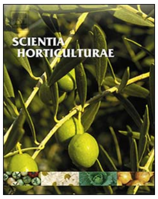
Scientia Horticulturae 225 (2017) 795–801

Contents lists available at ScienceDirect

Scientia Horticulturae

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



Genome-wide association analysis of free glutamate content, a key factor conferring umami taste in the bottle gourd [*Lagenaria siceraria* (Mol.) *Standl.*]

Xinyi Wu, Pei Xu, Xiaohua Wu, Baogen Wang, Zhongfu Lu, Guojing Li*

Institute of Vegetables, State Key Lab, Breeding Base for Sustainable Control of Plant Pest and Disease, Zhejiang Academy of Agricultural Sciences, Hangzhou, 310021, PR China

ARTICLE INFO

Keywords:
Bottle gourd
Umami taste
Glutamate
GWAS

ABSTRACT

The bottle gourd or calabash [*Lagenaria siceraria* (Mol.) *Standl.*] (2n = 2x = 22), also known as opo squash or long melon, is an important cucurbit crop worldwide. Commonly used as a vegetable, bottle gourd fruits have a peculiar umami taste that is important for their market value. To date, the umami chemical ingredients of bottle gourd fruits and their genetic determinants remain unidentified. In the current study, the relationship between umami flavor and free amino acid (FAA) contents was analyzed using a representative panel of twenty-eight accessions. Among the FAAs identified, free glutamate (Glu) was found to have the most significant association with umami taste, suggesting that free Glu is the main umami-conferring ingredient of bottle gourd. To further identify the genetic regions associated with umami taste, a genome-wide association study (GWAS) for free Glu content was conducted in a diversity panel consisting of 139 inbred lines, each sequenced with a restriction site-associated DNA sequencing (RAD-Seq) technology. Seventeen SNPs were identified as associated with free Glu content, each accounting for 7.5% to 15.1% of the phenotypic variation. The number of the favored alleles for each line varied from 0 to 13. Free Glu content exhibited a significant positive correlation with the number of the favored alleles. These results indicated that there is considerable genetic potential to develop new varieties with stronger umami taste through targeting more favored alleles of free Glu content. To our knowledge, this is the first GWAS study in bottle gourd. Our work reveals the genetic architecture of umami taste in the bottle gourd and will help facilitate molecular breeding of cultivars with improved flavor.

1. Introduction

Bottle gourd or calabash [*Lagenaria siceraria* (Mol.) Standl.] (2n = 2x = 22), also known as opo squash or long melon, is a diploid belonging to the genus *Lagenaria* of the *Cucurbitaceae* family (Beevy and Kuriachan 1996). Fresh fruit of bottle gourd is a common vegetable in many regions of Asia and Africa (Morimoto and Mvere 2004); dry fruits can be used as containers, pipes, floats, musical instruments, medicine, art, and even clothing accessories (Heiser 1979). Bottle gourd is also widely used as the rootstock in watermelon production to provide resistance against soil-borne diseases or tolerance to low soil temperatures (Lee 1994; Yetisir and Sari 2003).

Bottle gourds have a peculiar umami taste in the cooking process that makes them palatable and adaptable in different food forms and contributes greatly to consumer satisfaction and market profits. However, breeding for strong umami taste is challenging due to the difficulty in accurate evaluation of the bottle gourd umami taste. Sensory evaluation is the most common method used for taste evaluation, which, however, is time consuming and subjective due to the sensitivity of human physical and psychological conditions (Baldwin et al., 2011; Beullens et al., 2008; Phat et al., 2016). Objective indicators such as amino acid or nucleotide contents are preferable, but the key components underlying umami taste in bottle gourd are unknown, and a method for effective and objective quantification of umami taste intensity is also lacking.

Umami, in addition to sweet, salt, bitter and sour, is the fifth basic taste quality that humans can detect. Since the first discovery of monosodium glutamate (MSG) as the prototypic umami stimulus (Ikeda, 1908), a set of small molecules eliciting umami taste have been identified including amino acids, nucleotides (monophosphates of

---

Abbreviations: FAA, free amino acid; Glu, glutamate; GWAS, genome-wide association study; RAD-Seq, restriction site-associated DNA sequencing technology; MSG, monosodium glutamate; PCA, principal-component analysis; MLM, mixed linear model

* Corresponding author.

E-mail address: ligj@mail.zaas.ac.cn (G. Li).

http://dx.doi.org/10.1016/j.scienta.2017.08.015
Received 10 April 2017; Received in revised form 9 August 2017; Accepted 10 August 2017
0304-4238/ © 2017 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

inosinate or guanylate, inosine 5′-monophosphate, and guanosine-5′-monophosphate), short peptides and organic acids (Kuninka, 1960; Kurihara 2009). Among them, free amino acids have been found as the main umami ingredients in many vegetables. For instance, free glutamate exists in high concentrations in ripe tomatoes (140 mg/100 g) and gives the fruit its characteristic umami taste (Kenzo Kurihara 2009). Free glutamate and free aspartate are the two main umami ingredients in edible mushrooms (Zhang et al., 2013). Theanine (L-glutamate-c-ethylamide, Thea) is a unique free amino acid and the main compound responsible for the sweet umami taste of green tea (Kaneko et al., 2006). However, it is unclear which free amino acid contributes the most to the umami taste of bottle gourd.

Recently, significant progress has been made in the development of genomic resources in bottle gourd. Many ISSR, SSR and SNP markers have been released and a high-density genetic map has been constructed (Bhawna et al., 2014; Xu et al., 2011; Xu et al., 2014), allowing for the establishment of various marker-trait associations and the initiation of molecular breeding in bottle gourd. The objective of the present study was to identify the main umami ingredients in bottle gourd fruits, focusing on free amino acid contents. We then used this umami substance to represent the umami taste phenotype to perform whole genome-wide association analysis to detect genomic regions or markers associated with the umami trait and thus targets for molecular breeding in bottle gourd.

2. Materials and methods

2.1. Plant materials and growth conditions

A germplasm collection consisting of 135 bottle gourd accessions from China and 4 accessions from USDA ARS (United States Department of Agriculture, Agricultural Research Service) was used in the current study. All accessions are inbred lines. The 139 accessions were evaluated in the field houses of the Haining Experimental Station (30° N, 120° E) in the autumn of 2014 and 2015. Ten individuals of each accession were grown in 30 m rows spaced 0.5 m apart.

2.2. Samples preparation and free amino acids analysis

For amino acid analysis, three marketable immature fruits (8–12 days post pollination), were harvested for each accession and homogenized with equal weight to form a sample. All samples were stored at −80 °C until analysis.

Free amino acids were extracted from 1.5 g of each of the homogenates with 3% sulfosalicylic acid in a volume of 8 ml after vigorous shaking for 1 h. The suspension was centrifuged at 10,000*g* for 10 min at 4 °C. The supernatants were filtered with 0.45 μm syringe filters and then processed to measure free amino acid concentrations on an automatic amino acid analyzer (L-8900; Hitachi, Tokyo, Japan) that separates amino acids by cation-exchange chromatography. Quantification of the different amino acids was based on peak areas and calculated as equivalents of standard compounds. The analytical program EZChrom Elite was used for data analysis.

2.3. Sensory evaluation of umami taste

Twenty-eight accessions including landraces and cultivars from China were tasted and compared for umami flavor in the spring of 2014 and 2015. Marketable fruits, 8–12 days after pollination, were harvested and then fried or cooked. Ten tasters, 20–60 years of age, male and female, were requested to taste the cooked food’s flavor. A landrace, “Hangzhou Gourd” that has been recognized with a favorable flavor, was used as the control and recorded as 5. The umami taste metric used points between 1 and 10, and each taster scored independently.

2.4. RAD sequencing and SNP calling

Genomic DNA of the 139 bottle gourd accessions was extracted from young leaves of two-week old seedlings using a DNA extraction kit (TIANGEN Co. Ltd, Beijing) following the manufacturer’s instructions. The RAD libraries were constructed according to the protocol described by Recknagel et al. (2013); *EcoR* I and *Nla*III were used to double-digest the DNA. Paired-end (150 bp) sequencing was carried out using the Illumina HiSeq platform. Raw sequencing data was generated using the Illumina base calling software CASAVA v1.8.2 (http://support.illumina.com/sequencing/sequencing_software/casava.ilmn). SeqPrep (https://github.com/jstjohn/SeqPrep) was used to remove adaptors or primers (parameters: −q 20 −L 75 −B AGATCGGAAGAGCGTCGTGT −A AGATCGGAAGAGCACACGTC). The program Sickle (https://github.com/najoshi/sickle) was applied to perform read trimming to obtain “clean” data (high quality data) with the parameters “-n 5 −l 75”. The high quality reads were aligned to the Hangzhou Gourd reference genome sequence using BWA (http://bio-bwa.sourceforge.net/) software with default parameters. GATK “UnifiedGenotyper” function was used to detect SNPs. All the raw reads generated and used in this work have been deposited in the NCBI database with accession code SRR5234004- SRR5234142 for 139 bottle gourd accessions, SRR5194484- SRR5194490 for Hangzhou Gourd.

2.5. Statistical analysis and genome-wide association study

Calculation of phenotypic means, correlation coefficients and Student's *T-test* were evaluated in EXCEL; frequency distribution and broad-sense heritability (H^2) were evaluated using Tassel 5.0.

Dendrogram analysis (Neighbor-joining tree) and principal-component analysis (PCA) were used to investigate population structure of the 139 accessions. Two mixed linear models, accounting for population structure and kinship (MLM, PCA + K model) and Q matrix and kinship (MLM, Q + K model) were used for genome-wide association mapping of the free Glu content, as implemented by TASSEL 5.0. The relative distribution of observed $-\log_{10}$ P values for each SNP marker-trait association was compared individually with that of the expected distribution using the quantile–quantile plot of TASSEL 5.0.

3. Results

3.1. Free glutamate content is the main component for umami taste in bottle gourd

A total of 28 bottle gourd accessions with abundant fruit shape variation, representing the diversity panel of 139 accessions, were selected to taste their umami intensity by different tasters. Sensory evaluation of the twenty-eight accessions showed significant variations on their umami taste performances (Table 1). Correlation analysis between their free amino acid contents and sensory scores revealed that free glutamate (Glu) had the highest association with umami taste (Table 1). Using Hangzhou Gourd that had an umami taste score of 5 as the reference, the 28 accessions could be divided into two groups, weak group with umami taste score < 4.5 and strong group with umami taste score > 4.5. Comparing the FAA contents between the two groups, the most significant difference ($P \leq 0.0001$) was found on the free Glu content(Table 1). Therefore, free Glu content seemed to be the major factor affecting umami taste in bottle gourd and was thus used as an indirect indictor for umami flavor.

3.2. Diversity of free Glu content in the germplasm collection

The free amino acid contents, including Glu, of the 139 accessions were assayed in 2014 and 2015. As shown in Fig. 1, the free Glu content displayed a normal distribution in the two experiments. The free Glu content (μg/g) among the population varied significantly, ranging from

Table 1
The free amino acid content (μg/g) and umami taste in the twenty-eight accessions.

Accession	Taste score	Asp	Thr	Ser	Glu	Gly	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	g-ABA	Lys	His	Arg	Pro	total
J154-1①①	2.5	101.22	399.84	70.12	42.72	14.02	51.43	5.86	68.43	19.16	47.75	79.32	55.46	149.15	169.56	64.22	25.19	102.28	41.01	1506.71
G14①	3.33	29.18	157.11	15.31	35.77	3.64	15.60	5.22	7.93	1.56	5.58	9.39	7.17	15.52	48.73	7.77	3.20	16.15	2.62	387.47
J170-1①	3.5	20.85	240.81	17.97	34.18	6.23	28.43	5.76	14.48	2.59	12.70	13.27	11.12	22.74	33.02	9.35	5.35	32.05	2.86	513.77
Qingyaohulu	3.6	34.18	123.86	13.55	33.84	3.10	11.39	6.76	10.96	1.21	5.76	9.21	6.34	14.19	39.00	9.42	3.30	17.89	3.35	347.30
Duanguang137-2	3.67	54.39	393.88	30.99	137.29	7.30	21.67	6.62	13.51	3.89	11.64	16.15	5.90	22.90	71.20	15.39	5.25	39.04	3.54	860.55
J098-1②	3.84	90.63	357.92	41.49	50.69	9.03	47.49	1.99	37.04	6.98	26.17	37.99	13.00	55.11	103.77	35.61	12.10	69.85	17.33	1014.22
LS-1②①	3.85	97.33	482.13	55.35	75.83	11.82	57.69	36.13	54.95	34.04	34.80	49.62	12.57	87.12	67.20	77.33	19.67	83.92	17.23	1354.71
J083-1②	4	106.22	1110.24	101.15	266.04	19.39	61.77	4.13	70.73	14.26	51.83	71.22	38.70	87.02	188.85	50.67	25.56	175.12	26.06	2468.96
G32②①	4	75.10	508.99	48.24	79.51	10.10	38.42	2.43	36.01	6.55	29.87	32.33	10.86	50.15	87.24	23.82	14.59	56.85	11.43	1122.53
G120②	4	87.35	480.33	111.11	71.58	25.21	166.55	46.84	87.87	44.22	64.01	106.05	28.96	143.11	150.40	160.97	39.75	100.55	43.28	1958.15
I214	4.1	19.84	173.50	13.67	25.14	4.18	23.45	6.88	12.68	2.15	12.12	12.75	7.45	22.47	28.85	9.90	5.57	26.81	3.10	410.49
J160-1①④	4.18	31.09	143.72	14.33	27.02	3.83	12.23	5.57	8.72	0.71	7.73	9.00	7.97	17.11	38.70	6.18	3.21	18.67	1.98	357.76
J010①②	4.19	31.83	193.05	16.19	44.36	4.03	16.83	4.78	12.13	2.53	10.27	13.26	9.73	18.60	49.38	10.13	3.77	21.58	3.44	465.88
Yuanhu No1	4.29	53.66	741.00	51.45	151.69	12.41	67.01	34.28	50.76	31.29	37.19	42.86	8.55	78.22	75.31	56.98	20.08	99.81	5.77	1618.33
Zhenmu	4.47	53.30	795.20	89.26	185.02	16.99	129.81	9.77	73.03	12.67	57.15	55.31	19.78	116.66	99.87	39.04	43.35	92.76	14.60	1903.59
Qingpiyuanpu	4.7	37.42	356.17	49.72	56.07	9.29	75.53	34.71	48.32	31.87	31.52	41.00	10.96	95.56	73.69	67.50	19.63	75.35	14.77	1129.08
Niutuipu	4.78	132.33	338.47	63.27	121.46	11.98	40.44	33.13	40.22	31.38	29.51	35.40	3.90	78.45	101.60	76.75	20.16	49.12	4.80	1212.36
J084②②	4.8	102.45	965.02	132.31	390.03	33.92	113.19	4.46	97.68	17.66	71.03	87.52	36.89	101.52	136.98	63.73	30.56	172.70	29.29	2586.94
I82①	4.94	143.59	513.35	116.42	299.71	24.64	98.27	5.97	64.40	21.60	66.64	68.29	14.28	112.88	196.18	42.37	33.95	94.59	7.31	1924.45
Hangzhou Gourd	5	107.40	658.28	82.60	153.88	13.81	105.61	4.53	42.71	7.24	39.95	35.45	10.20	74.93	143.27	22.49	21.96	117.67	10.82	1652.79
J104-1⑥②	5.04	64.78	840.27	85.08	140.10	18.88	76.25	5.71	60.45	17.55	63.75	63.11	25.13	125.53	60.94	50.83	47.37	149.46	4.85	1900.03
Bianpu	5.08	96.13	787.14	123.33	277.12	23.37	146.30	39.01	79.90	37.61	57.87	99.70	26.12	108.19	119.43	134.70	29.00	109.23	36.35	2330.49
YD-6	5.22	124.13	1071.97	128.67	470.60	25.54	77.93	3.77	76.29	16.36	57.60	85.15	52.85	106.62	168.01	58.83	28.38	158.70	29.69	2741.08
YD-4	5.46	55.30	1093.08	68.99	241.45	20.32	115.96	7.12	50.47	7.62	40.74	44.82	26.82	68.49	133.84	32.90	23.85	147.69	9.78	2189.25
G6-3-5③	5.5	113.04	1593.36	149.62	431.55	26.92	222.74	49.35	126.06	47.44	150.90	148.30	28.50	177.84	273.06	108.91	113.98	212.42	23.53	3997.52
I77①	5.63	78.73	856.48	87.35	373.26	19.94	120.77	44.84	76.84	35.77	75.27	84.31	24.16	142.73	91.05	86.31	53.76	116.58	8.14	2376.28
Zhepu No.8	6.15	123.7	723.99	60.73	185.01	12.8	42.26	6.59	27.46	7.27	16.8	19.46	6.12	39.27	133.91	14.45	10.63	79.1	5.16	1514.71
J120③	6.25	83.65	935.20	83.05	203.12	20.11	118.54	37.76	70.76	37.68	51.70	70.77	13.68	106.14	97.63	96.64	32.96	108.72	19.24	2187.34
Correlation[a]		0.40	0.63	0.51	0.64	0.53	0.53	0.34	0.41	0.38	0.45	0.35	0.00	0.34	0.33	0.29	0.45	0.49	−0.04	0.59
t-test[b]		0.0037	0.0018	0.0006	0.0001	0.0006	0.0044	0.1512	0.0096	0.0270	0.0063	0.0181	0.3283	0.0125	0.0210	0.0704	0.0128	0.0020	0.6030	0.0007

a indicated the correlation between the umami score and each free amino acid content.
b indicated *t*-test from the weak umami group (taste score < 4.5) and strong umami group (taste score > 4.5).

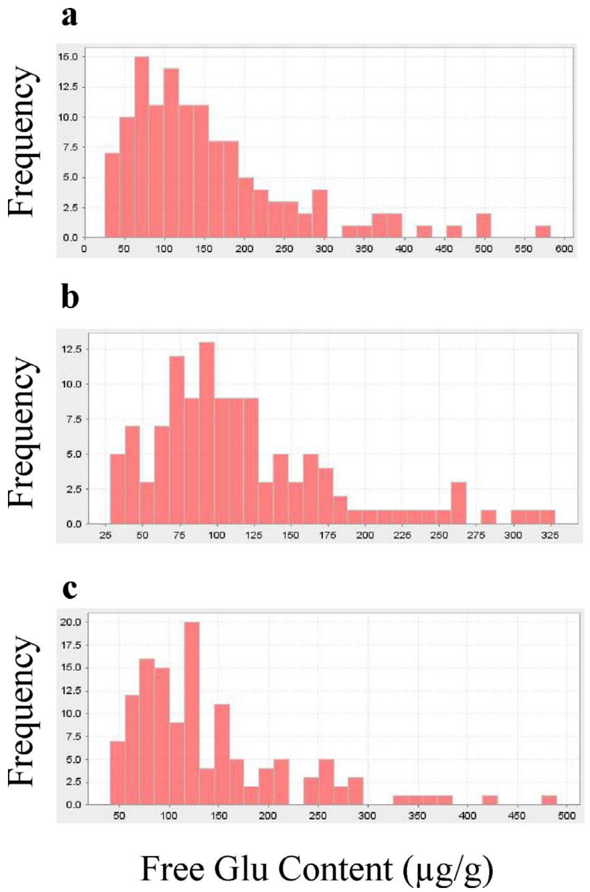


Fig. 1. The distribution frequency of the free glutamate content (μg/g) in 2014(a), 2015(b) and combined (c).

25.14 to 582.63 with an average of 155.67 in 2014 autumn and from 28.02 to 328.07 with an average of 117.39 in 2015 autumn. The heritability of free Glu content is 0.74 and 0.63 in the 2014 and 2015 data analysis, respectively. The phenotype correlation coefficient between the two years was lower (i.e., the value was only 0.55), indicating that free Glu content was greatly affected by the environment. As only one mixed sample for each accession were measured in each experiment, to make the phenotype data more representative, the two years' data was combined on average for further analysis, which showed that free Glu content ranged from 40.6 to 422.76 with an overall mean 132.54 (Fig. 1), where the heritability is 0.61 in combined analysis.

Wide range of natural variation in fruit shape and size is the most unique trait of bottle gourd. Growers have long recognized the round or calabash bottle gourd having higher umami taste than that of slender or straight varieties, but the latter has a larger market share than the former. Thus, the relationship between fruit shape (and size) and free Glu content was investigated. In our study, although the average Glu content of slender straight type varieties was relatively lower than that of other shape varieties, there were still some slender straight accessions with high free Glu content (Table 2). Indeed, broad range of Glu content variation was observed for each type, suggesting that fruit shape and size have no significant association with umami taste. Among the current major commercial varieties with slender straight shape, 85% had lower free Glu content than the control, suggesting lack of germplasms resources in this panel with strong umami taste for breeding slender straight varieties.

3.3. RAD-Seq and SNP calling

One hundred and thirty-nine bottle gourd accessions were genotyped using low coverage genome sequencing, which generated 40.22 Gb high-quality sequence data containing 335,666,250 pair-end reads, showing a minimum 96.21% Q20 and 91.95% Q30. For the single accession, the read number ranged from 1,431,582 to 5,757,524 with an average of 2,414,865.108; the RAD-tags number ranged from 706,411 to 1,517,777 with an average of 1,027,658. After reads aligning to the Hangzhou gourd reference genome, 19,226 SNPs were detected (on average, 0.059 SNPs per kb). Of them, 85.34% SNPs were located on the 11 chromosomes of bottle gourd and the SNPs number of each chromosome ranged from 622 to 2041 with an average of 1,491.64. Among the 139 accessions, five accessions with high heterozygous frequencies ($> 15\%$) and 4 accessions with high missing frequencies ($> 25\%$) were discarded in subsequent analysis. After removing the SNPs with missing call rates, heterozygous call rates $\geq 25\%$, and minor allele frequencies ≤ 0.01, 6222 SNPs remained for further analysis.

Table 2
Statistics of free Glu content (μg/g) for different fruit shape (and size) bottle gourds.

Fruit shape	Individuals	Range	Mean
slender straight	40	40.6–260.42	111.7
short cylinder	17	55.55–367.28	164.27
near round	7	104.18–422.76	181.4
pyriform	8	47.51–330.24	141.95
bracket type	22	59.08–373.26	160
waist hoists	11	49.2–489.84	130.79
long neck ball	3	123.04–348.71	209.01
long cylinder	22	68.14–257.17	139.14

3.4. Genome-wide SNP–trait associations

Prior to GWAS, the population structure of the 130 bottle gourd accessions was investigated using dendrogram analysis and PCA analysis. Both results indicated no significant subgrouping of the 130 accessions, except for 11 accessions that were distantly related to one another (Fig. 2). These results indicated that no primary factors, such as geographic distribution, affected the population structure of the 130 samples. Two MLM model approaches were used to search for SNP loci associated with free Glu content. Using the MLM model with corrections for PCA and kinship, 18 loci were detected exceeding a significant threshold ($-\log_{10} P \geq 2.50$). Using the MLM model with corrections for Q and kinship, 22 loci were detected using the same significance level. QQ plots from the two models are very similar (Fig. 3). Seventeen loci distributed across six chromosomes and one scaffold of the bottle gourd genome were detected by both models, accounting for 7.8% to 15.1% of the phenotypic variation (Table 3).

3.5. Favored alleles distribution analysis

Once the genetic loci governing a specific trait are determined, pyramiding favored alleles is important to develop elite cultivars. For this reason, the distribution of favored alleles in the free Glu associated loci was analyzed for each accession. The number of favored alleles in different accessions varied markedly, ranging from 0 to 13. Among them, 71.5% accessions showed no favored alleles, and 14.6% accessions carried only one favored allele. In 40 accessions with slender and straight fruit shapes, only three varieties had one favored allele. We also found linear correlations between free Glu content and favored alleles (Fig. 4), and the correlation coefficient between them reached 0.65 ($p < 0.001$). These results suggest that increasing the free Glu content of the current varieties via pyramiding favorable alleles of the current varieties is feasible.

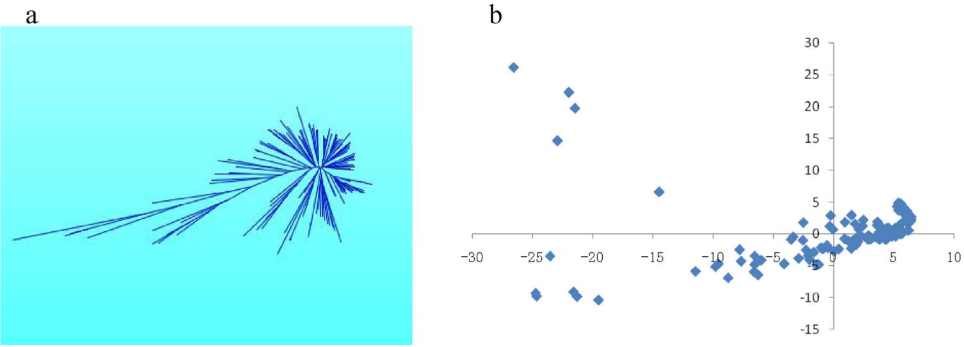


Fig. 2. Population structures of the bottle gourd germplasm. (a) Neighbor-joining tree of 130 accessions. (b) PCA plots of the 130 accessions.

4. Discussions

Umami is a pleasant savory taste elicited by three main substances (i.e., glutamate, inosinate, and guanylate) in a wide variety of foods including vegetables, meat, fish, seafood, and dairy products (Fuke and Ueda 1996; Uneyama et al., 2009). Free L-glutamate, usually in the form of monosodium glutamate (MSG), has been well recognized to provide umami taste in both animal and plant foodstuffs and is commonly used as an umami enhancer (France Bellisle 1999). As a functional amino acid, Glu interacts with specific taste cells in the tongue to release a signal that the brain identifies as umami taste (John and Margaret, 2013). Inosinate is only found in animal food products and guanylate has so far only been found in processed foods, most notably dried shiitake mushrooms (Yamaguchi 1967; Yamaguchi and Ninomiya, 2000; Osawa 2012). Here, we found that free Glu was the key component responsible for umami taste in bottle gourd. Other free amino acids may also contribute to the umami taste in bottle gourd, but according to our full amino acid content profile analysis, free Glu could

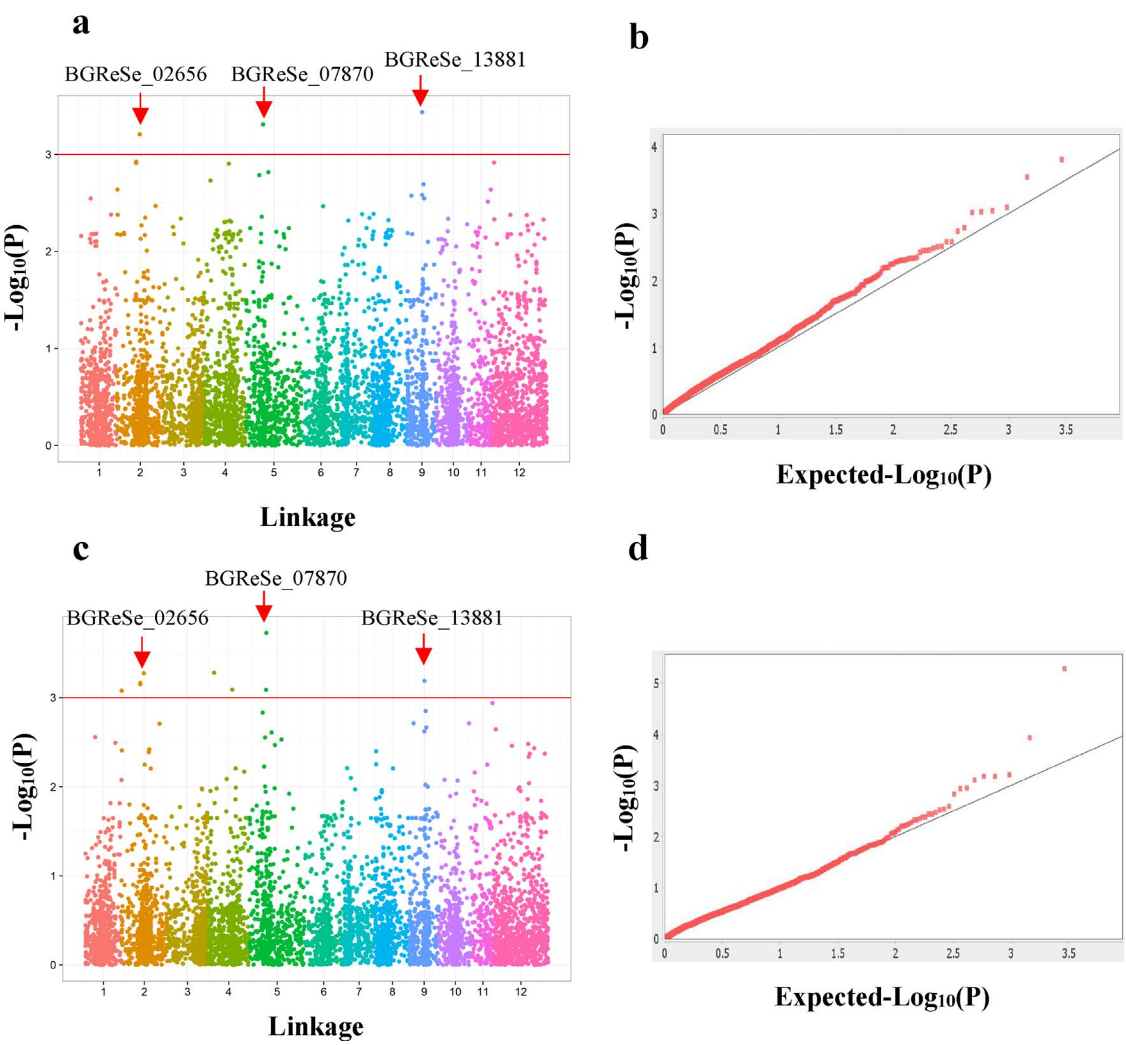


Fig. 3. Genome-wide association studies of free glutamate content. (a) Manhattan plots of the MLM (PCA + K) model. Negative $\log_{10}$-transformed P values from a genome-wide scan are plotted against position on 11 chromosomes and scaffolds, 12 indicates all the scaffolds. Red horizontal line indicates the genome-wide significance threshold. (b) Quantile-quantile plot of the MLM (PCA + K) model. (c) Manhattan plots of the MLM (Q + K) model, as in a. (d) Quantile-quantile plot of the MLM (Q + K) model. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Functional relevance of 17 genomic loci identified by GWAS.

Marker	Chr	Pos	SNP	Favored allele	MLM(PCA + K)		MLM(Q + K)	
					p	MarkerR^2(%)	*p*	MarkerR^2(%)
BGReSe_00233	1	6938814	C/T	T	2.84×10^{-3}	12.8	2.76×10^{-3}	12.8
BGReSe_01722	2	162191	C/G	C	2.30×10^{-3}	8.4	8.32×10^{-4}	10.1
BGReSe_02250	2	13566931	A/G	G	1.19×10^{-3}	13.9	6.81×10^{-4}	15.1
BGReSe_02254	2	13570856	C/T	C	1.22×10^{-3}	12.8	7.08×10^{-4}	13.9
BGReSe_02656	2	16172329	A/G	G	6.19×10^{-4}	14.5	5.30×10^{-4}	14.9
BGReSe_05631	4	4620748	C/T	T	1.85×10^{-3}	12.0	5.25×10^{-4}	14.5
BGReSe_06384	4	17798671	C/T	T	1.25×10^{-3}	9.0	8.09×10^{-4}	9.8
BGReSe_07671	5	9266893	C/T	T	1.64×10^{-3}	8.1	1.47×10^{-3}	8.3
BGReSe_07870	5	11924571	C/T	C	4.90×10^{-4}	10.2	1.87×10^{-4}	11.8
BGReSe_08221	5	15711773	A/G	G	1.52×10^{-3}	14.2	2.45×10^{-3}	13.1
BGReSe_13462	9	2837521	C/T	C	2.66×10^{-3}	7.3	1.93×10^{-3}	7.8
BGReSe_13860	9	10617120	A/C	A	2.61×10^{-3}	10.2	2.38×10^{-3}	10.3
BGReSe_13881	9	10672327	C/T	T	3.66×10^{-4}	11.7	6.47×10^{-4}	10.7
BGReSe_14034	9	11575954	A/G	A	2.03×10^{-3}	7.9	1.41×10^{-3}	8.5
BGReSe_14082	9	11955078	C/T	T	2.84×10^{-3}	10.1	2.15×10^{-3}	10.7
BGReSe_16342	11	14707964	A/T	A	2.30×10^{-3}	7.8	1.15×10^{-3}	8.9
BGReSe_16707	SCAFFOLD136	549253	A/G	G	1.21×10^{-3}	11.2	2.26×10^{-3}	10.1

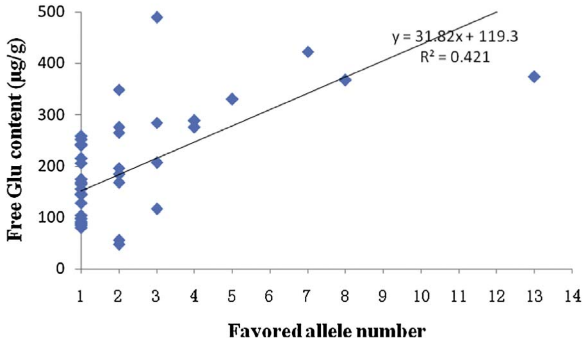


Fig. 4. Relation between free Glu content (μg/g) and favored allele number.

be readily used as an objective and quantitative indicator for umami phenotyping in breeding programs.

GWAS has emerged as a powerful approach for identification of genetic regions underlying complex traits in crop plants (Huang et al., 2010; Li et al., 2013; Lin et al., 2014; Yano et al., 2016). It is as time- and cost-effective as using existing germplasm populations and can detect many natural allelic variations simultaneously in a single study (Myles et al., 2009). However, GWAS has rarely been applied to genetic studies of flavor traits such as umami taste compared to agronomic traits. In this study, using free Glu content as an indicator of umami taste, we conducted a GWAS to detect the genetic basis underlying natural variation of this trait. A total of 17 association signals were identified and each explained approximately 10% of the phenotypic variation (Table 3). Our results first revealed the genetic architecture of umami taste in bottle gourd, suggesting that umami taste is a complex quantitative trait and probably controlled by multiple loci with small effects, similar to flowering time and grain yield in crops (Buckler et al., 2009; Huang et al., 2010). Our previous study showed a genome-wide LD decay rate for bottle gourd that was estimated to be over 100 kb, where the r^2 decreased to 0.2 (Xu et al., 2017, unpublished data). This means that if the distance between the two associations is less than 100 kb, they were probably represented as a single QTL or gene. Among the 17 loci, BGReSe_02250 is 3.925 kb distal to BGReSe_02254 on Chromosome 2 and BGReSe_13860 and BGReSe_13881 on Chromosome 9 are separated by 55.207 kb, suggesting these may represent two different association regions. We also investigated the relationship between the favored alleles and free glutamate content and found linear correlations between these variables (Fig. 4). These results indicated that additive effects were present in the association loci, and breeders could increase free Glu content by combining more favored alleles to develop new varieties with strong umami taste. Indeed, most accessions have no favored allele or have only 1–2 favored alleles; thus, there is great capability to increase free Glu content of current varieties. Therefore, it is feasible to increase umami taste in bottle gourd by marker-assisted selection.

As far as we know, there is no genetically mapped QTLs of free Glu content reported in bottle gourd, thus the identified SNPs loci can't be compared and validated with other studies. However, the genes included or near the associated SNPs in our study have been identified using Hangzhou gourd genome reference sequence, and two genes BG_GLEAN_10021173 and BG_GLEAN_10019012 have been found to involve the glutamate metabolism pathways. BG_GLEAN_10021173 is located downstream of BGReSe_02656 on the chromosome 2 with a 110 054 bp distal, and BG_GLEAN_10019012 located on the chromosome 4 has a 70 445 bp distal to BGReSe_05631, both the two genes have an amino acid transporter, transmembrane domain (IPR013057), which is found in many amino acid transporters that encodes a vesicular amino butyric acid (GABA) transporter, and GABA is mostly related to glutamate level in plants (Forde and Lea 2007, Sorrequieta et al., 2010). Given the LD distance over 100 kb in bottle gourd (Xu et al., 2017), the two genes may be not the causal variant itself but also indirectly validated the reliability of our results. Most of genes identified have unknown function or no significant function for glutamate synthesis or metabolism. The small population size, low genetic diversity panel, and low SNP resolution may be the main reasons to explain this phenomenon. Now that collecting more accessions worldwide for whole-genome resequencing is under way, associations from this broader sampling can be investigated in the future. In addition, we are also starting linkage analyses using several bi-parental populations to validate association results and clone the genes conferring umami taste. To our knowledge, this is the first report of GWAS in bottle gourd. Our results lay the foundation to discover the genetic architecture of the umami taste in bottle gourd and facilitate the molecular breeding of new varieties with strong umami taste.

Conflict of interest

The authors declare no competing financial interest.

Funding

This study was partially supported by the Natural Science Foundation of Zhejiang Province (LY14C150004), the National Natural

Science Foundation of China (31401880), the Major Science and Technology Project of Plant Breeding in Zhejiang Province (2016C02051), China Postdoctoral Science Foundation (2015M571900) and Youth Talent Training Project (to XY Wu) from Zhejiang Academy of Agricultural Sciences (2016R23R08E04).

Acknowledgements

We thank Dr. Yiqun Weng from University of Wisconsin-Madison for editing the manuscript. We thank Ye Tao from Biozeron Co. Ltd, Shanghai for technical support in bioinformatic analysis of data. We also thank Yaowen Hu, Jiandong Lin, and Xiaohong Yu for their assistance in sample preparation for free amino acid analysis.

References

Baldwin, E.A., Bai, J., Plotto, A., Dea, S., 2011. Electronic noses and tongues: applications for the food and pharmaceutical industries. Sensors 11 (5), 4744–4766.

Bhawna, A., Arya, M.Z., Saha, L., Sureja, D., Pandey, A.K., Verma, C., 2014. Population structure and genetic diversity in bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] germplasm from India assessed by ISSR markers. Plant Syst. Evol. 300, 767–773.

Beevy, S.S., Kuriachan, P., 1996. Chromosome numbers of south Indian Cucurbitaceae and a note on the cytological evolution in the family. J. Cytol. Genet. 1996 (31), 65–71.

Beullens, K., Mészáros, P., Vermeir, S., Kirsanov, D., Legin, A., Buysens, S., Cap, N., Nicolaï, B.M., Lammertyn, J., 2008. Analysis of tomato taste using two types of electronic tongues. Sens. Actuators, B: Chem. 131 (1), 10–17.

Buckler, E.S., Holland, J.B., Bradbury, P.J., Acharya, C.B., Brown, P.J., Browne, C., et al., 2009. The genetic architecture of maize flowering time. Science 325, 714–718.

Forde, B.G., Lea, P.J., 2007. Glutamate in plants: metabolism, regulation, and signalling. J. Exp. Bot. 58, 2339–2358.

France, B., 1999. Glutamate and the UMAMI taste: sensory, metabolic, nutritional and behavioural considerations: a review of the literature published in the last 10 years. Neurosci. Biobehav. Rev. 23, 423–438.

Fuke, S., Ueda, Y., 1996. Interactions between umami and other flavor characteristics. Trends Food Sci. Technol. 7 (12), 407e411.

Heiser, C.B., 1979. The Gourd Book. University of Oklahoma Press, Norman.

Huang, X.H., Wei, X.H., Sang, T., Zhao, Q., Feng, Q., Zhao, Y., Li, C.Y., Zhu, C.R., Lu, T.T., Zhang, Z.W., Li, M., Fan, D.L., Guo, Y.L., Wang, A.H., Wang, L., Deng, L.W., Li, W.J., Lu, Y.Q., Weng, Q.J., Liu, K.Y., Huang, T., Zhou, T.Y., Jing, Y.F., Li, W., Lin, Z., Buckler, E.S., Qian, Q., Zhang, Q.F., Li, J.Y., Han, B., 2010. Genome-wide association studies of 14 agronomic traits in rice landraces. Nat. Genet. 42, 961–967.

Ikeda, K. Japanese patent,1908, 4805.

John, T.B., Margaret, E.B., 2013. Glutamate: a truly functional amino acid. Amino Acids 45, 413–418.

Kaneko, S., Kumazawa, K., Masuda, H., Henze, A., Hofmann, T., 2006. Molecular and sensory studies on the umami taste of Japanese green tea. J. Agric. Food Chem. 54, 2688–2694.

Kuninka, A., 1960. Research on taste function of the nucleotides. J. Agric. Chem. Soc. Jpn. 34, 489–492.

Kurihara, K., 2009. Glutamate: from discovery as a food flavor to role as a basic taste (umami). Am. J. Clin. Nutr. 90, 719S–722S (suppl).

Lee, J.M., 1994. Cultivation of grafted vegetables I Current status, grafting methods and benefits. HortScience 1994 (29), 235–239.

Li, H., Peng, Z.Y., Yang, X.H., Wang, W.D., Fu, J.J., Wang, J.H., Han, Y.J., Chai, Y.C., Guo, T.T., Yang, N., Liu, J., Warburton, M.L., Cheng, Y.B., Hao, X.M., Zhang, P., Zhao, J.Y., Liu, Y.J., Wang, G.Y., Yan, J.B., 2013. Genome-wide association study dissects the genetic architecture of oil biosynthesis in maize kernels. Nat. Genet. 45, 43–50.

Lin, T., Zhu, G.T., Zhang, J.H., Xu, X.Y., Yu, Q.H., Zheng, Z., Zhang, Z.H., Lun, Y.Y., Li, S., Wang, X.X., Huang, Z.J., Li, J.M., Zhang, C.Z., Wang, T.T., Zhang, Y.Y., Wang, A.X., Zhang, Y.C., Lin, K., Li, C.Y., Xiong, G.S., Xue, Y.B., Mazzucato, A., Causse, M., Fei, Z.J., Giovannoni, J.J., Chetelat, R.T., Zamir, D., Städler, T., Li, J.F., Ye, Z.B., Du, Y.C., Huang, S.W., 2014. Genomic analyses provide insights into the history of tomato breeding. Nat. Genet. 46, 1220–1226.

Morimoto, Y., Mvere, B., 2004. Lagenaria siceraria. In: Grubben, G.J.H., Denton, O.A. (Eds.), Vegetables. Plant Resources of Tropical Africa 2. Backhuys Publishers/CTA, Wageningen/Leiden, pp. 353–358.

Myles, S., Peiffer, J., Brown, P.J., 2009. Association mapping: critical considerations shift from genotyping to experimental design. Plant Cell 21, 2194–2202.

Osawa, Y., 2012. Glutamate perception, soup stock, and the concept of umami: the ethnography, food ecology, and history of dashi in Japan. Ecol. Food Nutr. 51, 329–345.

Phat, C., Moon, B., Lee, C., 2016. Evaluation of umami taste in mushroom extracts by chemical analysis, sensory evaluation, and an electronic tongue system. Food Chem. 192, 1068–1077.

Recknagel, H., Elmer, K.R., Meyer, A., 2013. A hybrid genetic linkage map of two ecologically and morphologically divergent Midas cichlid fishes (Amphilophus spp.) obtained by massively parallel DNA Sequencing (ddRADSeq). G3 3, 65–74.

Sorrequieta, A., Ferraro, G., Boggio, S.B., Valle, E.M., 2010. Free amino acid production during tomato fruit ripening: a focus on L-glutamate. Amino Acids 38, 1523–1532.

Uneyama, H., Kawai, M., Sekine-Hayakawa, Y., Torii, K., 2009. Contribution of umami taste substances in human salivation during meal. J. Med. Invest. 2009 (56), 197–204.

Xu, P., Wu, X.H., Luo, J., Wang, B.G., Liu, Y.H., Ehlers, J.D., Wang, S., Lu, Z.F., Li, G.J., 2011. Partial sequencing of the bottle gourd genome reveals markers useful for phylogenetic analysis and breeding. BMC Genom. 12, 467.

Xu, P., Xu, S.Z., Wu, X.H., Tao, Y., Wang, B.G., Wang, S., Qin, D.H., Lu, Z.F., Li, G.J., 2014. Population genomic analyses from low-coverage RAD-Seq data: a case study on the non-model cucurbit bottle gourd. Plant J. 77, 430–442.

Xu, P.; Wu, X.H.; Sun, F.S.; Wu, R.L.; Wang, Y.H.; Wu, X.Y.; Xin, N.L.; Tao, Y.; Hu, Y.W.; Wang, B.G.; Huang, Y.P.; Zhou, W.; Zeng, L.; Lin, Q.; Lu, Z.F.; Li, G.J. Genome of the bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] and the genetic architecture of fruit shape variation. Under review in Nature Communication.

Yamaguchi, S., Ninomiya, K., 2000. Umami and food palatability. J. Nutr. 130 (4), 921S–926S.

Yamaguchi, S., 1967. The synergistic taste effect of monosodium glutamate and disodium 5'-inosinate. J. Food Sci. 32, 473–478.

Yano, K., Yamamoto, E., Aya, K., Takeuchi, H., Lo, P., Hu, L., Yamasaki, M., Yoshida, S., Kitano, H., Matsuoka, M., 2016. Genome-wide association study using whole-genome sequencing rapidly identifies new genes influencing agronomic traits in rice. Nat. Genet. 48, 927–934.

Yetisir, H., Sari, N., 2003. Effect of different rootstock on plant growth, yield and quality of watermelon. Aust. J. Exp. Agric. 2003 (43), 1269–1274.

Zhang, Y., Venkitasamy, C., Pan, Z.L., Wang, W., 2013. Recent developments on umami ingredients of edible mushrooms- A review. Trends Food Sci. Technol. 33, 78–92.