Comparative Study on Phytochemical Variations in Japanese F₁ Varieties of Bulb Onions and South-East Asian Shallot Landraces

Nur Aeni Ariyanti^{1,2}, Kotaro Torikai³, Rizky Psthika Kirana⁴, Sho Hirata¹,
Endang Sulistyaningsih⁴, Shin-ichi Ito^{1,3,5}, Naoki Yamauchi^{1,3,5}, Nobuo Kobayashi^{1,6}
and Masayoshi Shigyo^{1,3,5*}

¹The United Graduate School of Agricultural Sciences, Tottori University, Tottori 680-8553, Japan

²Department of Biology Education, Faculty of Mathematics and Sciences, Yogyakarta State University, Yogyakarta 55281, Indonesia

³Faculty of Agriculture, Yamaguchi University, Yamaguchi 753-8515, Japan

⁴Faculty of Agriculture, Gadjah Mada University, Yogyakarta 55281, Indonesia

⁵Graduate School of Sciences and Technology for Innovation, Yamaguchi 753-8515, Japan

⁶Faculty of Life and Environmental Science, Shimane University, Matsue 690-8504, Japan

Wide-ranging varieties and/or strains of bulb onions (*Allium cepa* Common onion group) and shallots (*A. cepa* Aggregatum group) were utilized to understand the variation in chemical compounds responsible for their taste. The bulb samples of 10 F₁ commercial onion varieties (seven short-day and three long-day varieties) from Japan and 12 shallot landraces from abroad (Vietnam: three landraces; Indonesia: nine landraces) were collected as plant materials once a year in 2014 and 2015. The contents of S-alk(en)yl-L-cysteine sulfoxides, total flavonoids, and soluble sugars—including fructose, glucose, sucrose, and fructans—were determined to find differences between bulb onions and shallots, as well as to detect variations among varieties and/or landraces. While a principal component analysis (PCA) based on the results from both 2014 and 2015 could clearly discriminate shallots from bulb onions from a phytochemical perspective, bulb onions mainly had higher monosaccharides than shallots. By contrast, shallots produced more disaccharides than bulb onions. In most cases, regression analyses using the numerical data of the chemical compounds found in bulb onions and shallots suggested year-year correlations between 2014 and 2015. The flavonoid and PeCSO (S-(1-propenyl)-L-cysteine sulfoxide: isoalliin) contents in shallots were higher than those detected in bulb onions, which indicated the stronger pungent and bitter taste could be attributable to excess amounts of these compounds in this tropical plant.

Key Words: bulb onion, cysteine sulfoxide, flavonoid, phytochemical, shallot, sugar.

Introduction

Bulb onions (*Allium cepa* Common onion group) and shallots (*Allium cepa* Aggregatum group) are two of more than 800 *Allium* species that have been used by humans for their unique characteristics of taste, odor, and health benefits. In the human diet, both bulb onions

and shallots are consumed raw or processed as a spice in foods or sauces because of their particular taste and their ability to increase the taste of other foods (Fattorusso et al., 2002; Kopsell and Randle, 1997). Although those two species differ in appearance, color, and taste, they may have similar biochemical, phytochemical, and nutraceutical contents (Benkeblia, 2004). The shallot is commonly used as a condiment in South-east Asian countries, including Indonesia, Malaysia, Vietnam, and the Philippines, whereas the bulb onion is widely used fresh or processed in Europe, America, and East Asia, including China and Japan. In Japan, there are two types of bulb onion cultivars, short day and long day (Shigyo and Kik, 2008), according to their photoperiod requirements for bulb formation, as sug-

Received; November 7, 2016. Accepted; February 15, 2017.

First Published Online in J-STAGE on March 31, 2017.

The first author gratefully acknowledge Yogyakarta State University for giving her a chance to pursue her study in The United Graduate School of Agricultural Sciences, Tottori University, with the financial support from Monbukagakusho Scholarship of The Japanese Ministry of Education, Culture, Sports, Science and Technology.

* Corresponding author (E-mail: shigyo@yamaguchi-u.ac.jp).

gested by Brewster (2008). A Japanese seed company developed three different F_1 varieties—‘Okhotsk 222’ (early season), ‘Kitamomiji 2000’ (mid-season), and ‘Super Kitamomiji’ (late season)—as long-day types suitable for the Hokkaido area. By using these three types through half of the year, farmers can harvest bulb onions from early to late September. In the same way, seven different F_1 varieties were developed as short-day types for the southwestern part of Japan, and can be harvested continuously from late April to early June before the rainy season. These leading varieties can be cultivated predominantly in the areas of main bulb onion production from north (Hokkaido) to south (Awaji and Saga) in Japan. Even so, no one knows the metabolomic profiles of the two complete sets for long- as well as short-day bulb onion varieties.

Consumer preferences for bulb onions and shallots depend on their eating quality. As mentioned by Kays and Yan (2000), the eating quality is generally considered to be the combination of odor, flavor, and taste. The amounts of some chemical compounds—including sulfur, soluble sugars, amino acids, and flavonoids—will determine the overall taste of the bulb onion or shallot. Sulfur compounds are responsible for the characteristics of flavors, odors, and taste in *Allium* species, including bulb onions and shallots (Randle and Lancaster, 2002). *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs), which produce volatile compounds, affect the flavor and pungency (Block, 1992). The quality of bulb onions and shallots, especially their sweet or bitter tastes, is affected by their soluble sugar and flavonoid contents (Tamaki et al., 2002). Southeast Asian consumers prefer a pungent taste and soft texture for raw consumption, such as in salads (Sulistyarningsih, personal communication), while sweetness and firmness are vital for quality when processed (Kimura et al., 2014).

Several studies have reported on the potentially important agronomic traits of shallots for plant-breeding purposes. Vu et al. (2012) discovered a novel gene of resistance to *Fusarium oxysporum*, the cause of *Fusarium* wilt in the Japanese bunching onion (*Allium fistulosum*), located on chromosome 2 of the shallot. Abdelrahman et al. (2015) estimated the responses of doubled haploid shallots (DHAs), doubled haploid onions (DHCs), and F_1 hybrids to some abiotic stresses by means of omics technology. Their results showed that several key genes and metabolites responsible for abiotic stress responses could be up-regulated in DHA and F_1 genotypes, as compared to those of DHC. Moreover, it is important to gather definite and reliable information on phytochemical contents for a wider range of varieties and/or landraces in order to reinforce the potential usefulness of *A. cepa*.

In this research, we obtained several groups of data sets for different kinds of chemical compounds from bulb onions and shallots in order to study the variation in chemical properties responsible for the taste of F_1 varieties of Japanese bulb onions and Southeast Asian shallot landraces.

Materials and Methods

Plant materials

This study was conducted in two years, 2014 and 2015. Ten F_1 varieties of Japanese bulb onion (*A. cepa* Common onion group), including seven short-day and three long-day bulb onion varieties, were examined. The short-day bulb onions were cultivated in Kagawa Prefecture (34° N, 134° E), and the long-day bulb onions were grown in Hokkaido Prefecture (43° N, 142° E). To maintain the originality of the samples, we collected identical shallot landraces from their original growing areas over two years. Twelve shallot (*A. cepa* Aggregatum group) landraces, divided into nine land-

Fig. 1. Unpeeled (upper) and peeled (lower) bulbs of eight Indonesian shallot landraces collected in 2015. Bar indicates 1 cm.

races from Indonesia (Bantul Region 7.9° S, 110.4° E; Probolinggo 7.7° S, 113.2° E; Nganjuk 7.6° S, 111.9° E) (Fig. 1) and three landraces from Vietnam (Ly Son Island 15.3° N, 109.1° E; Quảng Ngãi Province 15.0° N, 108.7° E; Sóc Trăng Province 9.6° N, 105.9° E), were collected from farmers or local markets. The names and origins of all samples, including cultivation periods, are indicated in Table 1, since a sufficient number of bulbs could be collected in some areas.

Extraction and determination of S-alk(en)yl-L-cysteine sulfoxides (ACSOs)

High-performance liquid chromatography (HPLC) was employed to determine the sulfur compounds of bulb onion varieties and shallot landraces. Three onion bulbs were usually used for each variety as biological replicates. Each onion bulb was cut into quarters from top to bottom. The first part was used for hot ethanol extraction, and the second part was used to determine the ACSO content using water extraction. For shallot samples, however, two to three bulbs of each of the usual three replicates were used for water extraction. The extraction method adopted was the same as that described by Vu et al. (2013).

To analyze *S*-2-propenyl (allyl)-L-cysteine sulfoxide (AICSO) and *S*-1-propenyl-L-cysteine sulfoxide (PeCSO), a 10-time dilution of the sample was filtrated

using a disposable membrane filter (DISMIC®-13 HP ADVANTEC; Toyo Roshi Kaisha, Ltd., Tokyo, Japan). A 25-μL sample was injected into the HPLC system for quantification. Twenty mg·mL⁻¹ of 1-propyl CSO and a mix of allyl (Al) and propenyl (Pe) CSO, produced by House Foods Corporation, Japan, was used as the standard. The HPLC system included a pump, a degasser, a column oven, a diode array detector set (220 nm, L-2450; HITACHI High-Technologies Corporation, Tokyo, Japan), a data collection system (EZChrom Elite™; HITACHI High-Technologies Corporation), and an AQUASIL column (4.6 mm Ø × 25 cm long). The solvent, 0.005% trifluoroacetic acid (TFA), flowed for 15 min at a flow rate of 0.6 mL·min⁻¹.

The *S*-methyl-L-cysteine sulfoxide (MeCSO) content was determined by using an amino acid analysis method because the method previously used to identify AICSO and PeCSO produces an overlapping peak of MeCSO, making it difficult to quantify. One hundred μL of the sample and 100 μL of the amino acid standard were dried in a vacuum using Spin Dryer Lite VC-36R (Taitec Co., Ltd., Saitama, Japan). For derivatization, 20 μL of freshly prepared methanol : H₂O : triethylamine : phenylisothiocyanate (7:1:1:1) was added to the dried sample and vortexed. The mixture was incubated for 20 min at room temperature for the reaction process before being dried again in a vacuum condition. The

Table 1. Plant material used in this study and relevant information.

Type	Variety or landrace	Abbreviation	Origin	Cultivation period	
				Sowing ^y	Harvesting
SDO (Short-day type of bulb onion)	‘Shippo wase 7 go’	SW	Kagawa prefecture (Japan)	September	April
	‘Advance’	AD	Kagawa prefecture (Japan)	September	April
	‘Answer’	AN	Kagawa prefecture (Japan)	September	April
	‘Shippo Ama 70’	SA	Kagawa prefecture (Japan)	September	May
	‘Tarzan’	TZ	Kagawa prefecture (Japan)	September	May
	‘Momijinokagayaki’	MK	Kagawa prefecture (Japan)	September	May
	‘Momiji 3 go’	MS	Kagawa prefecture (Japan)	September	May
LDO (Long-day type of bulb onion)	‘Okhotsk 222’	OK	Hokkaido prefecture (Japan)	March	August
	‘Kitamomiji 2000’	KM	Hokkaido prefecture (Japan)	March	September
	‘Super Kitamomiji’	SK	Hokkaido prefecture (Japan)	March	September
VNS (Vietnamese landraces of shallot)	‘Lyson’ ^z	LY	Ly Son island (Vietnam)	November	March
	‘Quangai’ ^z	QU	Quảng Ngãi province (Vietnam)	November	March
	‘Soc trang’ ^z	ST	Sóc Trăng province (Vietnam)	November	March
IDS (Indonesian landraces of shallot)	‘Biru’	BR	Bantul (Yogyakarta, Indonesia)	June	September
	‘Philip’ ^z	PH	Nganjuk (East Java, Indonesia)	June	September
	‘Tiron’	TR	Bantul (Yogyakarta, Indonesia)	June	September
	‘Thailand’	TH	Bantul (Yogyakarta, Indonesia)	June	September
	‘Bima Juna’	BJ	Tegal (Central Java, Indonesia)	June	September
	‘Bauji Bunder’	BB	Nganjuk (East Java, Indonesia)	June	September
	‘Bauji Plompong’	BP	Nganjuk (East Java, Indonesia)	June	September
	‘Crok Kuning’	CK	Bantul (Yogyakarta, Indonesia)	June	September
	‘Probolinggo’	PB	Probolinggo (Central Java, Indonesia)	June	September

^z Not analyzed in the second year (2015).

^y Seed sowing in bulb onions bulbs.

supernatant was dissolved in 100 μL of 5 mM sodium phosphate, pH 7.6, containing 5% acetonitrile. A quantitative analysis method using HPLC apparatus from Nihon Waters (Tokyo, Japan) followed the procedure described by Masamura et al. (2011), with some minor modifications. The injection volume was 50 μL , and the column temperature was 43°C. HPLC analysis was carried out by using the following solvent system. Solvent A: 19 g of sodium acetate trihydrate and 2 mL of triethylamine were dissolved in 1 L of high purity water. The solution was adjusted to pH 6.08 by the addition of glacial acetic acid. To make up a 10% acetonitrile solution, a 950-mL solution was supplemented by 50 mL of acetonitrile. Solvent B: 60% acetonitrile and 40% high purity water (v/v) were mixed. The transmission of the gradient elution and the flow rate were obtained as described by Masamura et al. (2011).

Extraction and determination of total flavonoid content

The total flavonoid content was determined by the colorimetric method using hot 70% ethanol extract. The method of flavonoid extraction was applied as described by Vu et al. (2013). A 500- μL sample and 500 μL of 100% hexane were mixed in the Eppendorf and then incubated until the mixture separated into two parts. Fifty μL of the down part was taken as a sample to analyze with 50 μL of 70% ethanol and 200 μL of 2% aluminum chloride. The mixture was homogenized thoroughly by pipetting on the microplate and incubated for one hour before analysis. Different concentrations of quercetin—5, 10, 20, and 40 $\mu\text{L}\cdot\text{mL}^{-1}$ —were used as standards. Moreover, a solution of 100 μL of 70% EtOH and 200 μL of 2% aluminum chloride was used as the blank. The total flavonoid content was quantified using the iMark Microplate Reader (Serial number 16548; BIO-RAD Laboratories, Tokyo, Japan) at 420 nm, and the data was read using Microplate Manager 6. To obtain the mean values, all chemical extractions were prepared for three biological replicates. Each extraction was applied to chemical determinations three times.

Extraction and determination of sugar content

The soluble sugar content—including fructose, glucose, and sucrose—was determined by the HPLC method using hot 70% ethanol extract. The method of sugar extraction adopted was the same as that described by Vu et al. (2013). The obtained extract was filtered using a 0.45- μm syringe filter (DISMIC®-13 HP ADVANTEC; Toyo Roshi Kaisha, Ltd.) before being analyzed using a Shimadzu Solusi LC (CBM-20A) HPLC machine equipped with an RI detector (Shimadzu, Kyoto, Japan), an LC-20AD pump (Shimadzu, 3.5 MPa Max 18.0 Min 0.0), and a Shimadzu SIL-20AC autosampler. Sugars from a 20- μL sample were separated on a LiChrospher 100 NH2 250-4.0 column (Kanto Chemical Co., Inc., Tokyo,

Japan). Separation was obtained with 80% acetonitrile with a 35°C column temperature and a flow rate of 0.8 $\text{mL}\cdot\text{min}^{-1}$.

The fructan content was determined using the thio-barbituric acid method (Percheron, 1962) from a 70% ethanol extract. Preparations for fructan analysis were carried out as described by Vu et al. (2013). Fructan quantification was achieved using a spectrophotometer (U-2001; HITACHI High-Technologies Corporation) at a 432-nm wavelength. Chemical extractions were prepared for three replications in order to obtain mean values. Each extraction was applied to triplicate chemical determinations.

Qualification of sugar content

Thin layer chromatography (TLC) was used to separate the sugar fraction of each sample. A hot 70% ethanol extract was spotted on TLC plates before development using a solvent system consisting of 1-butanol : acetic acid : distilled water (2:1:1) in a glass chamber. Sugar fractions were visualized by applying a coloring reagent for fructooligosaccharides (diphenylamine : aniline : 85% phosphoric acid : acetone (1:1:10:100), w/v/v/v) using an ink brush and were heated at 115°C, and 0.025% fructose, glucose, sucrose, 1-kestose, and nystose were used as standard chemicals.

Statistical analysis

All of the data sets obtained from content determinations were used to conduct an *F*-test together with Tukey's honestly significant difference (HSD) test. Principal component analysis (PCA) was conducted to obtain the similarities and differences among all bulb onion varieties and shallot landraces. Regression analysis was carried out to clarify the reliability of the data using two years of data from 2014 and 2015 data sets. All statistical analyses were performed using IBM SPSS statistics 19 (IBM, New York, USA).

Results

S-alk(en)yl-L-cysteine sulfoxides (ACSOs)

MeCSO in shallot landraces from Indonesia was difficult to identify using the first HPLC method. Overlapping peaks were found in the MeCSO peak area, making it impossible to determine which peak represented the MeCSO content. Therefore, the method of amino acid analysis was used to determine the MeCSO content in Indonesian shallot landraces. All of the data sets of three different ACSOs—MeCSO, AlCSO, and PeCSO—from the samples collected in 2014 and 2015 are shown in Table 2. The MeCSO of 2014 was not significantly different among short-day and long-day varieties of bulb onion. However, Indonesian shallot landraces produced higher MeCSO than shallot landraces from Vietnam and both bulb onion types. Data from the next year showed a different tendency. Short-day bulb onions produced significantly higher MeCSO

Table 2. Contents of ACSOs and flavonoid (mg·g⁻¹ FW) in bulb onions and shallots for 2014 and 2015.

Variety or landrace	2014				2015			
	MeCSO	AlCSO	PeCSO	Flavonoid	MeCSO	AlCSO	PeCSO	Flavonoid
<i>Short-day bulb onion</i>								
‘Shippo wase 7 go’	0.08±0.02 ^z de ^z	0.00±0.00 d	0.51±0.02 efg	0.00±0.00 ij	0.29±0.08 bcde	0.01±0.00 h	0.43±0.06 d	0.20±0.02 e
‘Advance’	0.07±0.01 de	0.01±0.00 cd	0.63±0.01 defg	0.00±0.00 ij	0.52±0.14 ab	0.02±0.00 efgh	0.58±0.01 cd	0.25±0.02 de
‘Answer’	0.18±0.02 de	0.00±0.00 d	0.78±0.05 cdefg	0.00±0.00 ij	0.46±0.09 abc	0.01±0.00 gh	0.65±0.02 bcd	0.28±0.02 cde
‘Shippo Ama 70’	0.15±0.01 de	0.01±0.00 cd	0.74±0.03 cdefg	0.00±0.00 ij	0.36±0.12 abcd	0.04±0.00 efg	0.69±0.06 bcd	0.19±0.01 e
‘Tarzan’	0.16±0.02 de	0.02±0.00 abcd	0.69±0.05 cdefg	0.01±0.00 hij	0.27±0.09 bcde	0.01±0.00 gh	0.36±0.01 d	0.28±0.02 cde
‘Momijinokagayaki’	0.14±0.02 de	0.01±0.00 cd	1.08±0.03 bcdef	0.00±0.00 ij	0.60±0.07 a	0.02±0.00 fgh	0.57±0.01 cd	0.27±0.05 cde
‘Momiji 3 go’	0.19±0.01 cd	0.01±0.00 cd	0.97±0.05 abcde	0.02±0.00 ghij	0.55±0.05 a	0.02±0.00 efgh	0.83±0.06 abcd	0.26±0.01 de
<i>Long-day bulb onion</i>								
‘Okhotsk 222’	0.03±0.00 de	0.02±0.00 abcd	0.92±0.09 abcdef	0.06±0.01 fghij	0.05±0.08 e	0.03±0.00 efgh	1.05±0.09 abcd	0.26±0.05 de
‘Kitamomiji 2000’	0.04±0.01 de	0.04±0.01 abcd	1.13±0.21 abcd	0.15±0.04 efghi	0.07±0.02 e	0.02±0.00 fgh	1.23±0.10 abcd	0.27±0.06 cde
‘Super Kitamomiji’	0.02±0.01 e	0.05±0.01 ab	1.23±0.25 abc	0.16±0.03 efgh	0.08±0.21 e	0.03±0.00 efgh	1.55±0.04 ab	0.33±0.04 bcde
<i>Vietnamese shallot</i>								
‘Lyon’	0.17±0.01 de	0.03±0.00 abcd	0.33±0.02 g	0.28±0.05 cde	na	na	na	na
‘Quangai’	0.14±0.01 de	0.03±0.01 abcd	0.40±0.04 fg	0.26±0.02 de	na	na	na	na
‘Soc trang’	0.18±0.02 cde	0.04±0.01 abcd	0.52±0.10 efg	0.16±0.03 efg	na	na	na	na
<i>Indonesian shallot</i>								
‘Biru’	0.43±0.06 ^x ab	0.03±0.01 abcd	1.01±0.01 abcde	0.46±0.05 ab	0.30±0.06 ^x bcde	0.07±0.00 ab	0.99±0.08 abcd	0.49±1.11 abcd
‘Philip’	0.54±0.05 ^x a	0.04±0.00 abcd	1.34±0.19 ab	0.57±0.05 a	na	na	na	na
‘Tiron’	0.47±0.06 ^x ab	0.02±0.00 abcd	0.69±0.26 cdefg	0.51±0.01 ab	0.26±0.04 ^x cde	0.07±0.01 ab	1.25±0.05 abcd	0.52±0.02 abc
‘Thailand’	0.35±0.02 ^x bc	0.04±0.01 abcd	1.42±0.24 a	0.44±0.02 ab	0.23±0.03 ^x cde	0.08±0.00 a	1.41±0.22 abc	0.34±0.03 abcde
‘Bima Juna’	0.56±0.01 ^x a	0.05±0.01 abc	0.97±0.13 abcde	0.41±0.02 bc	0.24±0.03 ^x cde	0.09±0.01 a	1.25±0.27 abcd	0.44±0.06 abcde
‘Bauji Bunder’	0.46±0.04 ^x ab	0.05±0.00 abcd	1.18±0.07 abc	0.43±0.02 abc	0.37±0.01 ^x abcd	0.05±0.01 b	0.60±0.08 cd	0.31±0.03 cde
‘Bauji Plompong’	0.47±0.04 ^x ab	0.06±0.01 a	0.99±0.02 abcde	0.38±0.03 bcd	0.26±0.05 ^x cde	0.03±0.01 efgh	0.96±0.10 abcd	0.44±0.06 abcde
‘Crok Kuning’	0.54±0.01 ^x a	0.05±0.01 a	0.85±0.16 bcdefg	0.47±0.04 ab	0.27±0.05 ^x bcde	0.04±0.01 cdef	0.86±0.22 abcd	0.58±0.06 ab
‘Probolinggo’	0.49±0.07 ^x ab	0.02±0.00 abcd	1.45±0.04 a	0.53±0.05 ab	0.17±0.03 ^x de	0.06±0.01 ab	1.64±0.54 a	0.42±0.03 abcde

^z Means with the same letter in a column are not significantly different according to Tukey’s test ($P < 0.05$).

^y Each value is the mean±SE (n=3).

^x Analyzed using amino acid analysis method.

na: not analyzed.

than the long-day bulb onions and Indonesian shallot landraces. Results of regression analysis showed that the MeCSO data of 2015 did not correlate with those of 2014 ($r = 0.016$) (Fig. 2A).

In the 2014 and 2015 trials, the AlCSO contents of bulb onion varieties were lower than those of shallot landraces. There were no significant differences in AlCSO content between bulb onion varieties. However, two shallot landraces from Indonesia, ‘Thailand’ and ‘Bima Juna’, produced significantly higher AlCSO (0.08 mg·g⁻¹ FW and 0.09 mg·g⁻¹ FW, respectively) than bulb onion varieties in 2015. There was a correlation of AlCSO content between the 2014 and 2015 trials ($r = 0.491^*$) (Fig. 2B). Table 1 shows that the PeCSO contents of bulb onion varieties were not significantly different from those of shallot landraces throughout the two years. As compared to bulb onion PeCSO contents, a relatively higher level of accumulation was observed in one Indonesian shallot landrace, ‘Probolinggo’ (1.45 mg·g⁻¹ FW in 2014 and 1.64 mg·g⁻¹ FW in 2015), as compared with short-day and long-

day bulb onions. Moreover, the PeCSO contents of long-day bulb onions were significantly higher than those of short-day bulb onions. Furthermore, the PeCSO data of 2014 were highly correlated with those of 2015 ($r = 0.800^{**}$) (Fig. 2C).

Flavonoid content

In the 2014 and 2015 trials, a significant difference in flavonoid content was detected between bulb onion varieties and shallot landraces (Table 2). In shallots, one Indonesian landrace, ‘Philip’ had a relatively higher level of flavonoid content (0.57 mg·g⁻¹ FW) than that of Vietnamese landraces. Although significant differences between varieties were not observed, long-day bulb onion varieties produced a higher amount of flavonoids than short-day varieties. Furthermore, shallots showed higher flavonoid contents than bulb onions. The same tendency was found in both experimental years, as the correlation test showed a highly significant correlation ($r = 0.863^{**}$) between the flavonoid data of 2014 and 2015 (Fig. 2D).

Soluble sugar content

The sweetness of bulb onions and shallots seems to be affected by the composition of the soluble sugar content. The total soluble sugar content—including fruc-

tose, glucose, and sucrose—was determined in this research using the HPLC method (Table 3). The statistical analysis data demonstrated that the fructose contents of short-day bulb onions were significantly higher than

Fig. 2. Relationships between data from 2014 and 2015 in MeCSO (A), AICSO (B), PeCSO (C), flavonoid (D), fructose (E), glucose (F), sucrose (G), and fructan (H) contents of bulb onions and shallots in * significant at $P < 0.05$ and ** significant at $P < 0.01$.

Table 3. Contents of soluble sugars ($\text{mg} \cdot \text{g}^{-1}$ FW) in bulb onions and shallots for 2014 and 2015.

Variety or landrace	2014				2015			
	Fructose	Glucose	Sucrose	Fructan	Fructose	Glucose	Sucrose	Fructan
<i>Short-day bulb onion</i>								
'Shippo wase 7 go'	$16.35 \pm 1.73^y \text{ a}^z$	$15.03 \pm 0.48 \text{ abc}$	$6.71 \pm 0.75 \text{ bcde}$	$0.78 \pm 0.13 \text{ h}$	$27.63 \pm 2.00 \text{ abc}$	$34.97 \pm 0.86 \text{ cde}$	$13.19 \pm 3.26 \text{ de}$	$0.12 \pm 0.03 \text{ f}$
'Advance'	$19.42 \pm 1.11 \text{ a}$	$16.43 \pm 0.66 \text{ ab}$	$2.85 \pm 1.22 \text{ e}$	$0.18 \pm 0.04 \text{ h}$	$22.14 \pm 0.72 \text{ cde}$	$31.28 \pm 1.83 \text{ ef}$	$5.75 \pm 3.57 \text{ e}$	$0.06 \pm 0.02 \text{ f}$
'Answer'	$18.83 \pm 0.70 \text{ a}$	$17.03 \pm 0.98 \text{ ab}$	$5.64 \pm 1.78 \text{ cde}$	$0.29 \pm 0.03 \text{ h}$	$28.98 \pm 3.07 \text{ abc}$	$27.30 \pm 1.99 \text{ f}$	$24.98 \pm 0.76 \text{ abcd}$	$0.15 \pm 0.05 \text{ f}$
'Shippo Ama 70'	$16.85 \pm 0.28 \text{ a}$	$15.88 \pm 0.78 \text{ abc}$	$4.13 \pm 0.68 \text{ de}$	$0.39 \pm 0.03 \text{ h}$	$30.81 \pm 2.46 \text{ abc}$	$31.79 \pm 0.87 \text{ def}$	$9.78 \pm 2.96 \text{ de}$	$0.05 \pm 0.02 \text{ f}$
'Tarzan'	$17.22 \pm 0.46 \text{ a}$	$18.18 \pm 0.87 \text{ a}$	$5.86 \pm 1.22 \text{ cde}$	$0.53 \pm 0.07 \text{ h}$	$36.23 \pm 1.32 \text{ a}$	$44.90 \pm 0.89 \text{ a}$	$20.72 \pm 1.23 \text{ abcde}$	$0.16 \pm 0.02 \text{ f}$
'Momijinokagayaki'	$9.33 \pm 1.15 \text{ b}$	$15.44 \pm 1.32 \text{ abc}$	$8.38 \pm 0.81 \text{ bcd}$	$0.91 \pm 0.09 \text{ h}$	$24.50 \pm 0.74 \text{ bcd}$	$38.28 \pm 2.65 \text{ abcde}$	$23.14 \pm 1.23 \text{ abcde}$	$0.69 \pm 0.11 \text{ ef}$
'Momiji 3 go'	$10.10 \pm 1.98 \text{ b}$	$15.69 \pm 1.16 \text{ abc}$	$7.21 \pm 0.82 \text{ bcde}$	$0.98 \pm 0.11 \text{ h}$	$33.77 \pm 4.78 \text{ ab}$	$36.60 \pm 2.64 \text{ bcde}$	$12.95 \pm 5.60 \text{ de}$	$0.22 \pm 0.14 \text{ f}$
<i>Long-day bulb onion</i>								
'Okhotsk 222'	$8.55 \pm 0.38 \text{ b}$	$12.82 \pm 0.21 \text{ c}$	$9.11 \pm 1.20 \text{ bc}$	$0.81 \pm 0.27 \text{ h}$	$15.21 \pm 1.73 \text{ defgh}$	$39.09 \pm 0.93 \text{ abcd}$	$17.84 \pm 4.91 \text{ bcde}$	$0.94 \pm 0.05 \text{ ef}$
'Kitamomiji 2000'	$7.84 \pm 0.14 \text{ bc}$	$15.06 \pm 1.07 \text{ abc}$	$8.59 \pm 0.78 \text{ bc}$	$1.01 \pm 0.22 \text{ h}$	$15.81 \pm 3.10 \text{ defg}$	$36.53 \pm 3.02 \text{ bcde}$	$21.22 \pm 2.39 \text{ abcde}$	$0.73 \pm 0.08 \text{ ef}$
'Super Kitamomiji'	$9.10 \pm 0.92 \text{ b}$	$13.64 \pm 0.61 \text{ bc}$	$8.53 \pm 1.02 \text{ bcd}$	$1.10 \pm 0.25 \text{ h}$	$17.14 \pm 1.75 \text{ def}$	$42.56 \pm 1.36 \text{ ab}$	$20.34 \pm 2.56 \text{ abcde}$	$1.10 \pm 0.13 \text{ ef}$
<i>Vietnamese shallot</i>								
'Lyson'	$8.57 \pm 0.62 \text{ b}$	$8.61 \pm 0.33 \text{ d}$	$6.32 \pm 0.18 \text{ bcde}$	$4.23 \pm 1.01 \text{ efg}$	na	na	na	na
'Quangai'	$9.21 \pm 0.54 \text{ b}$	$8.71 \pm 0.25 \text{ d}$	$6.84 \pm 0.27 \text{ bcde}$	$3.49 \pm 0.65 \text{ fg}$	na	na	na	na
'Soc trang'	$10.04 \pm 0.45 \text{ b}$	$8.25 \pm 0.32 \text{ d}$	$9.70 \pm 0.32 \text{ abc}$	$2.05 \pm 0.47 \text{ gh}$	na	na	na	na
<i>Indonesian shallot</i>								
'Biru'	$0.59 \pm 0.12 \text{ d}$	$0.11 \pm 0.04 \text{ e}$	$7.82 \pm 0.45 \text{ bcd}$	$14.80 \pm 0.54 \text{ a}$	$9.92 \pm 0.87 \text{ fgh}$	$2.18 \pm 0.48 \text{ g}$	$31.24 \pm 3.23 \text{ ab}$	$3.16 \pm 0.40 \text{ de}$
'Philip'	$0.74 \pm 0.05 \text{ d}$	$0.72 \pm 0.25 \text{ e}$	$5.91 \pm 0.22 \text{ cde}$	$13.14 \pm 0.93 \text{ ab}$	na	na	na	na
'Tiron'	$1.64 \pm 0.40 \text{ d}$	$0.62 \pm 0.04 \text{ e}$	$6.73 \pm 0.27 \text{ bcde}$	$12.49 \pm 0.29 \text{ b}$	$6.37 \pm 0.50 \text{ gh}$	$1.66 \pm 0.20 \text{ g}$	$17.36 \pm 3.19 \text{ bcde}$	$4.29 \pm 1.39 \text{ cd}$
'Thailand'	$3.46 \pm 0.08 \text{ d}$	$1.16 \pm 0.07 \text{ e}$	$13.84 \pm 0.67 \text{ a}$	$4.47 \pm 0.29 \text{ ef}$	$5.96 \pm 0.40 \text{ gh}$	$2.25 \pm 0.07 \text{ g}$	$35.16 \pm 2.52 \text{ a}$	$2.84 \pm 0.42 \text{ de}$
'Bima Juna'	$1.16 \pm 0.14 \text{ d}$	$1.07 \pm 0.09 \text{ e}$	$7.95 \pm 0.53 \text{ bcd}$	$6.19 \pm 0.20 \text{ de}$	$9.17 \pm 1.31 \text{ fgh}$	$2.01 \pm 0.24 \text{ g}$	$17.50 \pm 1.23 \text{ bcde}$	$5.20 \pm 0.84 \text{ cd}$
'Bauji Bunder'	$1.07 \pm 0.30 \text{ d}$	$0.52 \pm 0.16 \text{ e}$	$9.92 \pm 0.29 \text{ abc}$	$7.68 \pm 0.83 \text{ cd}$	$5.76 \pm 0.65 \text{ h}$	$1.16 \pm 0.36 \text{ g}$	$24.46 \pm 1.92 \text{ abcd}$	$8.20 \pm 0.75 \text{ ab}$
'Bauji Plompong'	$1.25 \pm 0.09 \text{ d}$	$0.61 \pm 0.20 \text{ e}$	$8.28 \pm 0.40 \text{ bcd}$	$9.28 \pm 0.25 \text{ c}$	$5.59 \pm 0.43 \text{ h}$	$2.11 \pm 0.28 \text{ g}$	$19.02 \pm 2.71 \text{ bcde}$	$9.30 \pm 0.72 \text{ a}$
'Crok Kuning'	$1.33 \pm 0.49 \text{ d}$	$0.53 \pm 0.06 \text{ e}$	$7.98 \pm 0.14 \text{ bcd}$	$7.83 \pm 0.29 \text{ cd}$	$10.16 \pm 0.82 \text{ fgh}$	$2.21 \pm 0.19 \text{ g}$	$29.21 \pm 1.91 \text{ abc}$	$3.98 \pm 0.33 \text{ d}$
'Probolinggo'	$1.47 \pm 0.17 \text{ d}$	$0.58 \pm 0.23 \text{ e}$	$9.29 \pm 0.27 \text{ bc}$	$7.98 \pm 1.13 \text{ cd}$	$12.50 \pm 1.18 \text{ efgh}$	$1.95 \pm 0.19 \text{ g}$	$24.20 \pm 0.93 \text{ abcd}$	$6.65 \pm 0.56 \text{ bc}$

^z Means with the same letter in a column are not significantly different according to Tukey's test ($P < 0.05$).

^y Each value is the mean $\pm$ SE ($n = 3$).

na: not analyzed.

those of long-day bulb onions and shallots. The same tendency was also observed in the glucose content of bulb onion and shallot samples. No significant differences were found in the sucrose contents of bulb onion varieties and shallot landraces from different areas. However, one Indonesian shallot landrace, ‘Thailand’ produced a higher sucrose content in the 2014 and 2015 observations. The same tendency was found throughout the two years, as regression analysis showed a significant correlation for fructose ($r=0.866^{**}$) (Fig. 2E), glucose ($r=0.959^{**}$) (Fig. 2F), and sucrose ($r=0.705^{**}$) (Fig. 2G) contents between 2014 and 2015.

More than 50% of the sugar contents of bulb onions were monosaccharides (fructose and glucose), and the major sugar content in shallot landraces was sucrose. This result was derived from the qualification of soluble sugar content using the TLC method for the 2014 samples. Figure 3 shows that bulb onion varieties had higher amounts of monosaccharides, and that shallot landraces were rich in disaccharides (sucrose) and polysaccharides (1-kestose and nystose). A significantly higher amount of fructans, known as fructose polymers,

could be detected in shallot landraces from Indonesia. The coefficient correlation of fructans contents between 2014 and 2015 was significantly high ($r=0.728^{**}$) (Fig. 2H).

Principal component analysis

The phytochemical properties—including ACSOs, flavonoids, and sugars—of 10 long-day bulb onion varieties, 3 short-day bulb onion varieties, 3 Vietnamese shallot landraces, and 9 Indonesian shallot landraces were analyzed in 2014 and 2015. The PCA of 2014 yielded 2 principal components, accounting for 83.9% of the total variations, divided into 68.3% of the PC1 and 15.6% of the PC2 (Fig. 4A). The loading values of fructose and glucose in the PC1 were found to be negative (-0.812 and -0.920 , respectively), as also observed in the PC1 of the 2015 PCA (-0.814 and -0.971 , respectively). The PC2 in 2014 consisted of sucrose (0.918), PeCSO (0.750), and AlCSO (0.577). However, in the PC2 of 2015, which was only 10.3% of the total variation, this component consisted of MeCSO (-0.925) and PeCSO (0.802). In the PCA analysis of

Fig. 3. TLC profile of soluble sugars in seven short-day bulb onions (SDO), three long-day bulb onions (LDO), three Vietnamese shallots (VNS), and nine Indonesian shallots (IDS). Fructose (F), glucose (G), sucrose (S), 1-kestose (K), and nystose (N) were used as standard compounds.

Fig. 4. Plot of the first and second principal components obtained from a data set of chemical composition analyses in bulb onions and shallots for 2014 (A) and 2015 (B).

2015, the PC1 explained 65.5% of the total variations (Fig. 4B). Both the PCA analyses of 2014 and 2015 could clearly discriminate short-day bulb onions from long-day bulb onions and shallots from a phytochemical point of view.

Discussion

The two groups of bulb onion varieties examined in this study could be differentiated by their photoperiod requirements for bulb formation. Short-day onions initiate bulbs with days 11–12 h long, while long-day onion need days longer than 16 h for bulb formation (Brewster, 2008). By employing two bulb onion types, Marotti and Piccaglia (2002) reported that the different photoperiods of the tested cultivars did not influence the bulb yield, but did affect the dry matter content. The same tendency was also found in this study where the dry matter content of long-day onions was higher ($\pm 11\%$) than that of short-day onions ($\pm 9\%$) (data not shown).

Organosulfur compounds, known as flavor precursors (Brewster, 2008), are related to the pungent taste of bulb onions and shallots (Block, 1992). In previous studies, MeCSO and PeCSO were found to be the main compounds correlated to pungency in bulb onions and shallots (Lee et al., 2009). However, AlCSO content did not lead to a stronger pungent taste as it was found at low concentrations or even undetected (Yoo and Pike, 1998). Lee et al. (2009) reported that the high pungency level achieved in their research occurred with $0.15 \text{ mg} \cdot \text{g}^{-1}$ FW of MeCSO and $0.60 \text{ mg} \cdot \text{g}^{-1}$ FW of PeCSO in bulb onions. Therefore, our shallot landraces, which possessed high levels of MeCSO ($0.49 \text{ mg} \cdot \text{g}^{-1}$ FW) and PeCSO ($1.45 \text{ mg} \cdot \text{g}^{-1}$ FW) contents were very pungent. Furthermore, the amounts of organosulfur compounds are affected by the variety, maturity, soil fertility, and other growing conditions (Saghir et al., 1965). In this study, long-day bulb onions, cultivated from March to September and grown during the summer, showed a higher PeCSO content than short-day bulb onions cultivated from September to March. It is reasonable to say that the level of pungency in bulb onions and shallots increases with temperature (Brewster, 2008). Shallots from Indonesia, cultivated in the dry season (June to September), also possessed high amounts of PeCSO and MeCSO. Regarding their edibility, Kimura et al. (2014) mentioned the importance of pungency for salad use of bulb onions and shallots. Actually, less pungent bulb onions are suitable for raw consumption, while strong pungency is needed for shallots, which are mainly used as a condiment.

Flavonoids are responsible for the bitterness of bulb onions and shallots (Astraya et al., 2007). Leighton et al. (1992) reported that, in addition to genetic background, environmental conditions also affect flavonoid production. Furthermore, the bulb onions and shallots used in this study were cultivated at different latitudes,

possibly leading to the different flavonoid contents produced (Jaakola and Hohtola, 2010). Differences in latitude may account for the differences in day length, light quality, UV radiation, and temperature. However, in agreement with the results of this research, it has been reported that shallots produce higher levels of flavonoids than bulb onions (Fattorusso et al., 2002; Leighton et al., 1992). In fact, shallots from Indonesia, which were cultivated near the equator and exposed to high UV radiation, produced the highest levels of flavonoids. Long-day bulb onions cultivated in Hokkaido also produced a higher amount of flavonoids than short-day types. Flavonoids play an important role in plant defense mechanisms against abiotic stresses such as high UV radiation (Harborne and Williams, 2000). The high flavonoid content found in Indonesian shallots makes them more bitter than other shallots and bulb onions. Moreover, long-day bulb onions, which are more suitable for processed food, also possessed a more bitter taste than short-day bulb onions. Further organoleptic analysis will be needed to explore the palatability of bulb onions as compared with shallots.

The quality of bulb onions and shallots as horticultural products depends on the dry matter contents of their bulbs. Darbyshire and Steer (1990) explained that most of the dry matter in bulb onions (65–80%) consists of non-structural carbohydrates, including fructose, glucose, sucrose, and fructans. As shown in Table 3, the *F*-test of this study detected fructose and glucose as major contents, followed by sucrose, in short-day bulb onions. This result was similar to that of Lee et al. (2009). Fructose and glucose contents in bulb onions were positively correlated with sweetness (Terry et al., 2005). However, the combination of high PeCSO and flavonoid contents with the low amount of monosaccharides in shallots may create a specific taste different from bulb onions. Such differences lead to different consumer preferences for bulb onions and shallots in the human diet. Therefore, it is recommended that the amino acid content be analyzed to better understand the difference in taste and flavor precursors in bulb onions and shallots. Furthermore, a high level of fructan content was also observed in shallots. This result is very interesting because Brewster (2008) reported that most species with high fructan accumulations are from temperate regions and not from tropical regions. However, Raines et al. (2009) reported a positive correlation between dry weight and fructan content in bulb onions. The shallots used in this study also had dry weights higher than those of bulb onions (data not shown). Moreover, the accumulation of fructans could be highly correlated with drought or cold stress tolerance (Livingston III et al., 2009). As a practical matter, transgenic tobaccos with a drought-resistant gene produce high levels of fructans due to an upregulatory effect that protects the membrane and other cellular components by inducing cell wall hardening and limiting cell growth to reduce

the water demand (Pilon-Smits et al., 1995). Therefore, we assumed that the higher production of fructans in the Indonesian shallots was closely related to cultivation conditions. It was suggested that the high fructan content of these shallots is a kind of plant-defense mechanism against fungal and pathogenic attacks (Kawakami and Yoshida, 2012; Van den Ende et al., 2004). Based on Brewster (2008), the degree of polymerization (DP) of fructans in bulb onions and shallots is up to 20. However, Yaguchi et al. (2009) reported that the DP of shallots was less than 12, as we observed in the lower part of sugar profiling using the TLC method. Further research will be needed to measure the effect of different climatic conditions on the production of fructans in shallots.

The results of PCA analyses clearly discriminate between bulb onions and shallots based on their area of cultivation. Indonesian shallots tended to accumulate soluble sugars in a polysaccharide form and produce high levels of flavonoids, as also reported by Leighton et al. (1992). Furthermore, in our previous report, we proved that 75–80% of shallot dry matter consisted of carbohydrates with a high level of sugars (Rabinowitch and Kamenetsky, 2002). Moreover, the fructan content represented 33–83% of the total sugars (Vu et al., 2013). In general, bulb onions accumulated high levels of monosaccharides, along with low levels of sulfur compounds and flavonoids, resulting in a mild and sweet taste. On the other hand, shallots produced a higher amount of polysaccharides, flavonoids, and ACSOs, leading to strong pungency and more bitter tastes. Between short-day and long-day bulb onion types, the PeCSO content became the main differentiating factor. Higher amounts of PeCSO content in long-day bulb onions clearly distinguished them from short-day bulb onions.

In conclusion, bulb onions and shallots possessed different taste characteristics based on their chemical compositions. The results of this research will be very important for producers and consumers in determining the quality of Japanese bulb onion varieties and shallot landraces in production fields, as well as at markets. Moreover, these results also underscore the importance of developing an F_1 hybrid between shallots and bulb onions to improve the taste quality of bulb onions. The similarities in the morphological characteristics of inflorescence and flowers, karyotype, and chromosome behaviors in the meiosis of the shallot and the bulb onion suggest indirectly that they are closely related (Tashiro et al., 1982). There is no serious internal barrier between shallots and bulb onions, as F_1 and F_2 plants exhibited hybrid vigor and full fertility. Further study of an F_1 hybrid between a cytoplasmic male sterile shallot with *A. galanthum* cytoplasm and the common onion reported three morphological characteristics of haploid plants—shallots, hybrid, and common onion types—and double haploid plants displayed integrated charac-

teristics between shallots and bulb onions (Sulistyaningsih et al., 2002). The future challenge in onion breeding is to produce an F_1 bulb onion hybrid with a common shape and size, but with higher eating quality derived from the shallot. All in all, we have elucidated here the partial metabolomic profile of all leading bulb onion varieties in Japan, together with some representative shallot landraces for Southeast Asia, which will allow us to provide a novel indicator for characterizing some genotypes of *A. cepa*. Further research related to amino acid content and intermediate forms of flavor precursors in bulb onions and shallots will be beneficial in better understanding the regulatory system for taste and flavor.

Literature Cited

- Abdelrahman, M., Y. Sawada, R. Nakabayashi, S. Sato, H. Hirakawa, M. El-Sayed, M. Y. Hirai, K. Saito, N. Yamauchi and M. Shigyo. 2015. Integrating transcriptome and target metabolome variability in doubled haploids of *Allium cepa* for abiotic stress protection. *Mol. Breed.* 35: 195.
- Astraya, G., L. García-Ríob, J. C. Mejuto and L. Pastranac. 2007. Chemistry in food: flavours. *Electronic J. Environ. Agric. Food Chem.* 6: 1742–1762.
- Benkeblia, N. 2004. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *LWT-Food Sci. Technol.* 37: 263–268.
- Block, E. 1992. The organosulfur chemistry of the genus *Allium*—implications for the organic chemistry of sulfur. *Angew. Chem. Int. Ed. Engl.* 31: 1135–1178.
- Brewster, J. L. 2008. Onions and other vegetable *Alliums*, 2nd Ed. CABI, Wallingford.
- Darbyshire, B. and B. T. Steer. 1990. Carbohydrate biochemistry. Onions and allied crops 3: 1–16.
- Fattorusso, E., M. Iorizzi, V. Lanzotti and O. Tagliatela-Scafati. 2002. Chemical composition of shallot (*Allium ascalonicum* Hort.). *J. Agric. Food Chem.* 50: 5686–5690.
- Harborne, J. A. and C. A. Williams. 2000. Advances in flavonoid research since 1992. *Phytochemistry* 55: 481–505.
- Jaakola, L. and A. Hohtola. 2010. Effect of latitude on flavonoid biosynthesis in plants. *Plant Cell Environ.* 33: 1239–1247.
- Kawakami, A. and M. Yoshida. 2012. Graminase breakdown by fructan exohydrolase induced in winter wheat inoculated with snow mold. *J. Plant Physiol.* 169: 294–302.
- Kays, S. J. and W. Yan. 2000. Thermally induced flavor compounds. *HortScience* 35: 1002–1012.
- Kimura, Y., K. Okazaki, D. Yanagida and T. Muro. 2014. Cultivar and regional differences in the metabolite composition of onion (*Allium cepa*). *Sci. Hortic.* 168: 1–8.
- Kopsell, D. E. and W. M. Randle. 1997. Onion cultivars differ in pungency and bulb quality changes during storage. *HortScience* 32: 1260–1263.
- Lee, E. J., K. S. Yoo, J. Jifon and B. S. Patil. 2009. Characterization of shortday onion cultivars of 3 pungency levels with flavor precursor, free amino acid, sulfur, and sugar contents. *J. Food Sci.* 74: 475–480.
- Leighton, T., C. Ginther, L. Fluss, W. K. Harter, J. Cansado and V. Notario. 1992. Molecular characterization of quercetin and quercetin glycosides in *Allium* vegetables: their effects on malignant cell transformation. p. 220–238. In: ACS symposium series (USA).
- Livingston III, D. P., D. K. Hinch and A. G. Heyer. 2009.

- Fructan and its relationship to abiotic stress tolerance in plants. *Cell. Mol. Life Sci.* 66: 2007–2023.
- Marotti, M. and R. Piccaglia. 2002. Characterization of flavonoids in different cultivars of onion (*Allium cepa* L.). *J. Food Sci.* 63: 1229–1232.
- Masamura, N., S. Yaguchi, Y. Ono, T. Nakajima, S. Masuzaki, S. Imai, N. Yamauchi and M. Shigyo. 2011. Characterization of amino acid and *S*-alk(en)yl-L-cysteine sulfoxide production in Japanese bunching onion carrying an extra chromosome of shallot. *J. Japan. Soc. Hort. Sci.* 80: 322–333.
- Percheron, F. 1962. Dosage colorimétrique du fructose et des fructo-furanosides par l'acide thiobarbiturique. *C. R. Acad. Sci.* 255: 2521–2522.
- Pilon-Smits, E. A. H., M. J. M. Ebskamp, M. J. Paul, M. J. W. Jeuken, P. J. Weisbeek and S. C. M. Smeekens. 1995. Improved performance of transgenic fructan-accumulating tobacco under drought stress. *Plant Physiol.* 107: 125–130.
- Rabinowitch, H. D. and R. Kamenetsky. 2002. Shallot (*Allium cepa*, *Aggregatum* Group). p. 404–430. In: H. D. Rabinowitch and L. Currah (eds.). *Allium Crop Science: Recent Advances*. CABI Publishing, Wallingford.
- Raines, S., C. Henson and M. J. Havey. 2009. Genetic analyses of soluble carbohydrate concentrations in onion bulbs. *J. Amer. Soc. Hort. Sci.* 134: 618–623.
- Randle, W. M. and J. E. Lancaster. 2002. Sulfur compounds in *alliums* in relation to flavour quality. p. 329–356. In: H. D. Rabinowitch and L. Currah (eds.). *Allium Crop Science: Recent Advances*. CABI Publishing, Wallingford.
- Saghir, A. R., L. K. Mann and M. Yamaguchi. 1965. Composition of volatiles in *Allium* as related to habitat, stage of growth, and plant part. *Plant Physiol.* 40: 681.
- Shigyo, M. and C. Kik. 2008. Onion. p. 121–159. In: J. Prohens and F. Nuez (eds.). *Handbook of Plant Breeding, Vegetables*, Vol II. Springer, New York.
- Sulistyaningsih, E., K. I. Yamashita and Y. Tashiro. 2002. Haploid induction from F_1 hybrids between CMS shallot with *Allium galanthum* cytoplasm and common onion by unpollinated flower culture. *Euphytica* 125: 139–144.
- Tamaki, M., M. Ukai, M. Murata and S. Homma. 2002. Cooking and proceeding quality of onions (*Allium cepa*) cultivated in Hokkaido. *J. Japan. Assoc. Food Preserv. Sci.* 28: 291–298.
- Tashiro, Y., S. Miyazaki and K. Kanazawa. 1982. On the shallot cultivated in the countries of Southeastern Asia. *Bull. Fac. Agr. Saga Univ.* 53: 65–73.
- Terry, L. A., K. A. Law, K. J. Hipwood and P. H. Bellamy. 2005. Non-structural carbohydrate profiles in onion bulbs influence taste preference. p. 33–40. In: *The proceedings of the 5th Fruit, Nut and Vegetable Production Engineering Symposium (FRUTIC 05)*, September 12–16, 2005, Montpellier.
- Van den Ende, W., B. De Coninck and A. Van Laere. 2004. Plant fructan exohydrolases: a role in signaling and defense? *Trends Plant Sci.* 9: 523–528.
- Vu, Q. H., M. El-Sayed, S. Ito, N. Yamauchi and M. Shigyo. 2012. Discovery of a new source of resistance to *Fusarium oxysporum*, cause of *Fusarium* wilt in *Allium fistulosum*, located on chromosome 2 of *Allium cepa* *Aggregatum* group. *Genome* 55: 797–807.
- Vu, Q. H., T. T. M. Hang, S. Yaguchi, Y. Ono, T. M. P. Pham, N. Yamauchi and M. Shigyo. 2013. Assessment of biochemical and antioxidant diversities in a shallot germplasm collection from Vietnam and its surrounding countries. *Genet. Res. Crop Evol.* 60: 1297–1312.
- Yaguchi, S., T. T. M. Hang, H. Tsukazaki, V. Q. Hoa, S. Masuzaki, T. Wako, N. Masamura, S. Onodera, N. Shiomi, N. Yamauchi and M. Shigyo. 2009. Molecular and biochemical identification of alien chromosome additions in shallot (*Allium cepa* L. *Aggregatum* group) carrying extra chromosome(s) of bunching onion (*A. fistulosum* L.). *Genes Genet. Syst.* 84: 43–55.
- Yoo, K. S. and L. M. Pike. 1998. Determination of flavor precursor compound *S*-alk(en)yl-L-cysteine sulfoxides by an HPLC method and their distribution in *Allium* species. *Sci. Hortic.* 75: 1–10.