



Classification and Screening of Baby-leaf Vegetables on the Basis of Their Yield, External Appearance and Internal Quality

Masayoshi Takahama^{1,2}, Koji Kawagishi³, Akito Sugawara⁴, Kazuya Araki⁵,
Shinya Munekata⁶, Silvana Nicola⁷ and Hajime Araki^{8*}

¹Graduate School of Environmental Science, Hokkaido University, Sapporo 060-0810, Japan

²Donan Agricultural Experiment Station, Agricultural Research Development, Hokkaido Research Organization, Hokuto 041-1201, Japan

³Ornamental Plants and Vegetables Research Center, Agricultural Research Development, Hokkaido Research Organization, Takikawa 073-0026, Japan

⁴Hokkaido Nuclear Energy Environmental Research Center, Kyowa 045-0123, Japan

⁵Kitami Agricultural Experiment Station, Agricultural Research Development, Hokkaido Research Organization, Kunneppu 099-1496, Japan

⁶Central Agricultural Experiment Station, Agricultural Research Development, Hokkaido Research Organization, Naganuma 069-1395, Japan

⁷Vegetable Crops & Medicinal and Aromatic Plants, Department of Agricultural, Forest and Food Sciences, University of Turin, 10095 Grugliasco (Turin), Italy

⁸Field Science Center for Northern Biosphere, Hokkaido University, Sapporo 060-0811, Japan

“Baby-leaf vegetables” is a new category of leafy vegetables that are used in juvenile stage mixtures of different types of leafy vegetables. They include Brassicaceae, Asteraceae, Amaranthaceae, and other crops. The emergence periods, growing periods, total cultivation periods, from sowing to harvest, as well as the yields, SPAD values, ascorbic acid and nitrate concentrations, leaf characteristics and post-harvest shelf lives of 22 baby-leaf crops were investigated in April 2010 and April 2011. A principal component analysis, based on the total cultivation periods, yields, SPAD values, as well as the ascorbic acid and nitrate concentrations, indicated three main groups: Brassicaceae crops, which had short total cultivation periods and high ascorbic acid concentrations; Asteraceae crops (except for ‘Italian Red’ chicory), which had long total cultivation periods and low ascorbic acid concentrations; Amaranthaceae crops, which had comparatively high yields and low nitrate concentrations. ‘Italian Red’ chicory did not fall into any of these three groups. The yield had very limited effects on the grouping. The higher-yield crops tended to have more leaves and lower dry matter ratios, and the crops with higher SPAD values tended to have thicker leaves. An ideal assortment of baby-leaf crops was proposed based on an evaluation of the yield, external appearance and internal quality of each crop.

Key Words: ascorbic acid, cultivation period, nitrate, SPAD value.

Introduction

“Baby-leaf vegetables” is a new category of leafy vegetables that are used in juvenile stage mixtures of

different types of leafy vegetables. They include Brassicaceae (e.g. mizuna, mustard, rucola); Asteraceae (e.g. romaine lettuce, endives); Amaranthaceae (e.g. spinach, table beet according to the Angiosperm Phylogeny Group III (APG III) system); and other vegetable leaves. These crops have a wide range of leaf shapes, colors, textures, and flavors (Fujime, 2005). In 2016, the Japanese market scale of baby-leaf vegetables was ca. 10 billion yen (90 million USD) and the annual Japanese consumption was 18 g per capita (ca. 2,160 metric tons in total) (Sato, 2016), reflecting the fact that

Received; August 6, 2018. Accepted; March 2, 2019.

First Published Online in J-STAGE on May 25, 2019.

No conflicts of interest declared.

This work was supported by Hokkaido University Research and Education Center for Robust Agriculture, Forestry and Fisheries Industry.

* Corresponding author (E-mail: araki@fsc.hokudai.ac.jp).

baby-leaf vegetable production in Japan was much smaller than in the USA (more than 110,000 metric tons) in 2008 (Hardesty, 2015) and in Europe (303,000 metric tons in Italy, the UK, France, and Germany) in 2015 (Nicola et al., 2016). However, the distribution of baby-leaf vegetables in the Tokyo Metropolitan Central Wholesale Market has been increasing over the years, and the market is expected to grow to 30 billion yen, that is, Japanese consumers will consume the same volume as Europeans (50 g per capita) (Sato, 2016). In fact, the importance of baby-leaf vegetable cultivation has recently increased due to increased market demand in the country. For this reason, the establishment of production in Hokkaido, which is located in the northern subarctic area of Japan (Dfa or Dfb, according to the Köppen [1936] classification), has been anticipated as a new production area in addition to the original production area in the south Japan (Cfa, according to the Köppen [1936] classification).

Very little technical information is available on the cultivation of baby-leaf vegetables in Japan, although there are some guidelines and reports in North America and Europe (Grahn et al., 2015a; Nicola et al., 2016). As far as agricultural practices are concerned, it is important to standardize the crop cycles in order to simultaneously harvest several different crops with the optimal timing: an early harvest leads to a low yield, while a late harvest leads to a non-commercial leaf size, as leaves become larger and older and are considered outside the category of “baby-leaf” vegetables.

On the other hand, because baby-leaf vegetables are eaten fresh at home and in restaurants, the external appearance and internal quality are very important for marketing (Tokita, 2005). More than 20 crops are recognized as baby-leaf crops. However, when baby-leaf vegetables are served to consumers, different types are selected and mixed considering volume, flavor and color. It is important to investigate many quality characteristics in a variety of leafy crops from a mix-use system point of view. There are some reports on the qualities of baby-leaf crops: some examples refer to the ascorbic acid concentrations in mizuna (Martínez-Sánchez et al., 2008), watercress (Martínez-Sánchez et al., 2008; Pignata et al., 2016), leaf lettuce (Kroggel et al., 2011; Samuolienė et al., 2012), rucola (Hall et al., 2015; Martínez-Sánchez et al., 2008; Nordmark et al., 2014), spinach (Morgen et al., 2012), corn salad (Nordmark et al., 2014), and komatsuna (Kroggel et al., 2011); the nitrate concentrations in green leaf lettuce (Aires et al., 2013), red leaf lettuce (Aires et al., 2013), watercress (Aires et al., 2013), rucola (Aires et al., 2013; Nicola et al., 2015), chard (Aires et al., 2013), corn salad (Aires et al., 2013; Fontana et al., 2004), garden cress (Nicola et al., 2015), spinach (Nicola et al., 2015), and bladder campion (Nicola et al., 2015); and the shelf lives of spinach (Conversa et al., 2014; Medina et al., 2012) and rucola (Nicola et al., 2003).

However, fewer than 5 crops were checked in terms of quality measurements, even though yield research was carried out by using 10 to 20 crops (Borrelli et al., 2013; Fujime, 2005).

The aim of the present study was to clarify the characteristics of the growth and quality at the juvenile stage of 22 leafy crops belonging to three botanical families for comparative evaluation in the southern part of Hokkaido, Japan. Five characteristics, namely, the SPAD value, ascorbic acid and nitrate concentrations, leaf characteristics and shelf life, were measured to evaluate quality. Similarities were analyzed by principal component analysis (PCA) among the 22 leafy vegetables. The obtained information will be useful for decision making regarding baby-leaf vegetable assortments by farmers.

Materials and Methods

Baby-leaf crops and experimental design

The experiments were carried out in the same unheated plastic greenhouse in the Donan Agricultural Experiment Station, Hokkaido, Japan (41°53'11" N, 140°39'14" E and 25 m a.s.l.) in 2010 and 2011. The ventilation started to work when the internal temperature reached 20°C. The air temperature was recorded every hour during each experimental period at about 20 cm above the ground, near the expanding plant leaves, using TR71S thermometer recorders (T and D Corp., Nagano, Japan). The sunshine duration was obtained from AMeDAS (Automated Meteorological Data Acquisition System) in Hokuto, Hokkaido, Japan. An aliquot of 1.2 kg of N, 1.0 kg of P₂O₅, and 0.8 kg of K₂O per are was applied to the brown lowland soil (loam) in the greenhouse and the soil was adjusted from pH 6.0 to 6.5 by adding calcium carbonate. Ten Brassicaceae, nine Asteraceae and three Amaranthaceae leafy crops were grown in the greenhouse (Table 1; Fig. 1). All the seeds were purchased from Nakahara Seed (Fukuoka, Japan), except for ‘Kiwame Chuba Shungiku’ (crown daisy, CD), which was purchased from Takii & Co., Ltd. (Kyoto, Japan). Sowing was performed on 25 March 2010 and 24 March 2011, respectively. All crops were sown in rows 0.1 m apart. One hundred seeds per meter were manually cast within each row. The sowing density, which was based on standard local production practices, was 1,000 seeds·m⁻², and each plot area was 1.0 m². Water was supplied when the pF at 10 cm depth under the ground reached between 2.5 and 2.6. The experimental design was a randomized complete block design with 22 crops over two years and included two plots. Two-way ANOVA was conducted on the below-mentioned items with families and years as factors. Tukey-Kramer’s multiple comparison was conducted within each family when there was no significant interaction for two-way ANOVA. Statistical analysis was performed with EZR (Saitama Medical Center, Jichi Medical University,

Table 1. Leaf shapes and colors of materials used in this study and harvesting criteria based on market research.

Scientific name	Commercial name	Abbreviation	Blade shape ^z	Color (Blade/Vein)	Harvesting criteria	
					Leaf length (mm)	Leaf area (mm ²)
Brassicaceae						
<i>Brassica rapa</i> var. <i>laciniifolia</i>	Wase Mizuna	WM	Spathulate incised	Green/Green	120	1,000–1,500
<i>Brassica juncea</i> ssp.	Green Karashi Mizuna (mustard)	GKM	Spathulate incised	Green/Green	120	1,000–1,500
<i>Brassica rapa</i> var. <i>perviridis</i>	Pino Green (komatsuna)	PG	Elliptic	Green/Green	80	1,000–1,500
<i>Brassica rapa</i> var. <i>rosularis</i>	Tat-Soi	TS	Elliptic	Green/Green	80	1,000–1,500
<i>Brassica oleracea</i> var. <i>acephala</i>	Green Kale	GK	Elliptic dentate	Green/Green	80	1,000–1,500
<i>Brassica juncea</i> ssp.	Green Mustard	GM	Elliptic crenate	Green/Green	80	1,000–1,500
<i>Eruca vesicaria</i> ssp.	Rucola	RC	Elliptic lyrate	Green/Green	80	1,000–1,500
<i>Brassica oleracea</i> var. <i>acephala</i>	Red Kale	RK	Spathulate incised	Green/Red	80	1,000–1,500
<i>Brassica juncea</i> ssp.	Red Karashi Mizuna (mustard)	RKM	Spathulate incised	Red/Red	120	1,000–1,500
<i>Brassica juncea</i> ssp.	Red Mustard	RM	Elliptic crenate	Red–green/Red	80	1,000–1,500
Asteraceae						
<i>Lactuca sativa</i> var. <i>longifolia</i>	Green Romaine	GR	Spathulate	Green/Green	80	1,000–1,500
<i>Lactuca sativa</i> var. <i>crispa</i>	Lollo Green	LG	Spathulate undulate	Green/Green	<80	<2,000
<i>Cichorium endivia</i>	Endive	EN	Spathulate undulate	Green/Green	80	1,000–1,500
<i>Glebionis coronarium</i>	Kiwame Chuba Shungiku (crown daisy)	CD	Lyrate	Green/Green	80	1,000–1,500
<i>Lactuca sativa</i> var. <i>crispa</i>	Green Oak	GO	Lyrate	Green/Green	80	1,000–1,500
<i>Lactuca sativa</i> var. <i>longifolia</i>	Red Romaine	RR	Spathulate	Red/Red	80	1,000–1,500
<i>Lactuca sativa</i> var. <i>crispa</i>	Lollo Rossa	LR	Spathulate undulate	Red/Red	<80	<2,000
<i>Lactuca sativa</i> var. <i>crispa</i>	Red Oak	RO	Lyrate	Red/Red	80	1,000–1,500
<i>Cichorium intybus</i>	Italian Red (chicory)	IR	Spathulate	Green/Red–green	80	1,000–1,500
Amaranthaceae						
<i>Spinacia oleracea</i> ssp.	Green Spinach	GS	Elliptic	Green/Green	80	1,000–1,500
<i>Spinacia oleracea</i> ssp.	Red Spinach	RS	Sagittate	Green/Red	80	1,000–1,500
<i>Beta vulgaris</i>	Detroit (table beet)	DT	Oblong	Green/Red	80	1,000–1,500

^z Terminologies of leaf shapes are quoted from Garden Plant Encyclopedia (Tsukamoto, 1994).

Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). EZR is actually a modified version of R commander, which was designed to add the statistical functions that are frequently used in biostatistics and statistics software.

Determination of the cultivation period

The day when more than 50% of the seeds had emerged was recognized as the emergence day for each crop. The emergence period was calculated as the period from the sowing date to the emergence date. The growing period was considered to be the difference between the harvest date, as defined below, and the emergence date. The total cultivation period was computed as the sum of the days necessary for emergence and the growing period.

Harvesting method

The leaf size of each crop in eight different commercial baby-leaf packages was first measured to determine the optimum size for use as a criteria to harvest the leaves in the experiments (Takahama et al., unpublished data). The optimal leaf length was found to be about

8 cm, and a leaf area of around 1,500 mm² was considered optimal (Table 1). True leaves were harvested using scissors between 11am and 2pm. The fresh weight (FW) per 0.1 m² was measured to determine the yield (g·m⁻²). The yield/total cultivation period was used as an index of additional productivity. These measurements were carried out for each plot.

SPAD value

The SPAD value was measured using a SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan) on the first day of the post-harvest evaluation, as explained hereafter.

Ascorbic acid concentration

Five g FW of leaves from each plot were cut into small pieces and homogenized with 45 or 20 mL of 5% metaphosphoric acid at 10,000 rpm for 30 s. Each homogenate was filtered through No. 5A quantitative filter paper (Advantech, Tokyo, Japan). An aliquot of each extract was analyzed with an RQflex plus reflectometer (Merck, Millipore, MA, USA) and a Reflectoquant Ascorbic Acid Test (Merck, Millipore, MA, USA). These measurements were carried out for each plot.

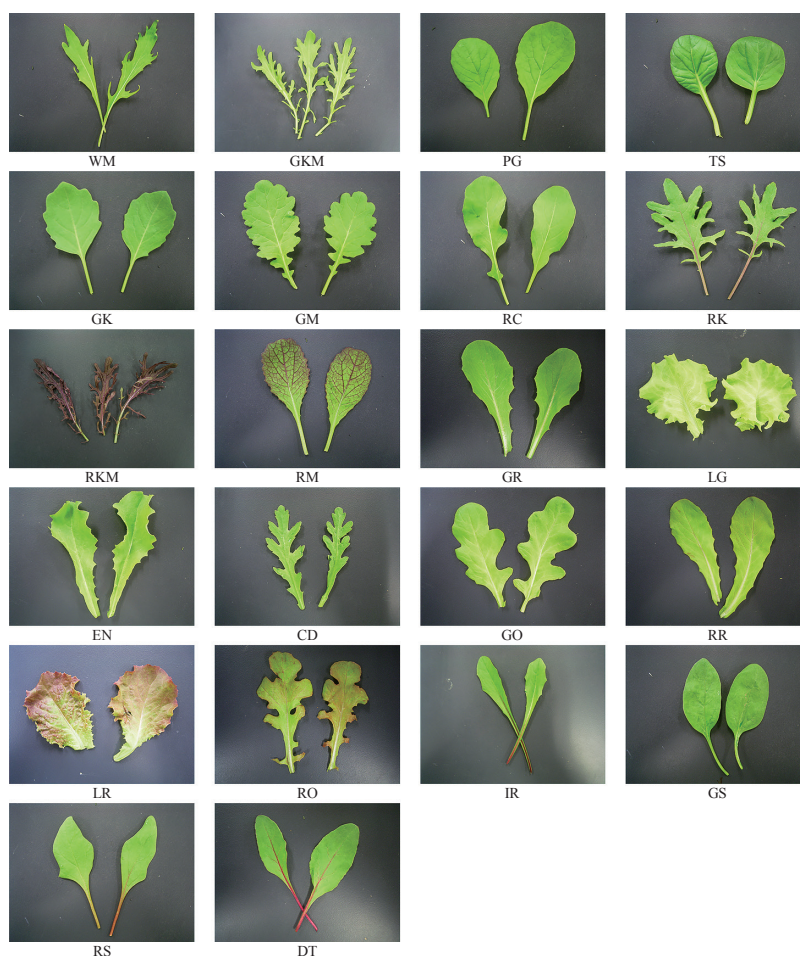


Fig. 1. Appearances of the 22 baby-leaf crops. See Table 1 for the abbreviations.

Nitrate concentration

Five g FW of leaves from each plot were cut into small pieces and homogenized with 45 mL of distilled water at 10,000 rpm for 30 s. Each homogenate was filtered through No. 5A quantitative filter paper. One mL of each extract was diluted in 4 mL of distilled water and analyzed with an RQflex plus reflectometer and a Reflectoquant Nitrate Test (Merck, Millipore). These measurements were carried out for each plot.

Leaf characteristics

Aliquots of 20 large homogenous leaves were prepared for each crop at harvest, and the leaf weight, leaf length, leaf width and leaf area were measured. The leaf area was calculated by comparing the number of pixels on the scanned leaf image with a control image, for which the area had been established, using a GT-8700F scanner (Seiko Epson, Nagano, Japan) and Photoshop Element software (Adobe Systems, CA, USA). The leaf weight/leaf area ratio was considered to be an indicator of leaf thickness (Wright and Westoby, 2002). The number of leaves was obtained by dividing the yield by the mean leaf weight. More than 30 g of harvested leaves were dried at 60°C using a drying oven until a

constant weight was reached in order to calculate the dry matter ratio. These measurements were carried out for each plot.

PCA characteristics of the 22 crops

PCA was conducted using data on the total cultivation periods, yields, SPAD values, and ascorbic acid and nitrate concentrations obtained in 2010 and 2011 to reveal the characteristics of the 22 baby-leaf crops. Statistical analysis was performed with the above-mentioned EZR.

Correlations of yield and SPAD value with leaf characteristics

Correlation coefficients of yield and SPAD value were calculated using the leaf weight, leaf number, leaf length, leaf width, leaf area, leaf weight/leaf area and dry matter ratio. Statistical analysis was performed with the above-mentioned EZR.

Post-harvest evaluation

The post-harvest evaluation started from 22 April 2010 or 19 April 2011 in the 10 Brassicaceae crops, and from 28 April 2010 or 24 April 2011 in the nine

Asteraceae and three Amaranthaceae crops, respectively. Samples of FW leaves (20 g) were collected from the two plots. The samples were divided into 3–4 g FW lots and packed in five commercial plastic punnets (158 mm × 110 mm internal dimensions, 67 mm depth), which were then wrapped in a plastic film. The packages were kept in the dark at 5°C and at 70–80% relative humidity using an MIR-153 incubator (Sanyo, Osaka, Japan). The external appearance was judged by opening the punnets every two days. Shelf life was determined as the number of days that passed until the day at which the following conditions were observed: (1) more than half of the samples had wilted (the leaf tips drooped when the leaf stalks were held); (2) more than half of the samples were turning yellow (SPAD value reduced by about 50% compared to that of the harvest time); and (3) more than two leaves in the samples had decayed. In addition, the condition at the end of shelf life was investigated in each sample. Two-way ANOVA and Tukey-Kramer's multiple comparison test were conducted on the shelf life, and the χ^2 test was conducted on the ratio of occurrence of the above-mentioned conditions.

Results

Environmental conditions during the cultivation period

The greenhouse environmental conditions were compared over the two years of the experiments (Fig. 2). The mean temperatures for 35 days from sowing in 2010 (from 25 March to 28 April) and 2011 (from 24 March to 27 April) were 10.8 and 11.3°C, respectively. The daily minimum temperature for one week after sowing in 2010 was lower than that in 2011 (−2.6 and −1.3°C, respectively). The total sunshine durations for the first 25/35 days from sowing in 2010 and 2011 were 135/182 and 167/191 hours, respectively.

Cultivation periods

Significant interactions between families and years were observed in the emergence period ($P=0.028$) (Table 2). All the crops emerged earlier in 2010 than in 2011. 'Green Romaine' (GR), CD, 'Green Oak' (GO),

and 'Detroit' (DT) took more than three days longer to emerge in 2010 (9.0, 10.5, 9.0, and 11.0 days, respectively) than in 2011 (6.0, 6.5, 6.0, and 8.0 days, respectively). The Brassicaceae crops (6.7 in 2010 and 5.6 days in 2011, respectively) tended to emerge earlier than the Asteraceae crops (8.5 in 2010 and 6.2 days in 2011, respectively) and Amaranthaceae crops (8.6 in 2010 and 7.7 days in 2011, respectively). The Asteraceae crops (23.8 days) grew slower than the Brassicaceae and Amaranthaceae crops (19.2 and 18.2 days, respectively), and 'Italian Red' (IR, 27.3 days) tended to be the slowest-growing crop. The total cultivation period for the Brassicaceae crops was significantly shorter than that in the Asteraceae crops (25.4 and 31.2 days, respectively; $P < 0.001$). The difference between the earliest crop ('Pino Green' (PG), 23.3 days) and the latest (IR, 35.8 days) was 12.5 days. The growing period and total cultivation period showed no significant interaction ($P = 0.403$ and 0.158 , respectively), but were significantly longer in 2010 than in 2011 ($P < 0.001$ and < 0.001 , respectively).

Yields and productivities

No significant difference in yield was observed among the three families ($P = 0.272$), although some differences were observed among the crops (Table 3). The highest yields in the Brassicaceae crops were observed for 'Wase Mizuna' (WM, 882 g·m⁻²) and 'Tat-Soi' (TS, 870 g·m⁻²), and the lowest was for 'Red Kale' (RK, 478 g·m⁻²). The highest yields in the Asteraceae crops were for 'Lollo Green' (LG, 842 g·m⁻²) and 'Lollo Rossa' (LR, 801 g·m⁻²), and the lowest was for IR (379 g·m⁻²). The yield of each Amaranthaceae crop was more than 700 g·m⁻². The yield/total cultivation period ratios in the Asteraceae crops were significantly lower than those in the other families ($P = 0.002$). PG, WM, and TS (35.1, 34.9, and 34.2 g·m⁻²·day⁻¹, respectively) showed the highest values, and IR showed the lowest (10.6 g·m⁻²·day⁻¹).

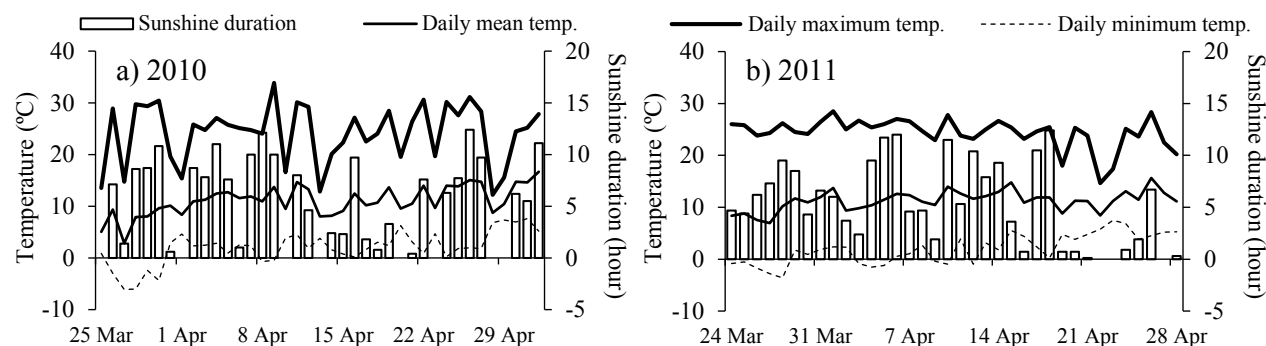


Fig. 2. Temperature lines and sunshine duration bars during the cultivations. The temperatures were measured inside the plastic greenhouse. The sunshine duration was obtained from AMeDAS (Automated Meteorological Data Acquisition System) in Hokuto, Hokkaido, Japan.

Table 2. Emergence, growing and total cultivation periods of 22 baby-leaf crops.

Abbreviation ^z	Emergence period (days)		Growing period (days)			Total cultivation period (days)		
	2010	2011	2010	2011	Mean ^y	2010	2011	Mean
Brassicaceae								
WM	5.5	5.0	20.5	19.5	20.0 ab ^x	26.0	24.5	25.3 ab
GKM	7.0	6.0	19.5	18.0	18.8 ab	26.5	24.0	25.3 ab
PG	5.5	5.0	19.0	17.0	18.0 b	24.5	22.0	23.3 b
TS	6.5	6.0	19.5	19.0	19.3 ab	26.0	25.0	25.5 ab
GK	7.5	6.0	23.5	20.0	21.8 a	31.0	26.0	28.5 a
GM	6.0	5.0	20.5	17.5	19.0 ab	26.5	22.5	24.5 ab
RC	7.0	5.0	19.5	19.0	19.3 ab	26.5	24.0	25.3 ab
RK	8.0	6.5	19.0	18.5	18.8 ab	27.0	25.0	26.0 ab
RKM	7.0	6.0	21.5	18.0	19.8 ab	28.5	24.0	26.3 ab
RM	7.0	5.5	18.5	17.0	17.8 b	25.5	22.5	24.0 ab
Mean	6.7	5.6	20.1	18.4	19.2	26.8	24.0	25.4
SE	0.08	0.18	1.49	1.03	1.13	1.80	1.28	1.42
Asteraceae								
GR	9.0	6.0	24.0	22.5	23.3 ad	33.0	28.5	30.8 ab
LG	7.5	5.5	25.5	23.0	24.3 ad	33.0	28.5	30.8 ab
EN	8.0	6.0	30.0	23.0	26.5 ab	38.0	29.0	33.5 ab
CD	10.5	6.5	21.5	22.5	22.0 cd	32.0	29.0	30.5 ab
GO	9.0	6.0	23.0	21.0	22.0 cd	32.0	27.0	29.5 ab
RR	7.5	5.5	23.5	22.0	22.8 bcd	31.0	27.5	29.3 ab
LR	8.0	6.5	26.0	25.0	25.5 ac	34.0	31.5	32.8 ab
RO	8.0	6.0	21.5	20.5	21.0 d	29.5	26.5	28.0 b
IR	9.0	8.0	29.0	25.5	27.3 a	38.0	33.5	35.8 a
Mean	8.5	6.2	24.9	22.8	23.8	33.4	29.0	31.2
SE	0.32	0.25	3.04	1.64	2.18	2.91	2.22	2.40
Amaranthaceae								
GS	8.5	8.0	16.5	16.0	16.3 c	25.0	24.0	24.5 b
RS	8.0	7.0	17.5	18.0	17.8 b	25.5	25.0	25.3 b
DT	11.0	8.0	21.0	20.0	20.5 a	32.0	28.0	30.0 a
Mean	8.6	7.7	18.3	18.0	18.2	27.5	25.7	26.6
SE	0.93	0.33	2.36	2.00	2.16	3.91	2.08	2.98
P (ANOVA)								
Family	<0.001			0.005		<0.001		
Year	<0.001			<0.001		<0.001		
Interaction	0.028			0.403		0.158		

^z See Table 1 for abbreviations.

^y See Table 2 for total cultivation period.

^x FW = fresh weight.

^w Based on the data in 2010 and 2011.

^v Means followed by a different letter within columns are not significantly the same at $P < 0.05$ by Tukey-Kramer's multiple comparison within each family.

Quality

1. SPAD values

The SPAD values of the Brassicaceae crops ranged from 24.4 ('Green Karashi Mizuna' (GKM) and 'Green Mustard' (GM)) to 40.4 (TS) (Table 3). The values of four *Brassica juncea* crops (GKM, 'Red Karashi Mizuna' (RKM), GM, and 'Red Mustard' (RM)) were all below 30. The SPAD values of most of the Asteraceae crops were below 30. LG and GO showed the lowest SPAD values (14.6 and 17.2, respectively).

Three Amaranthaceae crops had significantly higher SPAD values than the Asteraceae crops ($P < 0.001$). Furthermore, the SPAD values in 2010 were significantly higher than those in 2011 ($P < 0.001$).

2. Ascorbic acid concentrations

The ascorbic acid concentrations in the Brassicaceae crops tended to be higher than those in the Asteraceae crops both in 2010 and 2011 (Table 3). PG (635 in 2010 and 920 $\mu\text{g}\cdot\text{g}^{-1}$ FW in 2011, respectively) and 'Green Kale' (GK, 635 in 2010 and 905 $\mu\text{g}\cdot\text{g}^{-1}$ FW in 2011,

Table 3. Yields, SPAD values, and ascorbic acid and nitrate concentrations of 22 baby-leaf crops.

Abbreviation ^z	Yield	Yield/total cultivation period ^y			SPAD value			Ascorbic acid		Nitrate
	(g·m ⁻²)	(g·m ⁻² ·day ⁻¹)						(µg·g ⁻¹ FW ^x)		(µg·g ⁻¹ FW)
	Mean ^v	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
Brassicaceae										
WM	882 a ^v	34.2	35.6	34.9 a	30.4	23.1	26.7 d	565	745	4,563 a
GKM	821 ab	31.6	33.4	32.5 a	24.9	24.0	24.4 d	585	785	3,613 ab
PG	812 ab	33.0	37.2	35.1 a	38.4	36.3	37.4 ab	635	920	3,325 ab
TS	870 a	31.1	37.2	34.2 a	39.2	41.7	40.4 a	495	625	4,100 ab
GK	490 cd	16.2	18.4	17.3 c	40.1	34.2	37.1 ab	635	905	2,925 ab
GM	616 bd	21.4	29.5	25.4 ac	25.5	23.4	24.4 d	630	850	2,975 ab
RC	517 cd	18.3	22.9	20.6 bc	32.8	29.2	31.0 c	675	760	2,925 ab
RK	478 d	15.8	21.3	18.6 bc	35.4	34.0	34.7 b	635	865	3,888 ab
RKM	741 ab	29.3	27.0	28.1 ab	26.9	26.0	26.5 d	545	765	3,925 ab
RM	594 bd	22.7	27.1	24.9 ac	29.7	25.1	27.4 cd	580	825	2,300 b
Mean	682	25.4	29.0	27.2	32.3	29.7	31.0	598	805	3,454
SE	51	2.3	2.1	2.2	5.7	6.5	1.9	17	28	216
Asteraceae										
GR	648 ac	20.0	22.4	21.2 ab	31.8	30.9	31.3 ab	440	405	2,125 b
LG	842 a	27.1	27.8	27.5 a	14.9	14.3	14.6 e	265	350	2,075 b
EN	781 ab	18.9	29.1	24.0 a	30.3	24.9	27.6 bc	360	345	4,038 a
CD	744 ab	22.2	26.7	24.5 a	26.5	26.1	26.3 c	205	210	2,138 b
GO	734 ab	21.5	28.9	25.2 a	18.0	16.5	17.2 de	325	390	2,513 b
RR	766 ab	20.2	32.9	26.5 a	29.8	24.1	27.0 c	375	320	2,438 b
LR	801 a	22.3	26.8	24.5 a	20.8	20.5	20.7 d	175	155	1,975 b
RO	525 bc	15.0	22.9	18.9 ab	23.6	16.8	20.2 d	365	350	1,825 b
IR	379 c	10.7	10.5	10.6 b	35.8	27.7	31.8 a	340	380	4,838 a
Mean	691	19.8	25.3	22.5	25.7	22.4	24.1	317	323	2,663
SE	50	1.6	2.1	1.7	6.9	5.7	2.0	29	28	349
Amaranthaceae										
GS	717	25.8	32.8	29.3	36.0	32.1	34.1	565	465	1,813
RS	753	32.1	27.5	29.8	32.8	30.4	31.6	495	640	1,725
DT	848	27.0	29.7	28.3	36.0	30.3	33.1	495	335	2,163
Mean	772	28.3	30.0	29.1	34.9	30.9	32.9	518	480	1,900
SE	39	1.9	1.5	0.4	1.8	1.1	1.2	23	88	134
P (ANOVA)										
Family	0.272		0.002			<0.001		<0.001		<0.001
Year	0.535		0.026			<0.001		0.011		0.052
Interaction	0.716		0.634			0.283		<0.001		0.541

^z See Table 1 for abbreviations.^y See Table 2 for total cultivation period.^x FW = fresh weight.^v Based on the data in 2010 and 2011.^v Means followed by a different letter within columns are not significantly the same at $P < 0.05$ by Tukey-Kramer's multiple comparison within each family.

respectively) were the crops with the highest ascorbic acid concentrations, and LR (175 in 2010 and 155 µg·g⁻¹ FW in 2011, respectively) was the one with the lowest. However, a significant interaction between families and years was observed ($P < 0.001$). The ascorbic acid concentration in the Brassicaceae crops was higher in 2011 (805 µg·g⁻¹ FW) than in 2010 (598 µg·g⁻¹ FW), although there were few differences in ascorbic acid concentrations between the years in the

Asteraceae (317 in 2010 and 323 µg·g⁻¹ FW in 2011, respectively) and Amaranthaceae crops (518 in 2010 and 480 µg·g⁻¹ FW in 2011, respectively).

3. Nitrate concentrations

The nitrate concentrations in most of the Brassicaceae crops were above 2,500 µg·g⁻¹ FW, except for RM (2,300 µg·g⁻¹ FW) (Table 3). Most of the Asteraceae crops had values below 2,500 µg·g⁻¹ FW, although two *Cichorium* crops (IR and 'Endive' (EN))

had values above $4,000 \mu\text{g}\cdot\text{g}^{-1}$ FW. The nitrate concentrations in the three Amaranthaceae crops showed lower values and ranged from 1,725 ('Red Spinach' (RS)) to $2,163 \mu\text{g}\cdot\text{g}^{-1}$ FW (DT).

4. Leaf characteristics

The leaf weights ranged from 286 (IR) to $673 \mu\text{g}/\text{leaf}$ (PG) (Table 4). The leaves of the Amaranthaceae crops ($591 \mu\text{g}/\text{leaf}$) were generally heavier than those of the Brassicaceae and Asteraceae crops (505 and $410 \mu\text{g}/$

leaf, respectively). The leaf numbers ranged from less than 1,000 (GK) to more than $2,000 \text{ leaves}\cdot\text{m}^{-2}$ (WM, EN and 'Red Romaine' (RR)). In most crops, the leaf lengths ranged from 70 to 120 mm and the leaf areas ranged from 1,000 to $2,000 \text{ mm}^2$, respectively. The leaf lengths of TS, LG, and LR were less than 70 mm and were shorter than any other crops. There was no significant difference in leaf width or leaf area among the three families ($P = 0.387$ and 0.550 , respectively). LG

Table 4. Leaf characteristics of 22 baby-leaf crops.

Abbreviation ^z	Leaf weight ($\mu\text{g}/\text{leaf}$)	Leaf number ^y ($\text{leaves}\cdot\text{m}^{-2}$)			Leaf length (mm)	Leaf width (mm)	Leaf area (mm^2)	Leaf weight/ leaf area ($\mu\text{g}\cdot\text{mm}^{-2}$)	Dry matter ratio (%)
	Mean ^x	2010	2011	Mean	Mean	Mean	Mean	Mean	Mean
Brassicaceae									
WM	385 d ^w	2,093	2,529	2,311 a	117.0 a	27.0 d	1,047 b	0.37 ab	6.9 ab
GKM	510 bd	1,635	1,606	1,620 bc	114.3 a	34.6 bc	1,301 ab	0.40 a	7.0 ab
PG	673 a	1,141	1,285	1,213 cd	81.8 b	38.2 ab	1,720 a	0.39 ab	7.3 ab
TS	615 ab	1,326	1,501	1,414 cd	65.5 d	39.2 ab	1,537 ab	0.40 a	6.1 b
GK	534 ac	843	1,013	928 d	81.0 b	38.9 ab	1,558 ab	0.34 ab	8.6 a
GM	488 bd	1,110	1,439	1,275 cd	76.8 bc	36.8 bc	1,513 ab	0.32 ab	6.6 ab
RC	478 bd	990	1,182	1,086 cd	76.0 bd	32.1 cd	1,402 ab	0.34 ab	7.0 ab
RK	463 cd	983	1,070	1,026 d	79.9 bc	43.5 a	1,184 ab	0.39 ab	8.4 a
RKM	373 d	2,110	1,853	1,981 ab	115.5 a	32.1 cd	1,460 ab	0.26 b	6.9 ab
RM	534 ac	1,267	998	1,132 cd	70.0 cd	39.2 ab	1,653 a	0.32 ab	6.9 ab
Mean	505	1,350	1,448	1,399	87.8	36.2	1,438	0.35	7.2
SE	29	143	149	141	6.3	1.5	66	0.01	0.2
Asteraceae									
GR	479 ac	1,215	1,535	1,375	77.2 a	34.6 bc	1,633 ab	0.29 b	6.8 b
LG	526 ab	1,520	1,713	1,617	61.9 b	54.5 a	2,025 a	0.26 bc	5.3 b
EN	374 cd	1,608	2,805	2,206	77.8 a	27.5 cd	1,276 bd	0.29 b	6.9 b
CD	390 bd	1,800	1,992	1,896	78.3 a	25.2 d	986 d	0.40 a	6.5 b
GO	383 bd	1,647	2,266	1,957	77.3 a	38.5 b	1,670 ab	0.23 c	6.7 b
RR	356 cd	1,826	2,446	2,136	77.9 a	28.8 cd	1,297 bd	0.27 bc	5.7 b
LR	548 a	1,511	1,435	1,473	62.0 b	61.1 a	2,003 a	0.27 bc	5.5 b
RO	350 cd	1,150	1,936	1,543	79.9 a	37.9 b	1,514 bc	0.23 c	6.6 b
IR	286 d	1,389	1,244	1,316	86.2 a	23.1 d	1,091 cd	0.26 bc	9.2 a
Mean	410	1,518	1,930	1,724	75.4	36.8	1,499	0.28	6.6
SE	29	78	169	111	2.7	4.4	123	0.02	0.4
Amaranthaceae									
GS	631	976	1,307	1,141	90.2 a	33.9	1,600 a	0.40 b	6.5
RS	554	1,332	1,406	1,369	89.2 a	32.4	1,340 b	0.41 b	7.0
DT	588	1,430	1,451	1,440	77.6 b	31.4	1,240 b	0.47 a	6.5
Mean	591	1,246	1,388	1,317	85.7	32.6	1,393	0.43	6.7
SE	22	138	43	90	4.0	0.7	107	0.02	0.1
P (ANOVA)									
Family	<0.001		0.002		0.001	0.387	0.550	<0.001	0.051
Year	0.056		0.042		0.966	0.396	0.616	0.066	0.111
Interaction	0.615		0.265		0.939	0.882	0.227	0.568	0.072

^z See Table 1 for abbreviations.

^y Leaf number was obtained by yield/leaf weight.

^x Based on the data in 2010 and 2011.

^w Means followed by a different letter within columns are not significantly the same at $P < 0.05$ by Tukey-Kramer's multiple comparison within each family.

(54.5 mm and 2,025 mm², respectively) and LR (61.1 mm and 2,003 mm², respectively) showed the widest and largest leaves of the Asteraceae crops. The leaf weight/leaf area ratios were significantly higher in the Amaranthaceae crops than in the Asteraceae crops (0.43 and 0.28 µg·mm⁻², respectively; $P < 0.001$). DT showed the highest leaf weight/leaf area (0.47 µg·mm⁻²) of all the crops. The dry matter ratios in most of the crops ranged from 5 to 7%. IR (9.2%), GK (8.6%), and RK (8.4%) had the highest dry matter ratio of all the crops.

PCA based on the characteristics of the 22 baby-leaf crops

The component characteristics were shown in Table 5. Each eigenvalue in Component 1 and 2 was over 1.0, and the cumulative contribution ratio reached 68.4%. Strong positive correlations were observed for the ascorbic acid concentration and the SPAD value in Component 1, and a strong negative correlation was observed for the total cultivation period. A positive correlation with yield and a negative one with nitrate concentration were found for Component 2. Three groups were recognized in a scatterplot made up of Components 1 and 2 (Fig. 3). Group I contained the Brassicaceae crops and Group II contained most of the Asteraceae crops. Group III fell between Groups I and II, and the Amaranthaceae crops belonged to Group III. ‘Green Spinach’ (GS) and RS were positioned near Group I, while DT was located near Group II. All the groups overlapped the coordinate of Component 3 (data not shown). IR did not belong to any of the three groups.

Table 5. Factor loading, eigenvalue and contribution ratio of each principal component in PCA.

Characteristics	Component No.		
	1	2	3
Total cultivation period	-0.741	-0.619	-0.152
Yield	-0.226	0.680	-0.683
SPAD value	0.710	-0.235	-0.105
Ascorbic acid	0.946	0.120	0.106
Nitrate	0.470	-0.535	-0.622
Eigenvalue	2.22	1.20	0.90
Contribution	44.4	24.0	18.0
Cumulative contribution	44.4	68.4	86.4

Correlations of the yield and SPAD value with leaf characteristics

The correlation coefficients of yield and SPAD value with leaf characteristics are shown in Table 6. The yield had a significant positive correlation with leaf number ($r = 0.554$) and a significant negative correlation with dry matter ratio ($r = -0.702$). The SPAD value had the most positive correlation with leaf weight/leaf area ($r = 0.632$).

Post-harvest evaluation

The shelf life of the Amaranthaceae crops was significantly longer than that of the Brassicaceae crops (26.6 and 18.3 days, respectively; $P < 0.001$) (Table 7). The occurrence rates of wilting, yellowing and decay were different among the three families ($P < 0.001$). Yellowing only occurred in the Brassicaceae crops, except for WM. The shelf lives of the *Brassica juncea* crops (GKM, GM, RKM, and RM; 16.2, 15.6, 16.2, and 15.2 days, respectively) were shorter than the mean of the Brassicaceae crops (18.3 days). Decay occurred in all the Asteraceae crops, and the decay occurrence ratio of IR was the highest (70%). GR and RR had the longest shelf lives in the Asteraceae crops and had significantly longer ones than CD. There was no significant difference in shelf life among the three Amaranthaceae crops. The shelf lives of the Amaranthaceae crops were mainly determined by the occurrence of decay. The shelf

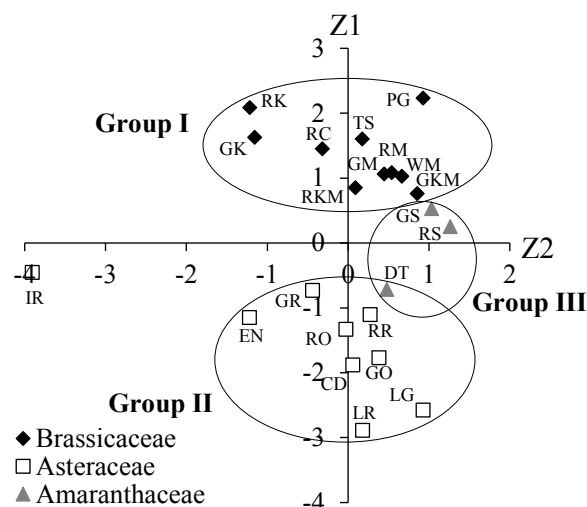


Fig. 3. Scatterplot of the principal component scores of the 22 baby-leaf crops. The Z1 and Z2 axes are defined by Components 1 and 2 in Table 5.

Table 6. Pearson product-moment correlation coefficients of the yield and SPAD value with leaf characteristics.

	Leaf weight	Leaf numbers	Leaf length	Leaf width	Leaf area	Leaf weight/leaf area	Dry matter ratio
Yield	0.346 NS ^z	0.554 **	0.121 NS	0.119 NS	0.159 NS	0.298 NS	-0.702 ***
SPAD value	0.432 *	-0.485 *	0.051 NS	-0.329 NS	-0.304 NS	0.632 **	0.483 *

^z NS, *, **, *** Not significant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

Table 7. Post-harvest evaluation of 22 baby-leaf crops.

Abbreviation ^z	Shelf life (days)			Occurrence (%) ^y		
	2010	2011	Mean ^x	Wilting	Yellowing	Decay
				Mean	Mean	Mean
Brassicaceae						
WM	20.0	22.4	21.2 ab ^w	60	0	40
GKM	14.4	18.0	16.2 c	40	60	0
PG	18.4	20.8	19.6 b	50	50	0
TS	20.4	22.4	21.4 ab	30	70	0
GK	22.4	25.6	24.0 a	30	70	0
GM	16.0	15.2	15.6 c	0	100	0
RC	16.8	20.0	18.4 bc	30	70	0
RK	13.2	17.2	15.2 c	0	100	0
RKM	15.6	16.8	16.2 c	10	90	0
RM	14.4	16.0	15.2 c	0	100	0
Mean	17.2	19.4	18.3	25	71	4
SE	1.0	1.1	1.0	7	10	4
Asteraceae						
GR	28.0	26.4	27.2 a	70	0	30
LG	18.4	20.8	19.6 bc	80	0	20
EN	20.4	19.6	20.0 bc	70	0	30
CD	18.8	17.2	18.0 c	70	0	30
GO	17.2	22.4	19.8 bc	90	0	10
RR	23.6	23.2	23.4 ab	70	0	30
LR	18.0	22.0	20.0 bc	60	0	40
RO	19.6	22.0	20.8 bc	60	0	40
IR	18.8	19.6	19.2 bc	30	0	70
Mean	20.3	21.5	20.9	67	0	33
SE	1.1	0.9	0.9	6	0	6
Amaranthaceae						
GS	23.6	26.8	25.2	100	0	0
RS	22.8	27.6	27.6	90	0	10
DT	24.8	29.2	27.0	80	0	20
Mean	23.7	27.9	26.6	90	0	10
SE	0.6	0.7	0.7	6	0	6
<i>P</i> (ANOVA)				<i>P</i> (χ^2 test)		
Family	<0.001			Family	<0.001	
Year	<0.001					
Interaction	0.089					

^z See Table 1 for abbreviations.

^y Evaluated at the end of shelf life in each sample.

^x Based on the data in 2010 and 2011.

^w Means followed by a different letter within columns are not significantly the same at $P < 0.05$ by Tukey-Kramer's multiple comparison within each family.

lives in general were significantly longer in 2011 than in 2010, especially for the Amaranthaceae crops (23.7 in 2010 and 28.0 days in 2011, respectively).

Discussion

Emergence and growth characteristics of baby-leaf crops

Farmers need to have information available concerning the cultivation period of each crop in order to en-

sure stable and sustainable production. In the present study, the total cultivation period of IR was more than 10 days longer than that of PG (Table 2). Therefore, it may be difficult to grow these two crops with the same timing.

The results on the total cultivation periods of the different families coincided with previous reports (Grahn et al., 2015b). Furthermore, it was revealed that the emergence and growth characteristics of the three fami-

lies used in our experiment were different: Brassicaceae crops emerged faster, Asteraceae crops grew more slowly and Amaranthaceae crops emerged more slowly and grow faster. On the other hand, the emergence, growing and total cultivation periods in most of the crops, especially the emergence periods in some *Lactuca sativa* crops (GR and GO) and the *Beta vulgaris* crop (DT), were longer in 2010 than in 2011 (Table 2). Such results were considered to have been influenced by the lower daily minimum temperature that occurred for one week after sowing in 2010 (Fig. 2). Previous reports mentioned that the minimum emergence temperatures of *Lactuca sativa* and *Beta vulgaris* were higher than those of some Brassicaceae crops and spinach (Bierhuizen and Wagenvoort, 1974). The lower temperature could have prevented the emergence of these crops in 2010. For actual spring production, temperature management could be very important in these crops.

Difference in yield among baby-leaf crops

The yield factor information regarding the different crops is considered the most important for farmers. Some research have reported the yields of baby-leaf vegetables in open fields (Grahn et al., 2015a) and hydroponic cultures (Nicola et al., 2016). We researched and compared the yields of a wide array of crops, that is, many more than the available reports related to unheated plastic greenhouses.

Grahn et al. (2015b) reported that *Brassica* crops grown in an open field had higher mean yields than lettuce or chenopod crops across seasons (spring and fall). However, in our study, no significant difference in yield was observed among the three families investigated (Table 3). Each Amaranthaceae crop showed a high yield and consistent productivity (Table 3). Therefore, these crops could be considered suitable for baby-leaf production. Nevertheless, their yields varied depending on the growing season and location (Grahn et al., 2015b). Our experiments were carried out in spring, which was suitable for growth evaluation considering leafy vegetable production in a greenhouse. However, yields will need to be studied in other growing seasons and at different locations.

Quality required by consumers

A market research questionnaire survey in 2009 (n = 73) indicated that 81% of the contacted Hokkaido consumers knew what baby-leaf vegetables were, and 67% of them had already eaten them (Takahama, unpublished data). Baby-leaf vegetables are probably more popular in Japan in 2019. The consumers (n = 73) stated that baby leaves were “tasty”, “colorful”, “fashionable”, and “healthy” (77%, 58%, 52%, and 37%, respectively) (Takahama, unpublished data). Therefore, it is important to consider the external appearance, as well as the internal quality, of baby-leaf vegetables, so here the

SPAD value was investigated as one of the external appearance quality aspects in addition to ascorbic acid and nitrate concentrations as internal qualities.

1. Differences among baby-leaf crops and effect of climate conditions on the SPAD value

Wang et al. (2005) reported that the visual-quality grades of these plants were closely correlated with the SPAD readings. In the present experiment, the SPAD values were substituted with the greenness of baby-leaf vegetables. A significant difference in SPAD value was found among families. LG and GO had pale green leaves (Table 3), and are considered useful for salad mix combinations together with other dark green leaf crops. Moreover, the SPAD values varied according to the year (Table 3). Junjun et al. (2014) reported that a lack of light caused a decrease in the SPAD value in rice. The short sunshine duration at the harvest time in 2011 (Fig. 2) could have caused the low SPAD values in some crops.

2. Differences among baby-leaf crops and effects of climate conditions on ascorbic acid concentration

Ascorbic acid is an important compound because of its antioxidant effect in humans (Bendich et al., 1986). The ascorbic acid concentrations in most of the Brassicaceae crops, except for RKM, were higher than those in the other tested families (Table 3). The results obtained in the present study were in agreement with the data of Szeto et al. (2002) and Wheeler et al. (1939). The rapidly growing Brassicaceae crops had higher ascorbic acid concentrations in 2011 than in 2010, although there were few differences between the years in the slowly growing Asteraceae crops. In general, the lower the light intensity during growth, the lower the ascorbic acid content of the plant tissues (Harris, 1975). The sunshine duration was longer until the middle of April 2011, in which most of the Brassicaceae crops were harvested, than in 2010 (Fig. 2). However, the sunshine duration was almost the same until late April, in which most of the Asteraceae crops were harvested in 2011 and in 2010. The significant interaction between families and years could have been influenced by the sunshine duration.

3. Nitrate concentration characteristics in baby-leaf crops

The nitrate concentration has been investigated in terms of human health. The maximum levels of nitrates in foodstuffs have been determined in the European Union (Gorenjak and Cencič, 2013), although the Food Safety Commission of Japan indicated that daily intake of nitrates was not harmful; information on nitrates in baby-leaf vegetables is necessary for marketing purposes. Santamaria (2006) and Aires et al. (2013) pointed out that Brassicaceae, Asteraceae and Chenopodiaceae crops (classified as Amaranthaceae according to APG III) had higher nitrate concentrations than other families. In the present experiment, the Brassicaceae crops showed significantly higher nitrate

concentrations than the Amaranthaceae crops (Table 3). However, RM could be a suitable crop for health-conscious consumers because it tended to have a lower nitrate concentration than any other Brassicaceae crops. The RC had a much lower nitrate concentration than the EU limits for the rucola crop group ($7,000 \mu\text{g}\cdot\text{g}^{-1}$ FW) (Gorenjak and Cencič, 2013). On the other hand, the nitrate concentration in EN exceeded $4,000 \mu\text{g}\cdot\text{g}^{-1}$ FW (Table 3), which is higher than the maximum level allowed for trading of endives in some European countries (Santamaria, 2006). Reducing the nitrate level of this crop through improvements in cultural practices is therefore advisable.

Classification based on the characteristics of 22 baby-leaf crops

The PCA divided the 22 baby-leaf crops into three main groups (Table 5; Fig. 3). The Brassicaceae crops had short total cultivation periods and high ascorbic acid concentrations; most of the Asteraceae crops had long total cultivation periods and low ascorbic acid concentrations, while the Amaranthaceae crops had high yields and low nitrate concentrations. IR did not fall into any of the three groups. Therefore, it is not considered suitable for use as a baby-leaf crop because of its longer total cultivation period and lower yield. Espíndola et al. (2015) reported the growth and yield of baby-leaf chicory not only under plastic tunnel and non-woven protection conditions, but also considering plant spacing; some improvements in the cultivation of IR, pertaining to the environmental conditions and sowing densities, were required to achieve earlier production and higher productivity.

There was a significant difference in yield among the Brassicaceae and Asteraceae crops (Table 4). Therefore, we analyzed the relationship between the yield and the leaf characteristics of the 22 crops, regardless of their families. The yield showed a significant positive correlation with leaf number, and a significant negative correlation with dry matter ratio (Table 6). For this reason, the fresh leaf yield was higher in the crops that had more leaves with a higher water content. Therefore, the Amaranthaceae crops were evaluated as high-yielding crops in this experiment along with the leafy WM and juicy TS, LG, and LR. Leaf number and dry matter ratio could be useful indicators to evaluate the yield of crops other than those used in our experiment.

Furthermore, crops with higher leaf weight/leaf area ratios tended to have higher SPAD values (Table 6). Because the leaf weight/leaf area ratio is one of the indicators used to establish leaf thickness (Wright and Westoby, 2002), leaf thickness could be related to the depth of leaf color. The positive relationship between leaf thickness and SPAD value was already observed for sorghum and pigeon pea (Yamamoto et al., 2002), although it was not seen in green-leaved foliage plants (Wang et al., 2005). This relationship may not be

common to all plants, but it could apply to baby-leaf vegetables. Crops with thicker leaves, such as Amaranthaceae, may be used as deeper-colored crops, and ones with thinner leaves, such as Asteraceae, may be used as paler-colored crops.

Screening of baby-leaf vegetables based on their yield, external appearance and internal qualities

Based on PCA, some crops located in the 1st or 4th quadrant of Figure 3 could be considered suitable baby-leaf vegetables from a yield point of view, while those located in the 1st or 2nd quadrant of Figure 3 had high ascorbic acid concentrations. On the contrary, those located in the 3rd quadrant of Figure 3, such as IR, could not be suitable baby-leaf vegetables in terms of both yield and internal qualities. Considering the above discussion and the SPAD values of individual crops, a possible assortment of baby-leaf crops could be; PG (shortest total cultivation period, higher yield, and higher ascorbic acid), RM (lower nitrate and red leaves), RS (higher yield, lower nitrate, and red vein leaves), LG (higher yield and paler green spathulate undulated leaves), and RR (higher yield and red leaves). A mixture of different shaped and colored leaves is also important to commercial value and is based on external appearance (Table 1). As a result, baby-leaf vegetables consisting similar types of crops can reduce commercial attractiveness. Furthermore, mustard (GKM, GM, RKM, and RM) and rucola (RC) have characteristic flavors. Therefore, farmers should select crops according to not only their growth characteristics and internal quality, but also flavor and external appearance in addition to the SPAD value.

Difference in shelf life and negative factors among baby-leaf crops

The shelf lives of 22 baby-leaf crops were assessed based on their external appearance (Table 7). A significant difference in shelf life was found among the three families. Furthermore, external appearance used to determine the shelf lives were yellowing in the Brassicaceae crops and decay in the Asteraceae crops. Some of these characteristics agreed with a previous report (Cantwell et al., 1998). On the other hand, the shelf lives in the Amaranthaceae crops seemed to be different between the years, despite the same post-harvest conditions. A previous report mentioned that high quantities of rainfall during the growing season reduced the storage potential of spinach by 40% on average (Johnson et al., 1989). Therefore, the difference in the water availability as a result of irrigation management in a plastic greenhouse could affect the shelf lives in the Amaranthaceae crops. On the other hand, shelf life is defined not only on the basis of wilting, yellowing and decay, but also according to the texture, aroma, weight loss, physiological disorders, microbial activity and internal qualities of crops (Ferrante et al., 2004; Martínez

and Artés, 1999; Nicola et al., 2006; Tokuchi et al., 2012). We recommend the use of GR, GS, RS, and DT as they are crops with longer shelf lives (27.2, 25.2, 27.6, and 27.0 days, respectively), but future investigations will concentrate on surveying and analyzing the weight loss and internal quality declines of baby-leaf crops.

Acknowledgements

The authors thank Dr. Toshiyuki Hirata Field Science Center for Northern Biosphere, Hokkaido University, for his useful advice concerning the statistical analysis.

Literature Cited

- Aires, A., R. Carvalho, E. A. Rosa and M. J. Saavedra. 2013. Effects of agriculture production systems on nitrate and nitrite accumulation on baby-leaf salads. *Food Sci. Nutr.* 1: 3–7.
- Bendich, A., L. J. Machlin, O. Scandurra, G. W. Burton and D. D. M. Wayner. 1986. The antioxidant role of vitamin C. *Adv. Free Rad. Biol. Med.* 2: 419–444.
- Bierhuizen, J. F. and W. A. Wagenvoort. 1974. Some aspects of seed emergence in vegetables. 1. The determination and application of heat sums and minimum temperature for emergence. *Sci. Hortic.* 2: 213–219.
- Borrelli, K., R. T. Koenig, B. M. Jaeckel and C. A. Miles. 2013. Yield of leafy greens in high tunnel winter production in the Northwest United States. *HortScience* 48: 183–188.
- Cantwell, M., J. Rovelo, X. Nie and V. Rubatzky. 1998. Specialty salad greens: postharvest physiology and shelf life. *Acta Hortic.* 467: 371–378.
- Conversa, G., A. Bonasia, C. Lazzizzera and A. Elia. 2014. Pre-harvest nitrogen and azoxystrobin application enhances raw product quality and post-harvest shelf life of baby spinach (*Spinacia oleracea* L.). *J. Sci. Food Agric.* 94: 3263–3272.
- Espíndola, J. S., R. F. Otto and G. C. Berusk. 2015. Growth and yield of baby-leaf chicory under different environmental conditions and plant spacing. *Interciencia* 40: 834–839 (In Portuguese with English abstract).
- Ferrante, A., L. Incrocci, R. Maggini, G. Serra and F. Tognoni. 2004. Colour changes of fresh-cut leafy vegetables during storage. *J. Food Agric. Environ.* 2: 40–44.
- Fontana, E., S. Nicola, J. Hoeberechts, D. Saglietti and G. Piovano. 2004. Managing traditional and soilless culture system to produce corn salad (*Valerianella olitoria*) with low nitrate content and lasting postharvest shelf life. *Acta Hortic.* 659: 763–768.
- Fujime, Y. 2005. Characteristics of baby leaves. *Agric. Hortic.* (Noko-to-Engei) 60: 162–166 (In Japanese).
- Gorenjak, A. H. and A. Cencič. 2013. Nitrate in vegetables and their impact on human health. A review. *Acta Aliment.* 42: 158–172.
- Grahn, C. M., C. Benedict, T. Thornton and C. Miles. 2015a. Baby-leaf salad green production guide for western Washington. *WSU Extension Bull.* 1–25.
- Grahn, C. M., C. Benedict, T. Thornton and C. Miles. 2015b. Production of baby-leaf salad greens in the spring and fall seasons of northwest Washington. *HortScience* 50: 1467–1471.
- Hall, M. K. D., J. J. Jobling and G. S. Rogers. 2015. Effect of nitrogen supply and storage temperature on vitamin C in two crops of baby-leaf rocket, and the potential of these crops for a nutrient claim in Australia. *J. Plant Nutr.* 38: 246–259.
- Hardesty, S. D. 2015. Spring mix case studies in the Sacramento MSA. p. 127–177. In: R. P. King, M. S. Hand and M. I. Gómez (eds.). *Growing local: case studies on local food supply chains*. Univ. Nebraska Press, Lincoln.
- Harris, R. S. 1975. Effects of agricultural practices on the composition of foods. p. 33–57. In: R. S. Harris and E. Karmas (eds.). *Nutritional evaluation of food processing*, 2nd ed. AVI, Westport.
- Johnson, J. R., J. R. McGuinn and J. W. Rushing. 1989. Influence of preharvest factors on postharvest quality of prepackaged fresh market spinach. *Appl. Agric. Res.* 4: 141–143.
- Junjun, H., Y. Jingping, Y. Hu, Z. Xing and Y. Xinyi. 2014. Effects of light intensity and nitrogen supply on the dynamic characteristics of leaf SPAD value of rice canopy. *J. Zhejiang Univ. Agric. Life Sci.* 40: 495–504 (In Chinese with English abstract).
- Köppen, W. 1936. Das geographische system der klimate. p. 1–44. In: W. Köppen and R. Geiger (eds.). *Handbuch der klimatologie*. Borntraeger, Berlin.
- Kroggel, M., W. Lovichit, C. Kubota and C. Thomson. 2011. Greenhouse baby leaf production of lettuce and komatsuna in semi-arid climate: seasonal effects on yield and quality. *Acta Hortic.* 952: 827–834.
- Martínez, J. A. and F. Artés. 1999. Effect of packaging treatments and vacuum-cooling on quality of winter harvested iceberg lettuce. *Food Res. Intl.* 32: 621–627.
- Martínez-Sánchez, A., A. Gil-Izquierdo, M. I. Gil and F. Ferreres. 2008. A comparative study of flavonoid compounds, vitamin C, and antioxidant properties of baby leaf *Brassicaceae* crops. *J. Agric. Food Chem.* 56: 2330–2340.
- Medina, M. S., J. A. Tudela, A. Marín, A. Allende and M. I. Gil. 2012. Short postharvest storage under low relative humidity improves quality and shelf life of minimally processed baby spinach (*Spinacia oleracea* L.). *Postharvest Biol. Technol.* 67: 1–9.
- Morgen, L., J. Reade and J. Monghan. 2012. Effects of environmental stress on ascorbic acid content in baby leaf spinach (*Spinacia oleracea*). *Acta Hortic.* 939: 205–208.
- Nicola, S., C. Egea-Gilabert, D. Niñirola, E. Conesa, G. Pignata, E. Fontana and J. A. Fernández. 2015. Nitrogen and aeration levels of the nutrient solution in soilless cultivation systems as important growing conditions affecting inherent quality of baby leaf vegetables. *Acta Hortic.* 1099: 167–177.
- Nicola, S., E. Fontana, C. Torassa and J. Hoeberechts. 2006. Fresh-cut produce: Postharvest critical issues. *Acta Hortic.* 712: 223–230.
- Nicola, S., J. Hoeberechts, E. Fontana and D. Saglietti. 2003. Cultural technique influences on post-harvest quality of rocket (*Eruca sativa* Mill.). *Acta Hortic.* 604: 685–690.
- Nicola, S., G. Pignata, M. Casale, P. E. Lo Turco and W. Gaino. 2016. Overview of a lab-scale pilot plant for studying baby leaf vegetables grown in soilless culture. *Hort. J.* 85: 97–104.
- Nordmark, L., U. Gertsson, K. Olsson and M. E. Olsson. 2014. Content of bioactive compounds in baby-leaves as affected by season and growth stage at harvest. *Acta Hortic.* 1040: 201–206.
- Pignata, G., D. Niñirola, M. Casale, P. E. Lo Turco, C. Egea-Gilabert, J. A. Fernández and S. Nicola. 2016. Inherent quality and safety of watercress grown in a floating system using *Bacillus subtilis*. *Hort. J.* 85: 97–104.
- Samuolienė, G., R. Sirtautas, A. Brazaitytė and P. Duchovskis. 2012. LED lighting and seasonality effects antioxidant properties of baby leaf lettuce. *Food Chem.* 134: 1494–1499.
- Santamaria, P. 2006. Nitrate in vegetables: toxicity, content, intake and EC regulation (review). *J. Sci. Food Agric.* 86: 10–17.

- Sato, K. 2016. The demand characteristics of baby leaves and the response to needs for year-round supply: the case study of HATAKE company Co., Ltd. *Vegetable Information (Yasai-Joho)* 152: 36–45 (In Japanese).
- Szeto, Y. T., B. Tomlinson and I. F. F. Benzie. 2002. Total antioxidant and ascorbic acid content of fresh fruits and vegetables: implications for dietary planning and food preservation. *Br. J. Nutr.* 87: 55–59.
- Tokita, I. 2005. The baby leaves situation in America. *Agric. Hortic. (Noko-to-Engei)* 60: 174–176 (In Japanese).
- Tokuchi, T., Y. Suzuki and H. Terai. 2012. Effects of 1-methylcyclopropene on the postharvest quality of komatsuna (*Brassica rapa* L. *perviridis* group). *Food Preserv. Sci.* 38: 153–158 (In Japanese with English abstract).
- Tsukamoto, Y. 1994. *Garden plants encyclopedia* (In Japanese). Shogakukan, Tokyo.
- Wang, Q., J. Chen, R. H. Stamps and Y. Li. 2005. Correlation of visual quality grading and SPAD reading of green-leaved foliage plants. *J. Plant Nutr.* 28: 1215–1225.
- Wheeler, K., D. K. Tressler and C. G. King. 1939. Vitamin C content of vegetables. XII. broccoli, cauliflower, endive, cantaloup, parsnips, New Zealand spinach, kohlrabi, lettuce, and kale. *J. Food Sci.* 4: 593–604.
- Wright, I. J. and M. Westoby. 2002. Leaves at low versus high rainfall: coordination of structure, lifespan and physiology. *New Phytol.* 155: 403–416.
- Yamamoto, A., T. Nakamura, J. J. Adu-Gyamfi and M. Saigusa. 2002. Relationship between chlorophyll content in leaves of sorghum and pigeonpea determined by extraction method and by chlorophyll meter (SPAD-502). *J. Plant Nutr.* 25: 2295–2301.