2006). In this work, we investigated the relationship between leaf nutrient and fruit FAA profile in longan, with the objective to evaluate fruit quality before fruit maturation via leaf nutrient diagnosis.

Materials and Methods

Longan tree studied. Five commercial longan orchards (lat. 21°35′–22°15′N and long. 109°27′–110°54′E) located in Guangdong and Guangxi province, southern China, were included in this study. Nine or 10 trees were selected in each orchard. In total, 49 longan trees, aged 20 to 25 years and with a plantation density of 180 to 330 plants per hectare, were used from these five orchards.

The longan variety was Chuliang, a typical main longan cultivar in South China. Generally, Chuliang longan is harvested from early July to mid-August in South China, and shoots twice after harvest, followed by dormancy during November to December. In the spring, the floral bud differentiates and then blossoms in late March to early May, followed by fruit development from late March to August.

Sample collection. Usually, the most appropriate leaf for nutrient diagnosis is newly mature and fully developed because it can reflect the true nutritional state of the plant without being affected by nutrient redistribution in plant tissues. Additionally, leaf diagnosis is performed when the plant is at peak physiological activity, such as during flowering or beginning of fruiting (Pradoa and Rozane, 2019). Therefore, foliar nutrient diagnosis is commonly undertaken at two growth stages for longan. The first diagnosis is carried out when the terminal shoot (the bearing shoot) is newly mature, and the second is performed at the initiation of fruit fast bulking owing to the key nutrient requirement for fruit development at this stage.

Forty-nine leaf samples, one sample each per tree, were gathered while the terminal shoot was newly mature from late November to mid-December in 2018, and another batch of 49 leaf samples at fruit bulking stage from early May to early June in 2019 in the five orchards. While sampling, the second pair of leaflet from the second compound leaf in the terminal shoot from different directions were picked up from each tree, and ≈40 to 50 leaves comprised one leaf sample. Forty-nine fruit samples were collected when the fruits grew with similar maturity from mid-July to early August. Approximately 2 to 3 kg of fruit were randomly collected up from one tree

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Table 1. Leaf nutrient concentrations at different growth stages during 2018–19 in longan (dry weight, n = 49).

	Terminal shoot maturing stage (2018)			Fruit bulking stage (2019)				
Nutrient	Range	Mean	CV (%)	Range	Mean	CV (%)	Significance[z]	
N (g·kg^{-1})	19.8–26.0	22.8 ± 1.5	6.7	18.7–25.3	21.9 ± 1.5	6.7	t = 2.621	F = 6.870*
P (g·kg^{-1})	1.21–2.10	1.60 ± 0.22	13.9	1.13–2.06	1.53 ± 0.21	13.8	t = 1.779	F = 3.164
K (g·kg^{-1})	5.3–11.9	9.7 ± 1.0	10.8	5.6–13.2	8.5 ± 1.5	17.2	Z = –4.978	$P < 0.001$
Ca (g·kg^{-1})	4.7–13.5	7.5 ± 1.8	24.1	5.6–26.9	14.8 ± 5.3	36.0	Z = –7.212	$P < 0.001$
Mg (g·kg^{-1})	0.40–1.63	1.05 ± 0.23	21.8	0.55–2.35	1.37 ± 0.46	33.4	Z = –3.596	$P < 0.001$
S (g·kg^{-1})	1.19–1.84	1.43 ± 0.14	10.1	1.27–2.29	1.65 ± 0.19	11.5	t = –7.254	F = 52.625***
Fe (mg·kg^{-1})	23.8–55.4	36.0 ± 7.4	20.4	27.3–81.4	44.8 ± 11.2	25.1	Z = –4.587	$P < 0.001$
Mn (mg·kg^{-1})	8.1–50.5	17.7 ± 9.0	50.9	10.5–81.0	35.1 ± 18.7	53.2	Z = –4.324	$P < 0.001$
Cu (mg·kg^{-1})	4.1–10.6	6.4 ± 1.3	20.4	3.4–9.0	5.7 ± 1.4	24.0	t = 2.787	F = 7.768**
Zn (mg·kg^{-1})	11.4–29.7	18.6 ± 4.6	24.5	13.9–45.5	28.1 ± 7.4	26.5	Z = –6.388	$P < 0.001$
B (mg·kg^{-1})	12.6–37.8	24.0 ± 6.1	25.6	13.7–42.3	26.0 ± 5.7	21.8	t = –1.966	F = 3.866

[z]Data were performed with the *t* test when the variance of two groups of foliar nutrient contents from two growth stages was equal, whereas the nonparametric test was used if the variance was unequal.
*, **, ***Significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2. Free amino acid profile in longan fruit (fresh weight, n = 49).

Free amino acid	Necessity	Taste	Range (mg·kg^{-1})	Mean (mg·kg^{-1})	CV (%)	Composition (%)
P-Ser			19.1–40.5	30.9	15.5	0.6
Tau			1.2–3.9	2.3	24.5	0.0
PEA			2.8–11.5	4.8	39.0	0.1
Asp		Umami	126.8–880.5	457.9	39.5	8.2
Thr	Essential	Sweet	27.2–75.8	48.0	18.1	0.9
Ser		Sweet	102.4–390.3	228.9	23.5	4.1
Asn			274.9–1566.7	598.6	35.4	10.7
Glu		Umami	525.1–1546.8	846.6	28.8	15.2
Gln			107.9–647.3	366.6	33.6	6.6
Sar			23.4–55.1	39.8	20.0	0.7
α-AAA			8.4–98.5	28.4	65.6	0.5
Gly		Sweet	28.8–83.2	59.8	18.8	1.1
Ala		Sweet	336.7–1899.5	1110.1	31.0	19.9
α-ABA			6.1–18.9	12.3	19.1	0.2
Val	Essential	Bitter	32.7–81.8	55.4	19.2	1.0
Cys			2.0–15.4	9.1	39.9	0.2
Met	Essential	Bitter	1.4–26.8	6.9	74.9	0.1
Ile	Essential	Bitter	25.5–97.3	34.4	33.2	0.6
Leu	Essential	Bitter	35.0–83.1	56.9	16.9	1.0
Tyr			12.5–34.8	22.4	23.9	0.4
Phe	Essential	Bitter	1.4–26.8	6.6	68.4	0.1
β-Ala			38.9–63.5	48.4	9.4	0.9
β-AiBA			1.2–5.5	2.4	36.5	0.0
γ-ABA			395.7–1273.9	976.1	20.1	17.5
Lys	Essential	Sweet, bitter	9.4–35.3	21.2	25.2	0.4
Mehis			5.3–27.4	12.0	41.7	0.2
His	Essential	Bitter	23.2–77.7	56.1	20.0	1.0
Arg		Bitter	34.7–721.1	303.5	56.5	5.4
Pro		Sweet, bitter	52.3–264.7	125.4	43.0	2.3
Σ essential			169.0–444.4	285.5	18.0	5.1
Σ umami			976.6–1673.5	1304.5	14.3	23.9
Σ sweet			568.3–2473.0	1593.4	24.4	28.3
Σ bitter			240.2–1098.8	666.4	29.1	11.7
Total			3083.0–7595.1	5571.7	16.9	100.0

Ala = alanine; Arg = arginine; Asn = asparagine; Asp = aspartic acid; Cys = cystine; Gln = glutamine; Glu = glutamic acid; Gly = glycine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Mehis = methylhistidine; Met = methionine; PEA = phosphoethanolamine; Phe = phenylalanine; Pro = proline; P-Ser = phospho serine; Sar = sarcosine; Ser = serine; Tau = taurine; Thr = threonine; Tyr = tyrosine; Val = valine; α-AAA = α-aminoadipic acid; α-ABA = α- amino-n-butyric acid; β-AiBA = β-aminoisobutyric acid; β-Ala = β-alanine; γ-ABA = γ-aminobutyric acid.

and combined as one fruit sample. All the leaf and fruit samples were immediately delivered to the laboratory.

Sample preparation and analysis. The leaves were rinsed with deionized water in the laboratory, then deactivated the enzymes at 120 °C for 20 min and further dried to the constant weight at 70 °C in the oven. The oven-dried leaf samples were ground into fine powders by a stainless pulverator for mineral nutrient determination. Leaf N was digested with concentrated sulfate and hydrogen peroxide and then detected using continuous flow-injection analyzer (Skalar San++, Netherlands). Leaf phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), and boron (B) were extracted with concentrated nitric acid and perchloric acid and determined by inductively coupled plasma-optical emission spectroscopy (Agilent Varian 710-ES, Malaysia). The reference material GBW07603, purchased from the National Standard Material Center of China, was used to guarantee the analysis quality.

The fruit was rinsed with deionized water, and then the peel and seed were removed. All the pulp from each fruit sample was mixed into a slurry using an agitator. The slurry was centrifuged at 4000 g_n for 15 min (Eppendorf Centrifuge 580R; Germany), and the supernatant was filtered through 0.45-μm film. One milliliter of the filtrate was diluted by 10 times with ultrapure water (Unique-R20; Xiamen, China), and 1 mL of the solution was mixed with 1 mL 15% salicylsulfonic acid and then placed for 1 h, followed by centrifugation at 14,000 g_n (4 °C) for 10 min. The supernatant was filtered by 0.22-μm film and then injected into an automatic amino acid analyzer immediately.

Table 3. Relation between fruit free amino acids (Y) and foliar nutrients at terminal shoot maturing stage in longan.

Y	Model	*P* value of variable	Partial R^2 of variable
P-Ser	Y = 0.909N + 10.988Mg – 50.526Mn	N: <0.0001	0.978
		Mg: 0.0006	0.004
		Mn: 0.0272	0.002
Tau	Y = 0.087N + 0.178K – 224.720Cu	N: 0.0038	0.947
		K: 0.0335	0.004
		Cu: 0.0007	0.007
PEA	Y = 5.132Mg – 22.794Mn	Mg: <0.0001	0.882
		Mn: 0.0182	0.013
Asp	Y = 9750.817Zn + 11816B	Zn: 0.0224	0.010
		B: 0.0010	0.903
Thr	Y = 2.10180N	N: <0.0001	0.968
Ser	Y = 5.814N + 90.272Mg	N: 0.0018	0.949
		Mg: 0.0182	0.006
Asn	Y = 26.304N	N: <0.0001	0.895
Glu	Y = 52.355N + 65.057Ca – 35685B	N: <0.0001	0.928
		Ca: 0.0061	0.008
		B: <0.0001	0.018
Gln	Y = 33.344K + 1336.022Mn	K: <0.0001	0.909
		Mn: 0.0405	0.008
Sar	Y = 2.572K + 631.355B	K: <0.0001	0.962
		B: <0.0001	0.010
α-AAA	Y = 4324.709Cu	Cu: <0.0001	0.716
Gly	Y = 3.841N – 19.536S	N: <0.0001	0.974
		S: 0.0401	0.002
Ala	Y = 69.388N – 203B	N: <0.0001	0.914
		B: 0.0151	0.010
α-ABA	Y = 0.322N + 4.618Mg	N: <0.0001	0.967
		Mg: 0.0038	0.005
Val	Y = 1.816N + 731.543Zn	N: <0.0001	0.963
		Zn: 0.0323	0.003
Cys	Y = 195.345Fe + 66.522Mn	Fe: <0.0001	0.008
		Mn: 0.0003	0.022
Met	Y = 4.82700S	S: <0.0001	0.646
Ile	Y = 1.501N	N: <0.0001	0.897
Leu	Y = 1.770N + 867.617Zn	N: <0.0001	0.972
		Zn: 0.0036	0.005
Tyr	Y = 0.984N	N: <0.0001	0.947
Phe	Y = 0.290N	N: <0.0001	0.684
β-Ala	Y = 2.128N	N: <0.0001	0.992
β-AiBA	Y = 0.103N	N: <0.0001	0.880
γ-ABA	Y = 55.511K + 18620B	N: <0.0001	0.959
		B: <0.0001	0.010
Lys	Y = 0.646N + 345.670Zn	N: <0.0001	0.950
		Zn: 0.0258	0.005
Mehis	Y = 1.229K	K: <0.0001	0.864
His	Y = 2.459N	N: <0.0001	0.962
Arg	Y = 186.532P	P: <0.0001	0.773
Pro	Y = 5366.849B	B: <0.0001	0.890
Σ EAA	Y = 12.508N	N: <0.0001	0.968
Σ umami	Y = 69.891N + 261.408Mg – 400.958S	N: <0.0001	0.983
		Mg: 0.0253	0.002
		S: 0.0115	0.002
Σ sweet	X = 69.904N	N: <0.0001	0.946
Σ bitter	Y = 407.307P	P: <0.0001	0.926
Σ total	Y = 244.287N	N: <0.0001	0.973

Ala = alanine; Arg = arginine; Asn = asparagine; Asp = aspartic acid; Cys = cystine; Gln = glutamine; Glu = glutamic acid; Gly = glycine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Mehis = methylhistidine; Met = methionine; PEA = phosphoethanolamine; Phe = phenylalanine; Pro = proline; P-Ser = phospho serine; Sar = sarcosine; Ser = serine; Tau = taurine; Thr = threonine; Tyr = tyrosine; Val = valine; α-AAA = α-aminoadipic acid; α-ABA = α- amino-n-butyric acid; β-AiBA = β-aminoisobutyric acid; β-Ala = β-alanine; γ-ABA = γ-aminobutyric acid.

The amino acid analyzer (L-8900; Hitachi, Japan) equipped with a chromatographic column (855-4507; Hitachi), operated with a pH-dependent lithium buffer gradient elution procedure at the column temperature of 135 °C under the waves of 570 and 440 nm within 148 min. The buffer solution flew at 0.35 and 0.30 mL·min^{-1} in the elution pump and the derivatization pump, respectively.

Standards and reagents. Chromatographically pure amino acid standard stock solution type PH (Sykam, Germany) containing 1.0 μmol·mL^{-1} ± 2% of each AA was used. Ninhydrin solution and its buffer solution were purchased from Wako Pure Chemical Industries, Ltd. (Japan), and all the other buffer solutions from Mitsubishi Chemical Co. (Japan). Analytical-grade salicylsulfonic acid (99%) was produced by Guangzhou Chemical Reagent Factory (China).

Data analysis and statistics. Data were expressed as mean ± SD. The essential AAs includes isoleucine (Ile), valine (Val), phenylalanine (Phe), leucine (Leu), threonine (Thr), lysine (Lys), histidine (His), and methionine (Met). The umami-AA (the sum of Asp and Glu), sweet-AA [the sum of Thr, serine (Ser), glycine (Gly), alanine (Ala), Lys, and proline (Pro)] and bitter-taste AA [the sum of Val, Met, Ile, Leu, Phe, Lys, arginine (Arg), and Pro] were classified as the method reported by Kato et al. (1989). Multiple stepwise regression analysis was performed between foliar nutrient and pulp FAA by SAS 9.2. The *t* test was conducted if the variance of two groups of foliar nutrient contents from two growth stages was equal, whereas the nonparametric test was performed if the variance was unequal.

Table 4. Relation between fruit free amino acids (Y) and foliar nutrients at fruit bulking stage in longan.

Y	Model	*P* value of variable	Partial R^2 of variable
P-Ser	Y = 1.281N + 7.359Mg – 285.086B	N: <0.0001	0.976
		Mg: <0.0001	0.011
		B: 0.0026	0.002
Tau	Y = 0.136N – 133.572Cu	N: <0.0001	0.949
		Cu: 0.0095	0.007
PEA	Y = 0.314N – 2.924P + 1.649Mg	N: 0.0005	0.016
		P: 0.0058	0.015
		Mg: 0.0010	0.889
Asp	Y = 35.106N – 329.211S + 8182.410Zn	N: 0.0002	0.882
		S: 0.0061	0.012
		Zn: 0.0116	0.014
Thr	Y = 1.364N + 627.875Zn	N: <0.0001	0.966
		Zn: 0.0002	0.009
Ser	Y = 4.710N + 3.601Ca + 2471.357Zn	N: 0.0006	0.945
		Ca: 0.0244	0.013
		Zn: 0.0250	0.004
Asn	Y = 38.338N – 44015Cu	N: <0.0001	0.882
		Cu: 0.0427	0.010
Glu	Y = 507.424P + 387.517Mg – 18003B	P: <0.0001	0.005
		Mg: <0.0001	0.022
		B: 0.0024	0.004
Gln	Y = –67.291Mg + 8206.757Zn + 8743.961B	Mg: 0.0181	0.006
		Zn: <0.0001	0.013
		B: <0.0001	0.934
Sar	Y = 1.020N – 0.586Ca – 10.301Mg + 364.958Zn + 465.564B	N: <0.0001	0.964
		Ca: 0.0443	0.002
		Mg: 0.0007	0.004
		Zn: 0.0168	0.003
		B: 0.0093	0.007
α-AAA	Y = 3.364K	K: <0.0001	0.721
Gly	Y = 1.646N + 0.745Ca + 278.982Fe	N: <0.0001	0.971
		Ca: 0.0081	0.005
		Fe: 0.0353	0.002
Ala	Y = 250.036Mg + 465.493S	Mg: 0.0114	0.011
		S: <0.0001	0.914
α-ABA	Y = 1.881Mg + 5.881S – 486.141Cu + 93.743Zn	Mg: 0.0071	0.013
		S: <0.0001	0.001
		Cu: 0.0484	0.002
		Zn: 0.0212	0.003
Val	Y = 1.537N + 0.652Ca + 413.588Zn	N: <0.0001	0.968
		Ca: 0.0006	0.007
		Zn: 0.0464	0.002
Cys	Y = 354.215B	B: <0.0001	0.905
Met	Y = 232.247Zn	Zn: <0.0001	0.632
Ile	Y = 1.550N	N: <0.0001	0.893
Leu	Y = 1.665N + 0.620Ca + 382.018Zn	N: <0.0001	0.975
		Ca: 0.0201	0.006
		Zn: 0.0369	0.002
Tyr	Y = 0.690N + 253.772Zn	N: <0.0001	0.944
		Zn: 0.0183	0.006
Phe	Y = 0.412Ca	Ca: <0.0001	0.684
β-Ala	Y = 1.869N + 5.169Mg	N: <0.0001	0.989
		Mg: 0.0007	0.002
β-AiBA	Y = 0.054Ca + 52.494Zn	Ca: 0.0357	0.010
		Zn: 0.0004	0.895
γ-ABA	Y = 37.066N – 154.102Mg + 13153Zn	N: <0.0001	0.965
		Mg: 0.0071	0.004
		Zn: 0.0005	0.005
Lys	Y = 0.860N – 1231.460Cu + 326.716Zn	N: <0.0001	0.950
		Cu: 0.0025	0.007
		Zn: <0.0001	0.014
Mehis	Y = 3.636S + 210.284Zn	S: 0.0347	0.014
		Zn: 0.0303	0.853
His	Y = 1.616N + 722.412Zn	N: <0.0001	0.962
		Zn: 0.0006	0.009
Arg	Y = 10749Zn	Zn: <0.0001	0.818
Pro	Y = 2766.076Fe	Fe: <0.0001	0.858
Σ EAA	Y = 7.900N + 3.345Ca + 2127.427Zn	N: <0.0001	0.968
		Ca: 0.0282	0.007
		Zn: 0.0421	0.002
Σ umami	Y = 44.402N + 238.164Mg	N: <0.0001	0.980
		Mg: <0.0001	0.007
Σ sweet	Y = 32.709Ca + 663.152S	Ca: 0.0013	0.012
		S: <0.0001	0.941

(*Continued on next page*)

Table 4. (*Continued*) Relation between fruit free amino acids (Y) and foliar nutrients at fruit bulking stage in longan.

Y	Model	*P* value of variable	Partial R^2 of variable
Σ bitter	Y = 10.681N + 15201Zn	N: 0.0160	0.007
		Zn: <0.0001	0.940
Σ total	Y = 163.666N + 69194Zn	N: <0.0001	0.970
		Zn: 0.0002	0.008

Ala = alanine; Arg = arginine; Asn = asparagine; Asp = aspartic acid; Cys = cystine; Gln = glutamine; Glu = glutamic acid; Gly = glycine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Mehis = methylhistidine; Met = methionine; PEA = phosphoethanolamine; Phe = phenylalanine; Pro = proline; P-Ser = phospho serine; Sar = sarcosine; Ser = serine; Tau = taurine; Thr = threonine; Tyr = tyrosine; Val = valine; α-AAA = α-aminoadipic acid; α-ABA = α- amino-n-butyric acid; β-AiBA = β-aminoisobutyric acid; β-Ala = β-alanine; γ-ABA = γ-aminobutyric acid.

Results and Discussion

Nutrient content in longan leaf. Nitrogen was the upmost macronutrient in longan leaf, ranging from 19.8 to 26.0 and 18.7 to 25.3 g·kg^{-1} with the mean concentrations of 22.8 and 21.9 g·kg^{-1} at stages of mature terminal shoot and fruit bulking, respectively (Table 1). Leaf K concentration (9.7 g·kg^{-1}) was 2-fold lower than N level, followed by Ca value (7.5 g·kg^{-1}) in matured terminal shoot, with similar low concentrations of P (1.60 g·kg^{-1}), S (1.43 g·kg^{-1}), and Mg (1.05 g·kg^{-1}). Fe (36.0 g·kg^{-1}) was the highest micronutrient, followed by B (24.0 g·kg^{-1}) and similar levels of Zn (18.6·kg^{-1}) and Mn (17.7 g·kg^{-1}), whereas Cu (6.4 mg·kg^{-1}) was the minimum detected element in matured leaf.

Foliar Ca, K, S, P, and Mg were 14.8, 8.5, 1.65, 1.53, and 1.37 g·kg^{-1} at fruit bulking stage, respectively. For foliar micronutrients, they were detected with descending order as Fe (44.8 mg·kg^{-1}), Mn (35.1 mg·kg^{-1}), Zn (28.1 mg·kg^{-1}), B (26.0 mg·kg^{-1}), and Cu (5.7 mg·kg^{-1}).

Foliar Mn differed with greater coefficients of variance (50.9% to 53.2%) in both stages, and foliar P, K, Ca, Mg, S, Fe, Cu, Zn, and B altered with moderate variation (CV 10.1% to 36.0%), whereas foliar N showed minimum variance (6.7%).

Additionally, significant differences for leaf N, K, Ca, Mg, S, Fe, Mn, Cu, and Zn were observed between both stages of terminal shoot maturing and fruit bulking, which confirms the necessity of foliar diagnosis based on growth stages in woody fruit crop.

FAA profile in longan pulp. Twenty-nine FAAs were determined in longan fruit (Table 2), similar to 28 FAAs detectable in dried longan pulp (Khan et al., 2018). However, the major FAA components included Ala (19.9%), γ-aminobutyric acid (γ-ABA, 17.5%), Glu (15.2%), and asparagine (Asn, 10.7%) in fresh longan pulp, which were different from those in dried longan pulp (Phe 29.7%, Ala 18.0%, Glu 9.7%, and Asp 8.0%). The variation of FAA profile between longan flesh and the dried product is ascribed to the following: 1) whereas total FAAs significantly decreased, all the individual FAAs decreased to greatly varying extents in longan pulp during drying and heating because of the Maillard reaction (Peng et al., 2020; Wen et al., 2015); and 2) FAA composition varied with different drying and heating techniques (Peng et al., 2020; Wen et al., 2015).

Specifically, γ-ABA is well known for its diverse biological functions, such as anxiety inhibition, antidepression, protection against liver injury, sleep promotion, blood pressure reduction, diabetes treatment, and immune enhancement in mammals (Diana et al., 2014; Nikmaram et al., 2017; Shabel et al., 2014). In our study, longan pulp contained 395.7 to 1273.9 mg·kg^{-1} of γ-ABA and averaged 976.1 mg·kg^{-1}, similar to litchi (Yang et al., 2014), whereas much higher than those in fresh or processed pulses (Nikmaram et al., 2017), germinated brown rice (Komatsuzaki et al., 2007), and more than one-half of the reported fermented foods (Xiang et al., 2019). It implies that longan flesh is a γ-ABA–abundant food and expected to be an excellent γ-ABA supplier for human. Although traditionally regarded as an umamic-taste AA, Glu is the largest contributor to the energy production of intestinal tract and one of the important AAs related to the growth and metabolism of intestinal mucosa (Fan et al., 2004). It attenuates the inflammatory bowel disease via antioxidative, antiapoptotic, and anti-inflammatory factors (Li et al., 2014; Lovasz et al., 2013) and mitigates the chronic visceral hypersensitivity by stimulation of cerebellum fastigial nucleus (Zhen et al., 2018). Dietary Glu supplementation is of functional and nutritional importance in intestinal mucosal growth and gut inflammation.

The total FAA concentrations varied from 3083.0 to 7595.1 mg·kg^{-1} and averaged 5571.7 mg·kg^{-1} in longan pulp (Table 2), similar to values reported previously (Zeng et al., 2015). Among them, eight EAAs, including Thr, Val, Met, Ile, Leu, Phe, Lys, and His, were detected with the total contents of 285.5 mg·kg^{-1}, accounting for 5.1% of the total FAAs. The umami-taste AAs (Asp, Glu) differed from 976.6 to 1673.5 mg·kg^{-1} and averaged 1304.5 mg·kg^{-1}. The sweet-taste AAs (Thr, Ser, Gly, Ala, Lys, Pro) varied within the range of 568.3 to 2473.0 mg·kg^{-1} with the mean of 1593.4 mg·kg^{-1}. The bitter-taste AAs (Val, Met, Ile, Leu, Phe, Lys, Arg, Pro) changed from 240.2 to 1098.8 mg·kg^{-1}, with the mean of 666.4 mg·kg^{-1}. The percent of umami-, sweet-, and bitter-taste AAs over total FAA was 23.9%, 28.3%, and 11.7%, respectively, indicating delicious flavor of longan fruit.

Relation between pulp FAA and foliar nutrient. Multiple regression analysis shows that pulp Thr, Asn, Ile, tyrosine (Tyr), Phe, β-alanine (β-Ala), β-aminoisobutyric acid (β-AiBA), and His were solely dependent on foliar N at the terminal shoot maturing stage based on the relation between foliar nutrients and fruit FAAs as presented in Table 3. Meanwhile, flesh phospho serine (P-Ser), taurine (Tau), Ser, Glu, Gly, Ala, α-aminoadipic acid (α-ABA), Val, Leu, and Lys were dominated by foliar N, and other nutrients, such as foliar B, Zn, Mg, K, Ca, and Mn had minor effect at this stage as well. Arg was solely dependent on foliar P. Glutamine (Gln), sarcosine (Sar), γ-ABA, and methylhistidine (Mehis) were dominated by foliar K, and foliar Mn and B were slightly affected by Gln and Sar. Foliar Mg-dependent phosphoethanolamine (PEA), foliar B-dependent Asp, foliar Cu-dependent α-aminoadipic acid (α-AAA), foliar S-dependent Met, and foliar B-dependent Pro were observed as well (Table 3). The essential AA, sweet-taste AA, and total FAA were solely determined by foliar N, whereas the umami-taste AA was primarily dependent on foliar N, and foliar Mg had positive effect and foliar S had negative influence. The bitter-taste AA was solely related to foliar P.

In contrast, pulp P-Ser, Tau, Asp, Thr, Ser, Asn, Sar, Gly, Val, Ile, Leu, Tyr, β-Ala, γ-ABA, Lys, and His were predominately affected by foliar N at the fruit bulking stage (Table 4). Some of the individual FAAs were also slightly influenced by foliar Mg, Zn, Ca, or B. Strong dependence between pulp PEA vs. foliar Mg, pulp Gln vs. foliar B, pulp α-AAA vs. foliar K, pulp Ala vs. foliar S, pulp cystine (Cys) vs. foliar B, pulp Met vs. foliar Zn, pulp Phe vs. foliar Ca, pulp β-AiBA vs. foliar Zn, pulp Mehis and Arg vs. foliar Zn, pulp Pro vs. foliar Fe were observed as well at this stage. Although well-fitting models were calculated for pulp Cys and foliar Mn and Fe in mature terminal shoot, and for pulp Glu and foliar Mg, P and B and for pulp α-ABA and foliar Mg, Cu, and S at the fruit bulking stage, the variance of the foliar nutrients accounted for <3.0% of the total variation of these three AAs, indicating that pulp Cys, Glu, and α-ABA were not well predicted by foliar nutrients at specific growth stages. The ΣEAA was jointly affected by foliar N, Ca, and Zn, whereas umami-taste AAs was governed by foliar N and Mg, sweet-taste AAs was regulated by foliar Ca and S, and bitter-taste AAs and total FAA were jointly dominated by foliar N and Zn while fruit was bulking.

The strong dependence of many individual AAs on foliar N either at terminal shoot maturing stage or at fruit developing stage in this work, further highlights the importance of N nutrition on AA synthesis in reproductive organs, as reported in previous studies (Barneix and Guitman, 1993; Blumenthal et al., 1990). However, many other nutrients, such as P, K, Mg, and S, modify FAA accumulation by regulation of N assimilation, AA phloem exudation or ethylene production (Kaack and Pedersen, 2014; Ruan et al., 1998; Veliz et al., 2017), leading to varied FAA profiles in plants. Meanwhile, the response of individual pulp AA to foliar nutrients differs as well. For example, application of N or N and Mg increased the concentrations of theanine, Gln, Arg, and Asn; however, other AAs, such as Ile, Leu, Asp, and Glu, in the xylem saps were unaffected by either N or Mg in tea leaf (Ruan et al., 2012). These investigations are helpful to explain the response difference of individual pulp AA to foliar nutrients in longan.

When phenology was considered, P-Ser, Tau, Thr, Ser, Asn, Gly, Val, Ile, Leu, Tyr, β-Ala, Lys, His, EAA, umami taste, and total FAA were primarily regulated by foliar N, and PEA was primarily determined by foliar Mg at both stages. However, Asp, Glu, Gln, Sar, α-AAA, Ala, Met, Phe, β-AiBA, γ-ABA, Mehis, Arg, Pro, and sweet- and bitter-taste AAs were influenced by various foliar nutrients at different growth stages. This may be partially linked to the different mobility of inorganic nutrients in both xylem and phloem (Epstein and Bloom, 2005). For example, K, N, Mg, P, B, and S are more mobile than Fe, Mn, Zn, and Cu, which are intermediate, and Ca and B, which are immobile in the phloem. Further, foliar nutrient concentrations vary annually with various patterns at different growth stages (Yang et al., 2015), which might alter the relationship between leaf nutrients and flesh AA at different phenophases. For example, γ-ABA is a temporary nitrogen store (Nikmaram et al., 2017). Glu and γ-ABA are produced during protein storage and mobilization as a means of recycling Arg-derived N and carbon (C) (Micallef and Shelp, 1989), thus, glutamic acid metabolism via γ-ABA transport is considerably important in the N cycle of plants (Shelp et al., 1999). This may explain why pulp γ-ABA was dominated by leaf N at fruit bulking stage, rather than at the terminal shoot maturing stage in longan.

Flesh AA level can be evaluated early in longan by foliar nutrient diagnosis, indicating that nutrient management in crop can be adopted to manipulate fruit FAA accumulation and generate target fruit taste or abundance in some functional AAs such as γ-ABA in practice. Theoretically, supplement of K and B for the terminal shoot and ample N and Zn nutrients for fruit development would promote γ-ABA accumulation in longan. Field experiments have shown that leaf N concentration increases with increasing N fertilizer dose in longan (Khaosumain et al., 2013), and advanced fertilization improves FAA profile in fruits of peach and mandarin orange (Jordan et al., 2012; Zhang et al., 2012). Further, the joint effect of N and other nutrients such as P, K, Ca, Mg, S, and Zn on pulp FAA highlights the importance of balanced fertilization of macronutrients, secondary nutrients, and micronutrients in longan.

Conclusion

Among the 11 foliar nutrients detected, N had the highest nutrient concentration at both growth stages in longan leaf. Twenty-nine FAAs were determined in longan pulp, and Ala, γ-ABA, Glu, and Asn were the primary components in the longan FAA profile. High dependency was calculated between individual FAA, EAAs, and taste AA vs. foliar nutrients, but the relation patterns between them differed among AAs. Most of the pulp FAAs were dominantly predicted by leaf N, and leaf B, Zn, P, K, Ca, Mg, and others played roles on pulp FAAs as well. However, some FAAs were solely dependent on specific foliar nutrients. Foliar nutrients are reliable indicators for preharvest evaluation of longan flesh FAAs, highlighting the feasibility of yielding specific FAA-target fruit or improving fruit quality through nutrient management during fruit production.

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