Title	Studies on the year-round production and quality improvement of baby-leaf vegetables
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Citation	北海道大学. 博士(環境科学) 甲第13747号
Issue Date	2019-09-25
DOI	10.14943/doctoral.k13747
Doc URL	http://hdl.handle.net/2115/82747
Type	theses (doctoral)
File Information	Masayoshi_TAKAHAMA.pdf

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2019

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Chapter 1

General Introduction

1. Characteristics of baby-leaf vegetables

‘Baby-leaf vegetables’ (BLV) is a new category in leafy vegetables with following three important features. The first feature is that several types of crops are contained in BLV. Crops used as BLV, baby-leaf crops (BLC), are included several lettuces (green and red romaine, green and red oak leaf, Lollo Rossa), table beet, spinach, swiss chard, perilla, frisee (endive), radicchio (chicory), tat-soi, mizuna, kale, rucola, watercress, orach, mache, chervil, dandelion, purslane, and others (Ryder, 2002; Saini et al., 2016). Every year new crops are added as BLC. Most of the BLC belongs to Brassicaceae, Asteraceae, and Amaranthaceae families. At present, more than 30 BLC are used in commercial production. These crops show wide ranges of leaf shapes, colors, textures and flavors (Fujime, 2005). There are no strict rules on assortment of BLC and the number of them.

The second feature is that BLV consist of small or juvenile whole leaves, not ones cut into bite-sized piece. Some reports suggested that BLV should be harvested when leaf lengths were 100 mm long, or less (Ryder, 2002) and others mentioned when heights ranged from 50 – 120 mm (Saini et al., 2016).

The third feature is that BLV are produced in a short period of time and many times within a year. Consumers expect a constant supply to ready-to-eat salad mix, such as BLV, more than any other seasonal vegetables. Above all, a stable year-round production is more important in the transactions between agricultural production companies and retailers than those through wholesale markets (Sato, 2016). On the other hand, Short-term BLV production is advantageous from the view point of stable production.

2. Status of BLV production in the world

The concept of BLV originated in mesclun, which come from the Provençal region in south France since 17th century and literally means a “mixture” (Grahn et al, 2015b; Hardesty, 2015; Jèhanno and Savage, 2009). Traditional mesclun is likely to represent leafy vegetable mixture, though Ryder (2002) defined that it consisted of chervil, arugula, lettuce, and endive in precise proportions. Currently, “mesclun” is the name used for the mixture of juvenile stage of different kinds of leafy vegetables, that is, BLV. Furthermore, BLV are sometimes called “spring mix” in North America.

Commercial BLV production as food service industry began in early 1980s, mainly in Europe and the USA. In Italy, one of the main producing countries in Europe, 100,000 metric tons of BLV were produced in 2012. In the UK, 91,000 metric tons of fresh-cut salads, including BLV, were produced, 69,000 metric tons in France, and 43,000 metric tons in Germany in 2013 (Nicola et al., 2016). In the USA, bagged ready-to-eat salad mixes were distributed in supermarkets in 1989, and were composed of mature heads of lettuce (*Lactuca sativa* L.; primarily iceberg type) cut into bite-sized pieces (Thompson and Wilson, 1999). At present, various leafy salad crops, including spinach (*Spinacia oleracea* L.), mustard (*Brassica juncea* L.), pak choi (*Brassica rapa* L.), kale (*Brassica oleracea* L.), arugula (*Eruca sativa* L. and *Diplotaxis tenuifolia* L.) and table beets (*Beta vulgaris* L.), are used as BLV. The main production regions are California, Arizona, and Florida. More than 150,000 metric tons (more than 300 million pounds) of BLV were produced in California, the largest production region in the USA in 2008 (Hardesty, 2015). Besides these major production countries, BLV are produced in Eastern and Northern Europe, Latin America, Oceania etc.

BLV are produced and supplied all year around in some countries. In Italy, one of the main production countries, production region changes depending on season: produced in south Italy from autumn to spring and in north region from spring to autumn. However, production areas vary according to season, especially less in summer.

There are two growing culture system in BLV production: soil culture and soilless culture.

Furthermore, soil cultures are carried out both in plastic house and in open field. There are less statistical data on these culture systems in BLV. Generally, soil culture systems, especially in open field, are traditional and are used in large scale production such as USA. However, soilless culture systems have been introduced recently for year-round production, and are characterized short production cycle, plant nutrition control, soil-borne disease avoidance, food safety etc. (Nicola et al, 2016). Therefore, the shift from soil culture system to soilless ones will continue to increase.

3. Baby-leaf vegetables production in Japan

Baby-leaf vegetables production began in late 1990s and increased considerably from around 2010 in Japan. On the other hand, BLV products are sometimes transacted between agricultural production companies and retailers directly without through wholesale markets. The Japanese market scale of BLV in 2016 was estimated 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). Hearing questionnaire revealed that the total volume produced in 6 major companies summed to ca. 2,730 metric tons in 2018. Furthermore, the distribution of BLV in the Tokyo Metropolitan Central Wholesale Market, which is the only public data in Japan, has been increasing from a long-term view (Fig. 1-1). These data shows that the BLV market grows gradually in Japan. Future market scale is expected to grow to 30 billion yen, that is, if Japanese consumers consume the same volume as Europeans (50 g per capita) (Sato, 2016).

The main BLV production regions were Kyushu, the south Japan, and Kanto, around Tokyo (Cfa, according to the Köppen classification; 1936). Above all, Kyushu is the earliest production region in Japan because it is a very warm region to produce BLV even in winter and is suitable from the view point of efficient production (Fig. 1-2A). However, it is too hot to produce BLV in summer in Kyushu or Kanto region. Therefore, BLV production in Hokkaido, which is located in the northern subarctic region of Japan (Dfa or Dfb, according to the Köppen classification; 1936), has been expected as a new production region. Baby-leaf vegetables production in Hokkaido

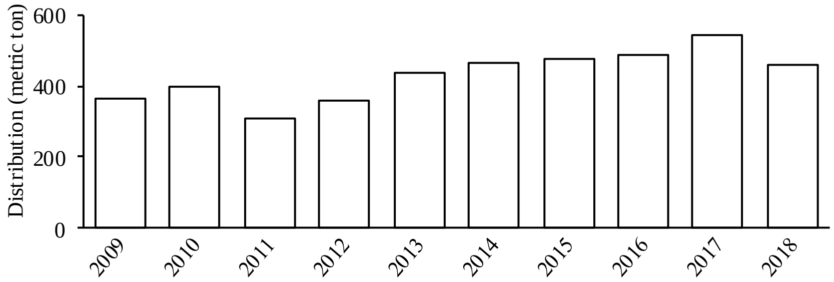

Fig.1-1 Shift of baby-leaf vegetables distribution in the Tokyo Metropolitan Central Wholesale Market.

Fig. 1-2 Baby-leaf vegetables production field in Japan. (A) soil culture in Kyushu region in winter and (B) soilless culture in Hokkaido in summer.

started in late 2000 and estimated production amount became 150 metric ton annually at present. However, winter temperature often depressed below freezing point even inside plastic house in Hokkaido. Therefore, heated cultivation is necessary for the winter crop production and takes vast fuel costs in general. For this reason, winter production in Hokkaido will be at an economic disadvantage.

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As to cultivation systems, BLV were produced mainly in soil culture in Kyushu and Kanto regions. However, BLV production in soilless culture system has been increasing in new production region recently. Most of the agricultural production companies induce the soilless culture systems for BLV in Hokkaido (Fig. 1-2B).

4. Knowledges and research interests related to BLV production

1) Characteristics demanded for BLV

Various kinds of leafy vegetables could be used as BLC. Therefore, attractive BLV are supplied for consumers depending on BLC assortment. However, there had few reports about what characteristics of BLV consumers' demand.

Meanwhile, it is important for producers to harvest in appropriate timing: an early harvest leads to a small yield, while a late harvest leads to a non-commercial leaf size, as leaves become larger and older and can be considered the outside category of "baby-leaf" vegetables. However, these leaf size criteria are different from BLC and even from consumers' demands. And there had not been reported the leaf size of commercial BLV, especially in Japan.

2) Characteristics of baby-leaf crops

BLV contained many kinds of crops. Therefore, it is important to investigate many characteristics in a lot of leafy crops from the view point of the mix-use system. However, only a few crops, less than 5 crops, were checked in most of BLV reports. Borrelli et al., (2013) investigated yields of 20 leafy crops in the same cropping season and Grahn et al., (2015) did ones in 9 leafy crops. However, they did not report other characteristics such as internal qualities. Hence, the author focused on evaluating the productivities and qualities in more kinds of BLC comprehensively.

3) Year-round production

(1) Spring to autumn production

Understanding of seasonal variation of BLV characteristics is important for stable year-round production. Some reports mentioned the seasonal variations: some examples refer to the yield in komatsuna (Grahn et al, 2015; Kroggel et al, 2011), lettuce (Grahn et al, 2015; Kroggel et al, 2011), and spinach (Grahn et al, 2015; Nicola et al, 2016); the ascorbic acid contents in komatsuna (Tamura, 2004), Qing gin cai (Ikeba et al., 2005), rucola (Nordmark et al., 2014), corn salad (Nordmark et al., 2014), and spinach (Tamura, 2004); the nitrate contents in Qing gin cai (Ikeba et al., 2005), and spinach (Yorifuji et al., 2005) etc. Therefore, the author investigated seasonal variation in another BLC from spring to autumn.

(2) Winter production in unheated plastic house

At present, vegetables are hardly produced in Hokkaido in winter. On the other hand, some report mentioned the cold tolerances of leafy vegetables. Maynard and Hochmuth (2006) have classified many leafy green vegetables as cool season-hardy (cabbage, kale, mustard, spinach) or half-hardy (Chinese cabbage, lettuce). Borrelli et al (2013) investigated the yield of 20 leafy crops grown in in unheated high tunnel winter production in the northwest United States where temperature sometimes depressed to -10ºC. Other reports revealed that lettuce could tolerate temperatures as low as -2ºC without damage and that spinach could do as low as -9ºC (Borrelli et al, 2013). In Japan, Tamura (2000) found that spinach and komatsuna could tolerate temperatures low as -10ºC by low temperature acclimation. Furthermore, as to qualities, cold temperature increased sugars and vitamin C contents in spinach and komatsuna (Tamura et al, 2003). By using these reports, high-quality spinach called ‘Chidimi Hourenso’ (winter sweet spinach) have been produced mainly in Tohoku region and sold in winter. Based on these reports, the author evaluated the productivity and qualities in unheated winter production of BLC in Hokkaido.

(3) Improvement qualities using salinity treatment

As to seasonal variations in BLV qualities, ascorbic acid contents decreased in high temperature conditions such as summer in spinach (Yorifuji et al., 2005) and komatsuna (Tamura, 2004). Therefore, the improvement of qualities is also necessary for stable year-round production of BLV.

Salt stress increases the generation of reactive oxygen species in plants (Abogadallah 2010), and scavenging of them depends on both enzymatic and nonenzymatic components. For this reason, the rises of the beneficial components such as antioxidants in some leafy crop can be obtained by exposing plants to salinity stress: some examples refer to the total phenolics contents in lettuce (Fernández, et al., 2016; Pérez-López et al., 2015), watercress (Fernández, et al., 2016; Kaddour et al., 2013); the glutathione contents in lettuce (Pérez-López et al., 2015). By contrast, salinity treatment depressed ascorbic acid contents in lettuce (Pérez-López et al., 2015) and watercress (Kaddour et al., 2013). However, the response to the salinity treatment were limited were observed in limited crops. Therefore, the effects of salinity treatment need to be investigated in various BLC.

5. Scope and objectives of this study

Increasing BLV production is expected in Japan, and Hokkaido is the progressing region for BLV production in summer season. However, it is necessary to solve the following problems for establishing a main production region:

1) Comprehension of consumer demands and supplies of BLV products

BLV is new-categorized by consumer demands, and consumer demands vary depending on gender, age etc. the information of BLV important for consumers has not been surveyed. Therefore, questionnaire survey of BLV was carried out on consumers.

On the other hand, there is no clear BLV definition and less marketing research in Japan. Therefore, BLV components and leaf sizes were revealed to establish standard of BLV by surveying commercial BLV products.

2) Understanding the characteristics of BLC

There are many BLC and some of them are selected and used as BLV mixtures. Twenty-two BLC were investigated for the cultivation periods, yields and qualities and classified based on its characteristics to show an ideal BLC assortment.

3) Investigating the seasonal variation of growth and quality

Twenty-two BLC characteristics were researched in each season from spring to autumn, which is the usual production season in Hokkaido. In addition, the relations between their characteristics and air temperatures, one of the most efficient factors, were investigated.

4) Establishing a BLV production in winter in Hokkaido

Baby-leaf vegetables were expected year-round production as market demands. However, the BLV production in severe winter in Hokkaido had not been carried out. The possibilities of unheated cultivation of BLV in winter were revealed to extend production season in Hokkaido.

5) Improving BLV qualities in summer production.

Hokkaido is expected as the production region in summer because of cool weather. However, the qualities are depressed in summer compared to spring or autumn, even in Hokkaido. On the other hand, there are some cases that salinity applications improved BLV qualities. An attempt of salinity treatment to BLV grown in soilless culture in hot season were performed to reveal the effects on their qualities.

By the solutions of these problems in this study, stable year-round BLV production will be established in Hokkaido.

Chapter 2

Research on demand and supply of commercial baby-leaf vegetables

Introduction

As above-mentioned in chapter 1, there are two main rules on BLV (Fujime, 2005; Ryder, 2002; Saini et al., 2016): (1) various kinds of crops are used; (2) they contain small or juvenile whole leaves. In general, the compositions and numbers of crops in BLV depends on producers taking leaf shapes, colors, textures and flavors in account. If BLC are selected by reflecting consumer demands of BLV, their marketability will rise. However, there were few reports on the BLV market research, especially for Japanese consumers. On the other hand, there are no definite criteria of leaf sizes of BLC, and producers harvest base on their own criteria. Furthermore, criteria might be different among BLC. Leaf sizes could correlate with total cultivation periods, from sowing to harvest and yields. Hence, it is important to make leaf size clear to establish harvest timing in the next chapters.

In this chapter a questionnaire survey of BLV for Hokkaido consumers and investigations on components of commercial BLV were carried out to understand the commercial demands of BLV.

Materials and Methods

Survey 1: Recognition survey on BLV to consumers

Questionnaire survey was carried out to two hundred forty three visitors (general consumers) at the public open event of Donan Agricultural Experimental Station, Hokkaido, Japan (41 ° 53'11" N, 140 ° 39'14" E and 25 m a.s.l.) in 3 August 2009. At first, visitors (ranged from teenager to 70’s; the ratio of male to female was 47 to 53%) tasted BLV produced in the Station to make them aware, and answered the following questionnaires:

Questionnaire 1: Have you ever known the new vegetable category of BLV?

(Already known / Never known / No answer)

Questionnaire 2: Which impression do you have to BLV?

(fashionable, colorful, tasty, characteristical taste, or healthy; multiple answers)

Survey 2: Survey on crop components of commercial BLV

Commercial BLV, which were 20–50 g fresh weight (FW) packed by plastic bags or punnets, were collected from six production groups (companies or cooperatives) in April and seven ones in June 2009 (Table 2-1 and Fig. 2-1). Five samples were used from every production group. All leaves were sorted out for every crop and the leaf length 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, whose area had been established, using a GT-8700F scanner (Seiko Epson, Nagano, Japan) and Photoshop Element software (Adobe Systems, CA, USA).

Results

Survey 1: Recognition survey on BLV to general consumers

The number of valid responses was 154. Around 58% of respondents replied as “already known” about BLV (Fig. 2-2). There were significant differences on recognition of BLV depending on sex and age. Female respondents to know BLV rather than male, and the recognition ratios of the people age ranged from 20’s to 50’s were higher than any other age respondents. Around 56, 51, 32, 28, and 25% of the respondents had “tasty”, “healthy”, “colorful”, “fashionable”, and “characteristical taste” impression to BLV (Fig. 2-3).

Survey 2: Survey on crop components of commercial BLV

Sixteen crops were used in total thirteen commercial BLV of different production groups and seasons (Table 2-2). Table beets was contained in all BLV and second most common crops were Mizuna and Rucola. Commercial BLV from each production group had 4 to 10 crops. Each

Table 2-1. Commercial baby-leaf vegetables samples used in market research.

Production groups (production region)	Survey date (2009)		Remarks
	Apr	Jun	
A (Assabu in Hokkaido)	27 April	22 June	30 g packed in plastic film bag
B (Sapporo in Hokkaido)	21 April	25 June	30 g packed in plastic film bag
C (Chitose in Hokkaido)	-	22 June	35 g packed in plastic punnet
D (Mukawa in Hokkaido)	-	26 June	25 g packed in plastic film bag
E (Ibaraki)	21 April	22 June	50 g packed in plastic film bag
F (Fukuoka)	21 April	23 June	35 g packed in plastic punnet
G (Sizuoka)	26 April	23 June	25 g packed in plastic film bag
H (Kumamoto)	29 April	-	20 g packed in plastic film bag

Fig. 2-1. Commercial baby-leaf vegetables from Assabu (A), Sapporo (B), Chitose (C), Mukawa (D), Ibaraki (E), Fukuoka (F), Sizuoka (G), and Kumamoto (H).

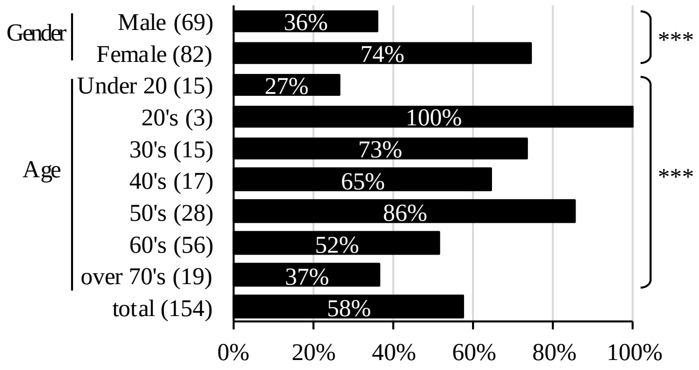


Fig. 2-2. Recognition ratio of consumers. The values given in parentheses are sample numbers. Total sample number contains gender or age unknown ones. *** Significant at $P < 0.001$ by χ^2-Test ($n = 154$).

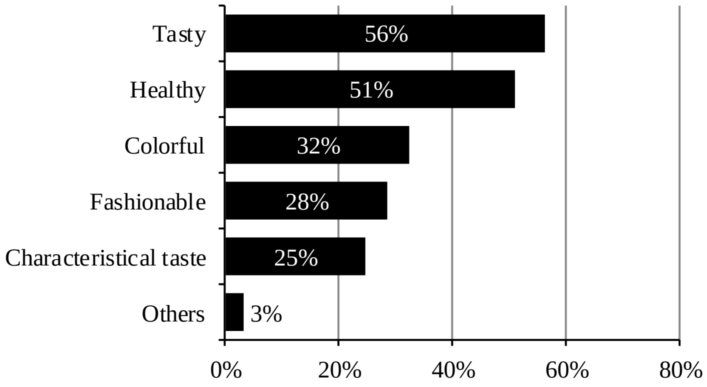


Fig. 2-3. Consumer's answer on impression to baby-leaf vegetables. $n = 151$. Multiple answer.

Table 2-2. Baby-leaf crops in the package from 8 production regions.

Leaf color type	Family	Crop	A		B		C	D	E		F		G		H
			Apr	Jun	Apr	Jun	Jun	Jun	Apr	Jun	Apr	Jun	Apr	Jun	Apr
Red[z]	Brassicaceae	Mustard	✓	✓				✓	✓	✓				✓	✓
		Karashi mizuna			✓	✓									
	Asteraceae	Leaf lettuce			✓	✓			✓	✓		✓			✓
		Romaine lettuce								✓		✓			✓
		Oak lettuce				✓	✓	✓		✓	✓	✓			
	Amaranthaceae	Table beet	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
		Swiss chard				✓									
	crop number		2	2	3	5	2	3	3	5	2	4	1	2	4
Green	Brassicaceae	Mizuna			✓		✓	✓	✓	✓	✓		✓	✓	✓
		Tat-soi					✓	✓	✓		✓	✓	✓	✓	✓
		Rucola	✓	✓			✓	✓	✓	✓		✓	✓	✓	
		Komatsuna		✓			✓			✓		✓	✓	✓	
		Mustard	✓	✓								✓			
		Kale						✓							
	Asteraceae	Endive	✓	✓	✓	✓			✓	✓		✓			
		Romaine lettuce	✓	✓					✓	✓		✓	✓	✓	
		Oak lettuce													✓
	crop number		4	5	2	1	4	4	5	5	2	6	5	5	3
Total crop number			6	7	5	6	6	7	8	10	4	10	6	7	7

[z] crops with red-colored leaf blades, sheathes, or veins.

Table 2-3 Leaf length and leaf area of each baby-leaf crops in commercial products.

Leaf shape	Crop (Leaf color type)	Leaf length (mm)			Leaf area (mm^2)		
		Apr	Jun	Means	Apr	Jun	Means
Elliptic	Tat-soi (Green)	81	82	81	1,132	1,150	1,141
	Komatsuna (Green)	97	79	82	1,539	1,231	1,282
	Mustard (Red)	79	80	79	1,291	1,333	1,315
	Mustard (Green)	107	75	85	1,119	842	934
	Kale (Red)		81	81		1,394	1,394
	Rucola (Green)	85	79	81	1,021	1,019	1,020
Oblong	Table beet (Red)	97	86	91	1,445	1,175	1,300
	Swiss chard (Red)		84	84		1,625	1,625
Lyrate	Oak lettuce (Red)	72	82	80	2,023	2,053	2,048
	Oak lettuce (Green)	78		78	1,069		1,069
Spatulate	Romaine lettuce (Red)	81	74	76	1,247	1,573	1,465
	Romaine lettuce (Green)	85	80	83	1,409	1,093	1,229
Spatulate undulate	Endive (Green)	98	84	90	1,347	1,010	1,155
	Leaf Lettuce (Red)	71	64	68	1,979	1,654	1,816
Spatulate incised	Mizuna (Green)	118	122	120	1,129	1,787	1,421
	Karashi Mizuna (Red)	126	91	108	1,320	516	918

commercial BLV contained green-colored crops and red one, which had red-colored leaf blades, sheathes, or veins. More green-colored crops tended to be contained in the BLV than red-colored ones, though not clearly. The crop components of BLV from production group A and G were almost the same in April and June. However, there were seasonal differences in the compounds among B, E, and F.

These sixteen crops were classified into six types based on the leaf shapes (elliptic, oblong, lyrate, spatulate, spatulate undulate, and spatulate incised) (Table 2-3). There were small differences in leaf lengths in most of crops except for green-colored mustard and red-colored karashi mizuna. The leaf length of most crops ranged around 80 to 90 mm except for ones with spatulate incised leaves. Red-colored leaf lettuce had somewhat shorter leaf length. The length of spatulate incised ranged from around 110 to 120 mm.

There were small differences in leaf areas in most of crops except for green-colored mustard, red-colored leaf lettuce, mizuna, and red-colored karashi mizuna. The leaf areas of most crops ranged around 1,000 to 1,500 mm^2. The leaf areas of red-colored oak lettuce and leaf lettuce were around 2,000 mm^2, which were larger than ones of any other crop.

Discussion

1. The qualities demanded for BLV

Baby-leaf vegetables production grew according to the increasing demands of ready-to-eat salad. However, other ready-to-eat salads have already existed such as iceberg lettuce cut into bite-sized pieces (Grahn et al., 2015b). Therefore, it is important for further BLV production to understand consumer demands unique to BLV that had different characteristics from any other ready-to-eat salads.

In this consumer survey conducted 10 years ago, the recognition ratio of BLV was less than 60% (Fig. 2-2). However, 10 years after the survey, BLV became more popular and are distributed in the market.

More than half of consumers had “tasty” and “healthy” impression (Fig. 2-3), which implies that many consumers expect internal qualities of BLV. The next important characteristics were “colorful” and “fashionable”, which were supported by more female respondents (*n* = 49) than male (*n* = 19). This result shows the importance of external appearances, which reflects that green and red-colored BLC were contained in all commercial BLV (Table 2-2).

Based on this consumer survey, it was decided to measure ascorbic acid and nitrate contents as internal qualities and Chlorophyll meter value (SPAD value) as external appearance in the following chapters. Ascorbic acid is an important content because of its antioxidant effect on humans (Bendich et al., 1986). Nitrate has been taken attention for human health, too. Ministry of Agriculture, Forestry and Fisheries certified nitrate functional component composition in 2018 (Watanabe, 2019). On the other hand, the maximum levels of nitrate contents in foodstuffs have been determined in the European Union (Gorenjak and Cencič, 2013). Some results have been already reported on these qualities of baby-leaf crops: some examples refer to the ascorbic acid contents 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 contents 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). 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 have been substituted with the greenness of BLV. Clarifying these internal qualities and external appearance could contribute to further BLV production and consumption.

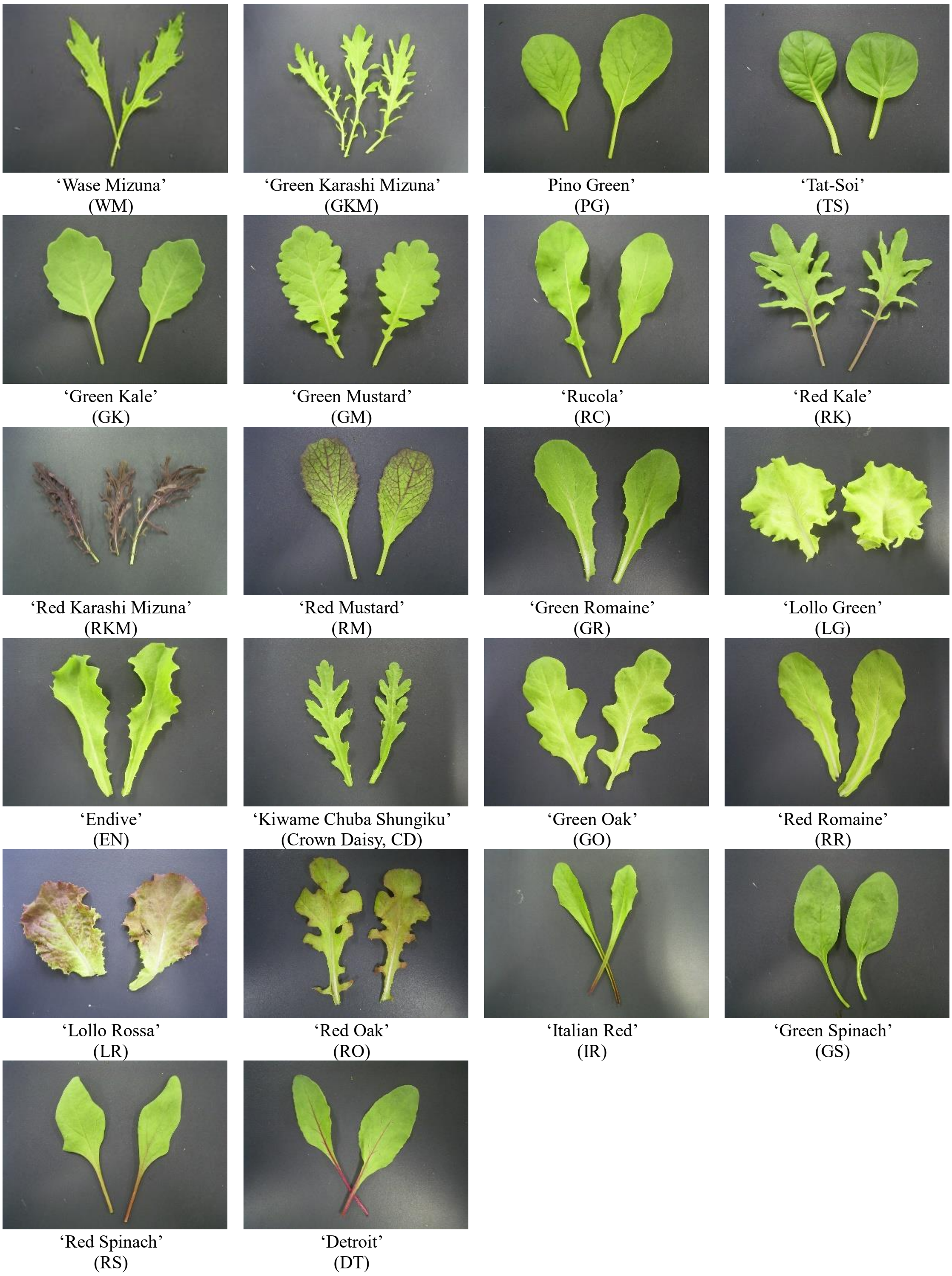
2. Establishment of harvest criteria

Farmers need to have information available concerning the cultivation period yield in order to ensure a stable and sustainable production. It is important to establish harvest criteria for evaluation of these items, especially in various BLC. In commercial BLV survey, leaf lengths of most of BLV were around 80 mm (Table 2-3). Lengths of BLC with larger leaf area were shorter, and ones of BLC with incised, less leaf area were longer. This result was consistent with the previous report (Saini et al., 2016). However, the leaf lengths and leaf areas based on leaf shapes of BLC were evaluated for the first time in this survey. Based on the survey results, the harvest criteria were established as to BLC used in following chapters (Table 2-4 and Fig. 2-4). The cultivation periods and yields were evaluated based on the harvest criteria.

Table 2-4. Leaf shapes and colors of materials used in the studies 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						
Brassica rapa var. *laciniifolia*	Wase Mizuna	WM	Spathulate incised	Green/Green	120	1,000–1,500
Brassica juncea ssp.	Green Karashi Mizuna (mustard)	GKM	Spathulate incised	Green/Green	120	1,000–1,500
Brassica rapa var. *perviridis*	Pino Green (komatsuna)	PG	Elliptic	Green/Green	80	1,000–1,500
Brassica rapa var. *rosularis*	Tat-Soi	TS	Elliptic	Green/Green	80	1,000–1,500
Brassica oleracea var. *acephala*	Green Kale	GK	Elliptic dentate	Green/Green	80	1,000–1,500
Brassica juncea ssp.	Green Mustard	GM	Elliptic crenate	Green/Green	80	1,000–1,500
Eruca vesicaria ssp.	Rucola	RC	Elliptic lyrate	Green/Green	80	1,000–1,500
Brassica oleracea var. *acephala*	Red Kale	RK	Spathulate incised	Green/Red	80	1,000–1,500
Brassica juncea ssp.	Red Karashi Mizuna (mustard)	RKM	Spathulate incised	Red/Red	120	1,000–1,500
Brassica juncea ssp.	Red Mustard	RM	Elliptic crenate	Red–green/Red	80	1,000–1,500
Asteraceae						
Lactuca sativa var. *longifolia*	Green Romaine	GR	Spathulate	Green/Green	80	1,000–1,500
Lactuca sativa var. *crispa*	Lollo Green	LG	Spathulate undulate	Green/Green	< 80	< 2,000
Cichorium endivia	Endive	EN	Spathulate undulate	Green/Green	80	1,000–1,500
Glebionis coronarium	Kiwame Chuba Shungiku (crown daisy)	CD	Lyrate	Green/Green	80	1,000–1,500
Lactuca sativa var. *crispa*	Green Oak	GO	Lyrate	Green/Green	80	1,000–1,500
Lactuca sativa var. *longifolia*	Red Romaine	RR	Spathulate	Red/Red	80	1,000–1,500
Lactuca sativa var. *crispa*	Lollo Rossa	LR	Spathulate undulate	Red/Red	< 80	< 2,000
Lactuca sativa var. *crispa*	Red Oak	RO	Lyrate	Red/Red	80	1,000–1,500
Cichorium intybus	Italian Red (chicory)	IR	Spathulate	Green/Red–green	80	1,000–1,500
Amaranthaceae						
Spinacia oleracea ssp.	Green Spinach	GS	Elliptic	Green/Green	80	1,000–1,500
Spinacia oleracea ssp.	Red Spinach	RS	Sagittate	Green/Red	80	1,000–1,500
Beta vulgaris	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).



‘Wase Mizuna’
(WM)

‘Green Karashi Mizuna’
(GKM)

Pino Green’
(PG)

‘Tat-Soi’
(TS)

‘Green Kale’
(GK)

‘Green Mustard’
(GM)

‘Rucola’
(RC)

‘Red Kale’
(RK)

‘Red Karashi Mizuna’
(RKM)

‘Red Mustard’
(RM)

‘Green Romaine’
(GR)

‘Lollo Green’
(LG)

‘Endive’
(EN)

‘Kiwame Chuba Shungiku’
(Crown Daisy, CD)

‘Green Oak’
(GO)

‘Red Romaine’
(RR)

‘Lollo Rossa’
(LR)

‘Red Oak’
(RO)

‘Italian Red’
(IR)

‘Green Spinach’
(GS)

‘Red Spinach’
(RS)

‘Detroit’
(DT)

Fig. 2-4. External appearances of the 22 baby-leaf crops.

Chapter 3

Classification based on productivity, external appearance and internal quality

Introduction

Very little technical information is available on the cultivation of BLV in Japan, although there are some guidelines and reports in North America and Europe (Grahn et al., 2015a; Nicola et al., 2016).

More than 20 crops are recognized as BLC. However, when BLV are served to consumers, some different kinds of the crops are selected and mixed considering volume, flavor and color tone. It is important to investigate many quality characteristics in a lot of BLC from the view of the mix-use system. Some reports already exist on the qualities of BLC such as ascorbic acid and nitrate, as mentioned in Chapter 2, and the shelf-life in spinach (Conversa et al., 2014; Medina et al., 2012) and rucola (Nicola et al., 2003). However, only a few crops, less than 5 crops, were investigated for quality measurements, even though yield researches were 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 juvenile stage by using 22 BLC belonging to 3 botanical families for comparative evaluation in the southern part of Hokkaido, Japan. Five characteristics, that is to say, SPAD value, ascorbic acid and nitrate contents, leaf characteristics and shelf life, were measured for the evaluation of quality. The similarity was analyzed by principal component analysis (PCA) among the used crops.

Materials and Methods

1. Baby-leaf crops and experimental design

The experiments were carried out at the same unheated plastic house 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, by means of TR71S thermometer recorders (T and D, 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 P_2O_5 and 0.8 kg of K_2O per are was applied to the brown lowland soil (loam) in the plastic house and the soil was adjusted from pH 6.0 to 6.5 by adding calcium carbonate. Ten Brassicaceae, 9 Asteraceae and 3 Amaranthaceae leafy crops were grown in the plastic house (Table 2-4 and Fig. 2-4). All the seeds were purchased from Nakahara Seed (Fukuoka, Japan), except for 'Kiwame Chuba Shungiku' (crown daisy, CD), which was purchased from Takii (Kyoto, Japan). Sowing was performed on 25 March 2010 and 24 March 2011, respectively. All the 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 the standard local production practices, was 1,000 $\text{seeds}\cdot\text{m}^{-2}$, and each plot area was 1.0 m^2. Water was supplied when the pF at 100 mm depth under the ground reached between 2.5 and 2.6. The experimental design consisted of a randomized complete block design with 22 crops which lasted 2 years and it had 2 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, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). EZR is in fact a modified version of R commander, which was designed to add the statistical functions that are frequently used in biostatistics and statistics software.

2. 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 estimated as the difference between the harvest date, as mentioned below, and the emergence date. The total cultivation period was computed as the sum of the days necessary for emergence and the growing period.

3. Harvest method

Harvest was carried out when most of leaves satisfied with harvest criteria in each crop (Table 2-4). The true leaves were harvested using scissors above cotyledons between 11 a.m. and 2 p.m. The fresh weight (FW) per 0.1 m^2 was measured to determine the yield ($g \cdot m^{-2}$). The yield/total cultivation period was calculated as a growth rate.

4. SPAD value

SPAD value was measured by using a SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan) on the first day of the post-harvest evaluation, as explained hereafter, on 22 April 2010 or 19 April 2011 in *Brassicaceae* crop, and on 28 April 2010 or 24 April 2011 in Asteraceae and Amaranthaceae crops.

5. Ascorbic acid content

Five g FW of leaves from each plot was 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). Aliquot of each extract was analyzed with an RQflex plus reflectometer (Merck, Millipore, MA, USA) and by means of a Reflectoquant Ascorbic Acid Test (Merck, Millipore, MA, USA).

6. Nitrate content

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 with 4 ml of distilled water and analyzed with an RQflex plus reflectometer and by means of a Reflectoquant Nitrate Test (Merck, Millipore, MA, USA).

7. Leaf characteristics

Aliquots of 20 well-developed 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, whose 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 calculated as an indicator of leaf thickness (Wright and Westoby, 2002). The number of leaves were obtained by dividing the yield by the mean leaf weight. More than 30 g of harvested leaves was dried at 60 °C, using a drying oven, for one week, in order to calculate the dry matter ratio.

8. PCA of the characteristics of 22 BLC

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

9. 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.

Results

1. Temperatures and sunshine durations during the cultivation period

The plastic house environmental conditions were compared over the 2 years of the experiments (Fig. 3-1). 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 1 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 35 days from sowing in 2010 and 2011 were 182 and 191 hours, respectively.

2. Cultivation periods

Significant interactions between families and years were observed in the emergence period ($P = 0.028$) (Table 3-1). The Brassicaceae crops (6.2 days) tended to emerge earlier than the Asteraceae (7.4 days) and Amaranthaceae crops (8.4 days). 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 crops. The total cultivation period in the Brassicaceae crops was shorter than that in the Asteraceae crops (25.4 and 31.2 days, respectively). The difference between the earliest crop ('Pino Green' (PG), 23.3 days) and the slowest (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).

3. Yields and productivities

No significant difference in yield was observed among the 3 families ($P = 0.272$), although some differences were observed among the crops (Table 3-1). The largest yields in the Brassicaceae crops were observed for 'Wase Mizuna' (WM, 882 $g \cdot m^{-2}$) and 'Tat-Soi' (TS, 870 $g \cdot m^{-2}$), and the smallest was for 'Red Kale' (RK, 478 $g \cdot m^{-2}$). The largest yields in the Asteraceae crops were for 'Lollo Green' (LG, 842 $g \cdot m^{-2}$) and 'Lollo Rossa' (LR, 801 $g \cdot m^{-2}$), and the smallest

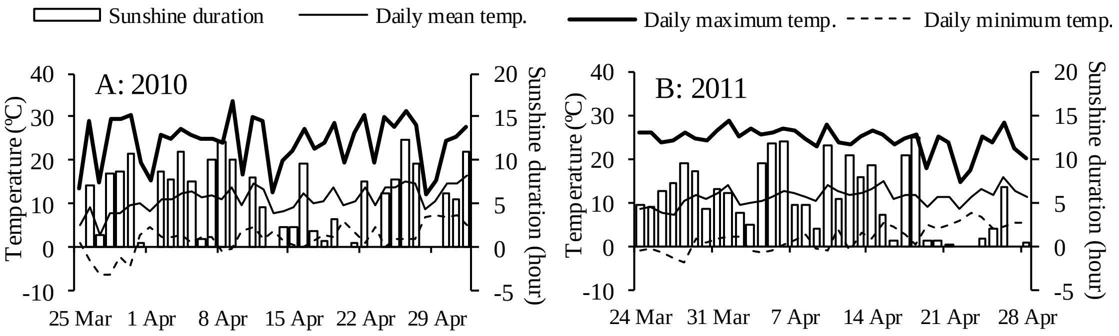


Fig. 3-1. Temperatures and sunshine durations during the cultivations in (A) 2010 and (B) 2011. The temperatures were measured inside the plastic house. The sunshine duration was obtained from AMeDAS (Automated Meteorological Data Acquisition System) in Hokuto, Hokkaido, Japan.

Table 3-1. Cultivation periods, yields, SPAD values ascorbic acid and nitrate contents of baby-leaf crops.

Abbreviation^{z}	Emergence period (days)	Growing period (days)	Total cultivation period (days)	Yield (g·m^{-2})	Yield/total cultivation period (g·m^{-2}·day^{-1})	SPAD value	Ascorbic acid (μg·g^{-1} FW)	Nitrate (μg·g^{-1} FW)
	Mean^{z}	Mean	Mean	Mean	Mean	Mean	Mean	Mean
Brassicaceae								
WM	5.3	20.0 ab^{x}	25.3 ab	882 a	34.9 a	26.7 d	655	4,563 a
GKM	6.5	18.8 ab	25.3 ab	821 ab	32.5 a	24.4 d	685	3,613 ab
PG	5.3	18.0 b	23.3 b	812 ab	35.1 a	37.4 ab	778	3,325 ab
TS	6.3	19.3 ab	25.5 ab	870 a	34.2 a	40.4 a	560	4,100 ab
GK	6.8	21.8 a	28.5 a	490 cd	17.3 c	37.1 ab	770	2,925 ab
GM	5.5	19.0 ab	24.5 ab	616 bd	25.4 ac	24.4 d	740	2,975 ab
RC	6.0	19.3 ab	25.3 ab	517 cd	20.6 bc	31.0 c	718	2,925 ab
RK	7.3	18.8 ab	26.0 ab	478 d	18.6 bc	34.7 b	750	3,888 ab
RKM	6.5	19.8 ab	26.3 ab	741 ab	28.1 ab	26.5 d	655	3,925 ab
RM	6.3	17.8 b	24.0 ab	594 bd	24.9 ac	27.4 cd	703	2,300 b
Mean	6.2	19.2	25.4	682	27.2	31.0	701	3,454
SE	0.7	1.1	1.4	161	2.2	1.9	66	216
Asteraceae								
GR	7.5	23.3 ad	30.8 ab	648 ac	21.2 ab	31.3 ab	423	2,125 b
LG	6.5	24.3 ad	30.8 ab	842 a	27.5 a	14.6 e	308	2,075 b
EN	7.0	26.5 ab	33.5 ab	781 ab	24.0 a	27.6 bc	353	4,038 a
CD	8.5	22.0 cd	30.5 ab	744 ab	24.5 a	26.3 c	208	2,138 b
GO	7.5	22.0 cd	29.5 ab	734 ab	25.2 a	17.2 de	358	2,513 b
RR	6.5	22.8 bcd	29.3 ab	766 ab	26.5 a	27.0 c	348	2,438 b
LR	7.3	25.5 ac	32.8 ab	801 a	24.5 a	20.7 d	165	1,975 b
RO	7.0	21.0 d	28.0 b	525 bc	18.9 ab	20.2 d	358	1,825 b
IR	8.5	27.3 a	35.8 a	379 c	10.6 b	31.8 a	360	4,838 a
Mean	7.4	23.8	31.2	691	22.5	24.1	320	2,663
SE	0.7	2.18	2.4	150	1.7	2.0	82	349
Amaranthaceae								
GS	8.3	16.3 c	24.5 b	717	29.3	34.1	515	1,813
RS	7.5	17.8 b	25.3 b	753	29.8	31.6	568	1,725
DT	9.5	20.5 a	30.0 a	848	28.3	33.1	415	2,163
Mean	8.4	18.2	26.6	772	29.1	32.9	499	1,900
SE	1.0	2.2	3.0	68	0.4	1.2	77	134
P (ANOVA)								
Family	< 0.001	0.005	< 0.001	0.272	0.002	< 0.001	< 0.001	< 0.001
Year	< 0.001	< 0.001	< 0.001	0.535	0.026	< 0.001	0.011	0.052
Interaction	0.028	0.403	0.158	0.716	0.634	0.283	< 0.001	0.541

^{z}See Table 2-4 for abbreviations.

^{y}Based on the data in 2010 and 2011.

^{x}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.

was for IR (379 $g \cdot m^{-2}$). The yield in each Amaranthaceae crop was more than 700 $g \cdot m^{-2}$. The yield/total cultivation period ratios in the Asteraceae crops were lower than those in the other families. PG, WM and TS (35.1, 34.9 and 34.2 $g \cdot m^{-2} \cdot day^{-1}$, respectively) showed the highest values, and IR showed the lowest (10.6 $g \cdot m^{-2} \cdot day^{-1}$).

4. 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 3-1). The values of the 4 *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 ‘Green Oak’ (GO) showed the lowest SPAD values (14.6 and 17.2, respectively). Three Amaranthaceae crops had significantly higher SPAD values than the Asteraceae crops ($P < 0.001$).

2) Ascorbic acid contents

The ascorbic acid contents in the Brassicaceae crops tended to be higher than those in the Asteraceae crops (Table 3-1). PG (778 $\mu g \cdot g^{-1}$ FW) and ‘Green Kale’ (GK, 770 $\mu g \cdot g^{-1}$ FW) were the crops with the highest ascorbic acid contents, and LR (165 $\mu g \cdot g^{-1}$ FW) was the one with the lowest. However, significant interaction between families and years was observed ($P < 0.001$).

3) Nitrate content

The nitrate contents in most of the Brassicaceae crops were above 2,500 $\mu g \cdot g^{-1}$ FW, except for RM (2,300 $\mu g \cdot g^{-1}$ FW) (Table 3-1). Most of the Asteraceae crops had values below 2,500 $\mu g \cdot g^{-1}$ FW of nitrate contents, although 2 *Cichorium* crops (IR and ‘Endive’ (EN)) had values above 4,000 $\mu g \cdot g^{-1}$ FW of nitrate. The nitrate contents in the all Amaranthaceae crops showed lower values and ranged from 1,725 (‘Red Spinach’ (RS)) to 2,163 $\mu g \cdot g^{-1}$ FW (‘Detroit’ (DT)).

4) Leaf characteristics

The leaf weights ranged from 286 (IR) to 673 $\mu g \cdot leaf^{-1}$ (PG) (Table 3-2). The leaves of the

Amaranthaceae crops (591 μg·leaf^{-1}) were generally heavier than those of the Brassicaceae and Asteraceae crops (505 and 410 μg·leaf^{-1}, respectively). The leaf numbers ranged from less than 1,000 (GK) to more than 2,000 leaves·m^{-2} (WM, EN and 'Red Romaine' (RR)). In almost used crops, the leaf lengths ranged from 70 to 120 mm and the leaf areas did from 1,000 to 2,000 mm^2, respectively. The lengths of TS, LG and LR were less than 70 mm and shorter than any other crop. There was no significant difference in leaf width or leaf area among the 3 families ($P = 0.387$ and 0.550, respectively). LG (54.5 mm and 2,025 mm^2, respectively) and LR (61.1 mm and 2,003 mm^2, respectively) showed the widest and the largest leaf in 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.

5. PCA on the basis of characteristics of the 22 baby-leaf crops

The component characteristics are shown in Table 3-3. 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 content and the SPAD value in Component 1, and strong negative one was also observed for the total cultivation period. A positive correlation with yield and a negative one with nitrate content emerged for Component 2. Three groups were recognized in the scatterplot made up of Components 1 and 2 (Fig. 3-2). 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 3 groups.

6. Correlations of the yield and SPAD value with leaf characteristics

Table 3-2. Leaf characteristics of 22 baby-leaf crops.

Abbreviation[z]	Leaf weight (μg/leaf)	Leaf number[y] (leaves·m^{-2})	Leaf length (mm)	Leaf width (mm)	Leaf area (mm^{2})	Leaf weight/ leaf area (μg·mm^{-2})	Dry matter ratio (%)
	Mean[x]	Mean	Mean	Mean	Mean	Mean	Mean
Brassicaceae							
WM	385 d[w]	2,311 a	117.0 a	27.0 d	1,047 b	0.37 ab	6.93 ab
GKM	510 bd	1,620 bc	114.3 a	34.6 bc	1,301 ab	0.40 a	7.04 ab
PG	673 a	1,213 cd	81.8 b	38.2 ab	1,720 a	0.39 ab	7.31 ab
TS	615 ab	1,414 cd	65.5 d	39.2 ab	1,537 ab	0.40 a	6.07 b
GK	534 ac	928 d	81.0 b	38.9 ab	1,558 ab	0.34 ab	8.57 a
GM	488 bd	1,275 cd	76.8 bc	36.8 bc	1,513 ab	0.32 ab	6.63 ab
RC	478 bd	1,086 cd	76.0 bd	32.1 cd	1,402 ab	0.34 ab	7.03 ab
RK	463 cd	1,026 d	79.9 bc	43.5 a	1,184 ab	0.39 ab	8.36 a
RKM	373 d	1,981 ab	115.5 a	32.1 cd	1,460 ab	0.28 b	6.91 ab
RM	534 ac	1,132 cd	70.0 cd	39.2 ab	1,653 a	0.32 ab	6.94 ab
Mean	505	1,399	81.0	36.2	1,438	0.36	7.18
SE	92	141	15.6	4.8	209	0.04	0.75
Asteraceae							
GR	479 ac	1,375	77.2 a	34.6 bc	1,633 ab	0.29 b	6.76 b
LG	526 ab	1,617	61.9 b	54.5 a	2,025 a	0.26 bc	5.32 b
EN	374 cd	2,206	77.8 a	27.5 cd	1,276 bd	0.29 b	6.85 b
CD	390 bd	1,896	78.3 a	25.2 d	986 d	0.40 a	6.49 b
GO	383 bd	1,957	77.3 a	38.5 b	1,670 ab	0.23 c	6.73 b
RR	356 cd	2,136	77.9 a	28.8 cd	1,297 bd	0.27 bc	5.68 b
LR	548 a	1,473	62.0 b	61.1 a	2,003 a	0.27 bc	5.51 b
RO	350 cd	1,543	79.9 a	37.9 b	1,514 bc	0.23 c	6.59 b
IR	286 d	1,316	86.2 a	23.1 d	1,091 cd	0.26 bc	9.16 a
Mean	410	1,724	75.4	36.8	1,499	0.28	6.57
SE	88	111	8.1	13.2	370	0.05	1.14
Amaranthaceae							
GS	631	1,141	90.2 a	33.9	1,600 a	0.40 b	6.53
RS	554	1,369	89.2 a	32.4	1,340 b	0.41 b	6.95
DT	588	1,440	77.6 b	31.4	1,240 b	0.47 a	6.52
Mean	591	1,317	85.7	32.6	1,393	0.43	6.67
SE	39	90	7.0	1.3	186	0.04	0.25
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 2-4 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.

The correlation coefficients of yield and SPAD value with leaf characteristics were shown in Table 3-4. 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 ($r = 0.632$).

Discussion

1. Emergence and growth characteristics in BLC

In the present study, the total cultivation period of IR was more than 10 days longer than that of PG (Table 3-1). Therefore, it may be difficult to grow these 2 crops under 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 the growth characteristics of the 3 families used in the experiment were different: Brassicaceae crops emerge faster; Asteraceae crops grow more slowly; Amaranthaceae crops emerge more slowly and grow faster.

2. Difference in yield among BLC

Grahn et al. (2015b) reported that *Brassica* crops grown in an open field had larger mean yields than lettuce or chenopod crops across seasons (spring and fall). However, in these experiments, no significant difference in yield was observed among the 3 families (Table 3-1). All Amaranthaceae crops showed a large yield and consistent growth rate. Therefore, these crops could be considered suitable for BLV production.

3. Difference in SPAD value among BLC

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 have been substituted with the greenness of BLV. A significant difference in SPAD value was found among

Table 3-3. Factor loading, eigenvalue and contribution ratio of each principal component in principal component analysis.

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

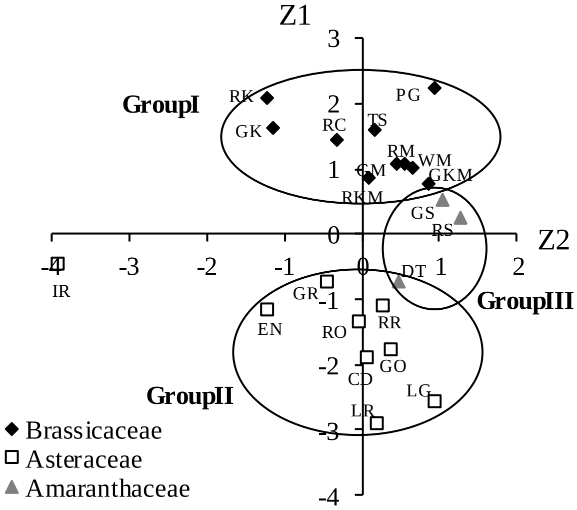


Fig. 3-2. Scatterplot of the principal component scores of the 22 baby-leaf crops. The Z1 and Z2 axes are defined by Components 1 and 2 in Table 3-3.

Table 3-4. Pearson product-moment correlation coefficient 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	0.554 **	0.121	0.119	0.159	0.298	-0.702 ***
SPAD value	0.432 *^{z}	-0.485 *	0.051	-0.329	-0.304	0.632 **	0.483 *

^{z}*, **, *** Significant at $P < 0.05$, 0.01, or 0.001, respectively ($n = 22$).

families. LG and GO showed pale green leaves (Table 3-1), and are considered useful for salad mix combinations together with other dark green leaf crops.

4. Emergence and growth characteristics in BLC

In the present study, the total cultivation period of IR was more than 10 days longer than that of PG (Table 3-1). Therefore, it may be difficult to grow these 2 crops under the same timing.

The result 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 the growth characteristics of the 3 families used in the experiment were different: Brassicaceae crops emerge faster; Asteraceae crops grow more slowly; Amaranthaceae crops emerge more slowly and grow faster.

5. Difference in yield among BLC

Grahn et al. (2015b) reported that *Brassica* crops grown in an open field had larger mean yields than lettuce or chenopod crops across seasons (spring and fall). However, in these experiments, no significant difference in yield was observed among the 3 families (Table 3-1). All Amaranthaceae crops showed a large yield and consistent growth rate. Therefore, these crops could be considered suitable for BLV production.

6. Difference in SPAD value among BLC

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 have been substituted with the greenness of BLV. A significant difference in SPAD value was found among families. LG and GO showed pale green leaves (Table 3-1), and are considered useful for salad mix combinations together with other dark green leaf crops.

7. Difference in ascorbic acid content among BLC

There are annual differences in ascorbic acid contents; however, the components in most of the Brassicaceae crops were higher than those in the other tested families, especially Asteraceae crops (Table 3-1). The results obtained in the present study were in agreement with the data of Szeto et al. (2002) and Wheeler et al. (1939). Therefore, Brassicaceae crops should be used in BLV assortment as ones with excellent qualities.

8. Characteristics of nitrate contents in BLC

Aires et al. (2013) and Santamaria (2006) pointed out that Brassicaceae, Asteraceae and *Chenopodiaceae* crops (classified as Amaranthaceae according to APG III) had higher nitrate contents than other families. In the present experiment, the Brassicaceae crops showed significantly higher nitrate content than the Amaranthaceae crops (Table 3-1). The ‘Rucola’ (RC) showed much lower nitrate content than the EU limits for the rucola crop group (7,000 $\mu g \cdot g^{-1}$ FW) (Gorenjak and Cencič, 2013). In contrast, the nitrate content in EN exceeded 4,000 $\mu g \cdot g^{-1}$ FW (Table 3-1), which is higher than the maximum level allowed for the trading of endives in some European countries (Santamaria, 2006). Reducing the nitrate level of this crop, through improvements in cultural practices, is therefore advisable.

9. Classification on the basis of the characteristics of 22 BLC

The PCA divided the 22 BLC into 3 main groups (Table 3-4 and Fig. 3-2). The Brassicaceae crops showed short total cultivation periods and high ascorbic acid contents; most of the Asteraceae crops showed long total cultivation periods and low ascorbic acid contents; the Amaranthaceae crops had large yields and low nitrate contents. IR did not fall into any of the 3 groups. Therefore, it is not considered suitable for use as a BLC because of its longer total cultivation period and smaller yield. Espíndola et al. (2015) reported the growths and yields of baby-leaf chicory not only under plastic tunnel and non-woven protection conditions, but also considering the plant spacing. Some improvements in the cultivation of IR, pertaining to the environmental conditions and sowing densities, are required to achieve an earlier production and higher productivity.

There was a significant difference in yield among crops in the Brassicaceae or Asteraceae family rather than among families (Table 3-1). On the other hand, Yield could be affected leaf characteristics such as leaf shapes. Therefore, we analyzed the relationship between the yield and the leaf characteristics of 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 3-4). For this reason, the fresh leaf yield was larger in the crop that had more leaves with a higher water content. Therefore, not only were the Amaranthaceae crops evaluated as large-yielding crops in the experiment, but also the leafy WM and juicy TS, LG and LR. Leaf number and dry matter ratio could be useful indicator to evaluate yield of crops other than ones used in the experiment.

Furthermore, crops with higher leaf weight/leaf area ratios tended to have higher SPAD values (Table 3-4). Because the leaf weight/leaf area ratio is one of indicators used to establish leaf thickness (Wright and Westoby, 2002), leaf thickness could be related to the depth of the leaf color. The positive relationship between leaf thickness and SPAD value had already been observed for sorghum and pigeon pea (Yamamoto et al., 2002), though it had not been found in green-leaved foliage plants (Wang et al., 2005). This relationship might not be common to all plants, but it could apply to BLV. 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.

Chapter 4

Seasonal variation of productivities and qualities from spring to autumn

Introduction

The experiments in Chapter 3 were carried out in spring, suitable season for growth evaluation, considering the leafy vegetable production in the plastic house. On the other hand, crop yields varied, depending on the seasons and locations (Grahn et al., 2015b). Ascorbic acid and nitrate contents could be changed by air temperature in spinach (Tamura, 2004), komatsuna (Tamura, 2004) and Qing gin cai (Ikeba et al., 2005).

In general, BLV are produced continuously during snowless season, from spring to autumn in Hokkaido. The productivities and qualities of BLC were investigated from spring to autumn to clarify the relation between these characteristics and air temperature, one of the important environmental factors in Hokkaido.

Materials and Methods

1. Baby-leaf crops and experimental design

The experiments were carried out for 8 times from 2009 to 2011 including 2 experiments in Chapter 3 (Table 4-1). All experiments were done in the same unheated plastic house in the Donan Agricultural Experiment Station same as Chapter 3. Experimental designs was generally same as Chapter 3. However, plastic house was covered with 50% shading screen in hot season, that is July 2009, October 2009, and August 2011, to prevent the excessive rise of air temperature in plastic house. Almost the same crops were used in Chapter 3, though some crops were not used in some experiments. Dates for sowing and harvests were shown in Table 4-1.

2. Determination of the cultivation period

The measurements were carried out same as Chapter 3. The total cultivation period was

Table 4-1. Sowing dates and harvest dates in 8 seasons.

Harvest season		Sowing	Harvest	Remarks[z]
May	2010	25 Mar	18 Apr-2 May	Measured SPAD value in 22 Apr and 28 Apr.
	2011	24 Mar	15 Apr-27 Apr	Measured SPAD value in 19 Apr and 24 Apr.
Jun	2010	7 May	25 May-2 Jun	Measured SPAD value in 27 May and 2 Jun.
Jul	2009	15 Jun	1 Jul-8 Jul	Covered greenhouse with 50% shading screen. Measured SPAD value in 2 Jul and 6 Jul.
Aug	2011	20 Jul	2 Aug-8 Aug	Covered greenhouse with 50% shading screen. Measured SPAD value in 9 Augl.
Oct	2009	10 Sep	25 Sep-6 Oct	Covered greenhouse with 50% shading screen. Measured SPAD value in 28 Sep and 5 Oct.
Nov	2010	30 Sep	18 Oct-27 Oct	Measured SPAD value in 22 Oct and 29 Oct.
	2011	3 Oct	22 Oct-2 Nov	Measured SPAD value in 25 Oct and 27 Oct.

[z] SPAD value was measured divisionally twice in each season, except for Aug 2011. SPAD measurement in Brassicaceae crops was carried out in first time and those in Asteraceae and Amaranthaceae crops were done in latter time.

calculated for the period from the sowing to the harvest.

3. Harvest method

The harvests were carried out same as Chapter 3. The true leaves were harvested using scissors above cotyledons between 11 a.m. and 2 p.m. The fresh weight (FW) per 0.1 m^2 was measured to determine the yield ($g \cdot m^{-2}$). The yield/total cultivation period was calculated as a growth rate.

4. SPAD value

SPAD value was measured using a SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan) divisionally twice in every season, except for August 2011: Brassicaceae crops were used at first measurement and Asteraceae and Amaranthaceae crops at second (Table 4-1).

5. Ascorbic acid content

The measurements were carried out same as Chapter 3. Five g FW of leaves from each plot was 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). Aliquot of each extract was analyzed with an RQflex plus reflectometer (Merck, Millipore, MA, USA) and by means of a Reflectoquant Ascorbic Acid Test (Merck, Millipore, MA, USA).

6. Nitrate content

The measurements were carried out same as Chapter 3. Five g FW of leaves from each plot was 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 with 4 ml of distilled water and analyzed with an RQflex plus reflectometer and by means of a Reflectoquant Nitrate Test (Merck, Millipore, MA, USA).

7. Leaf characteristics

The measurements were carried out same as Chapter 3. Aliquots of 20 well-developed 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, whose 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 calculated as an indicator of leaf thickness. The number of leaves were obtained by dividing the yield by the mean leaf weight. More than 30 g of harvested leaves was dried at 60°C, using a drying oven, for one week, in order to calculate the dry matter ratio.

8. Correlations between growth/quality and air temperature

Correlation coefficients of germination period, growing period and total cultivation period were calculated using the daily mean air temperature during each period in each crop. The coefficients of yield, yield/total cultivation period, SPAD value, ascorbic acid, nitrate contents and every leaf characteristic was calculated using the daily mean air temperature during total cultivation period in each crop. The coefficients of ascorbic acid and nitrate contents were calculated using the daily mean, daily maximum and daily minimum air temperature during total cultivation period and one week before harvest in each crop. Statistical analysis was performed with the above-mentioned EZR.

Results

1. Temperatures and sunshine durations during the cultivation period

The plastic house environmental conditions of the 6 seasons, except for ones in May 2010 and 2011, were compared (Fig. 4-1 and Table 4-2). The daily mean, maximum, and minimum air temperatures for total cultivation periods in WM ranged from 10.9 (May 2010) to 24.4°C (August 2011), from 25.2 (May 2010) to 31.9°C (August 2011), and from 0.8 (May 2011) to 19.1 (August 2011), respectively. The total sunshine durations for total cultivation periods in WM ranged 80.2 in August 2011 to 173.4 hours in May 2011.

2. Cultivation periods

There tended to be longest total cultivation periods in May (25.3, 30.8, and 26.6 days in Brassicaceae, Asteraceae, and Amaranthaceae, respectively) in all BLC (Fig. 4-2A). By contrast, the total cultivation periods in August were shortest in all BLC (14.8, 16.9, and 16.3 days in mean of 2010 and 2011, respectively).

3. Yields and productivities

The yields were larger in May than in August in all BLC (Fig. 4-2B). The yields in May and August showed 682 and 319 $g \cdot m^{-2}$ in mean in Brassicaceae, respectively; 691 and 307 $g \cdot m^{-2}$ in mean in Asteraceae, respectively; 772 and 356 $g \cdot m^{-2}$ in mean in Amaranthaceae, respectively. There were smaller differences among seasons in yields of IR and RK (82 and 73 $g \cdot m^{-2}$ standard deviation, respectively; data not shown). The yield/total cultivation period were also higher in May than in August in all BLC (Fig. 4-2C). The yield/total cultivation periods was higher in May (27.2, 22.5, and 29.1 $g \cdot m^{-2} \cdot day^{-1}$ in mean in Brassicaceae, Asteraceae, and Amaranthaceae, respectively) than August (21.7, 18.2, and 22.0 $g \cdot m^{-2} \cdot day^{-1}$ in Brassicaceae, Asteraceae, and Amaranthaceae, respectively) except for RK (18.6 in May and 24.3 $g \cdot m^{-2} \cdot day^{-1}$ in August, respectively) and ‘Green Romaine’(GR, 21.2 in May and 21.8 $g \cdot m^{-2} \cdot day^{-1}$ in August, respectively). However, seasonal tendency of the yield/total cultivation period was not as clearly as those in yield.

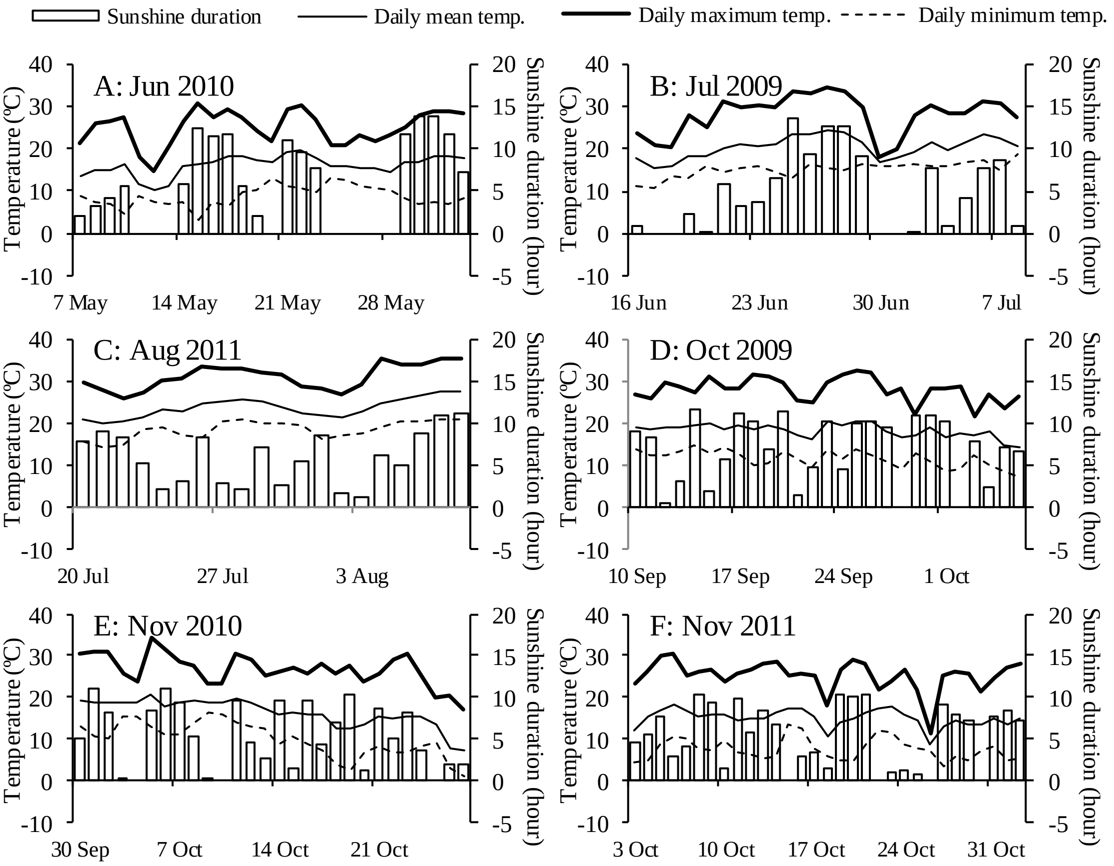


Fig. 4-1. Temperatures land sunshine durations during 6 seasons. The temperatures were measured inside the plastic house. Plastic house was covered with 50 % shading screen in July 2009 (B), August 2011 (C) and October 2009 (D). The sunshine duration was obtained from AMeDAS (Automated Meteorological Data Acquisition System) in Hokuto, Hokkaido, Japan.

Table 4-2. Air temperature and sunshine duration of 'Wase Mizuna' (WM)-total cultivation period.

Harvest season		Daily mean (℃)	Daily maximum (℃)	Daily minimum (℃)	Total sunshine duration (hour)
May	2010	10.9	25.2	1.1	138.5
	2011	11.6	26.4	0.8	173.4
Jun	2010	16.6	25.8	9.3	92.0
Jul	2009	20.0	27.5	14.5	80.2
Aug	2011	24.4	31.9	19.1	75.4
Oct	2009	20.0	30.6	13.1	130.3
Nov	2010	18.4	29.0	11.7	117.6
	2011	16.3	27.2	8.4	116.0

[z] Plastic house were covered with 50% shading screen.

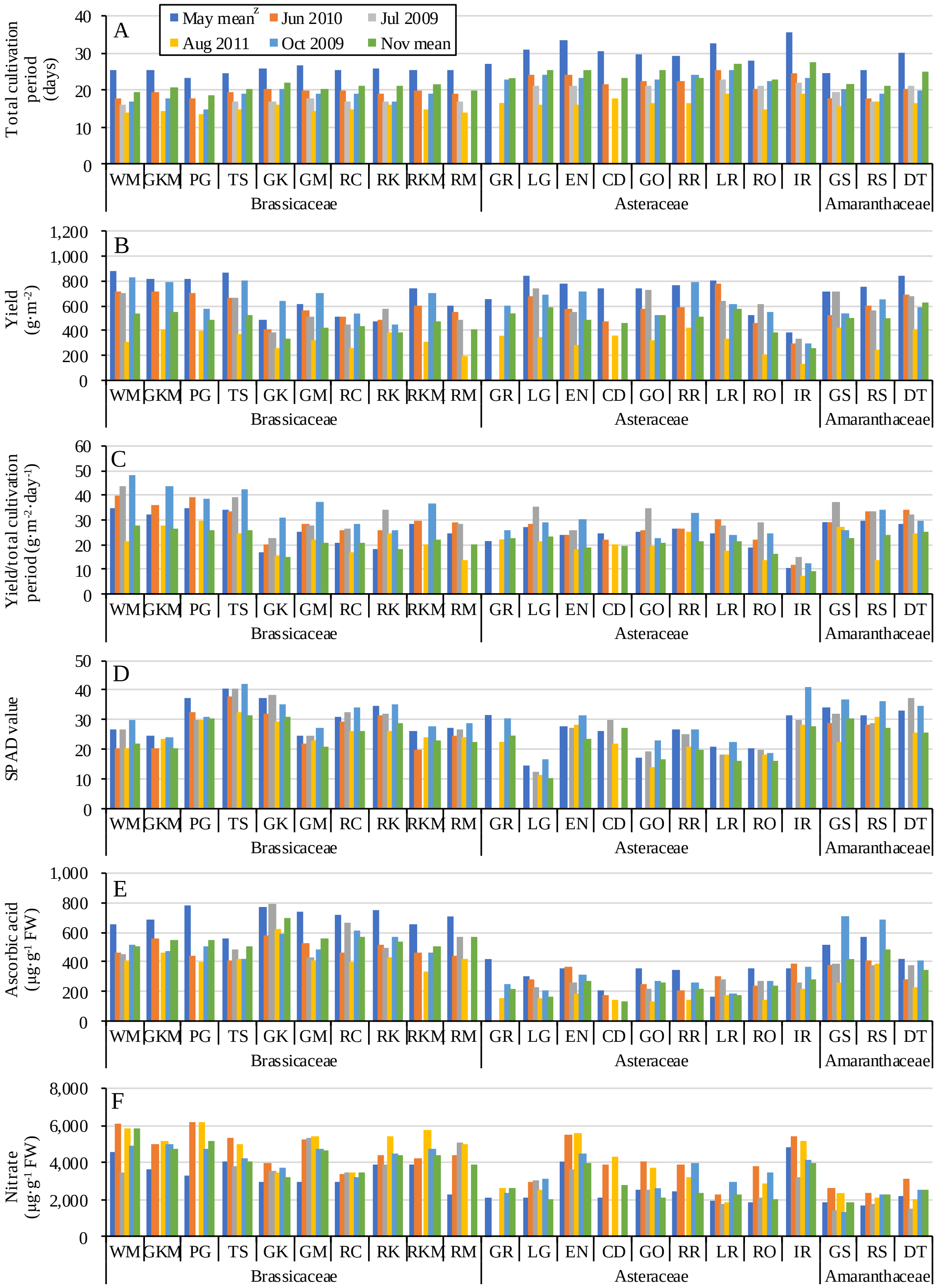


Fig. 4-2. Seasonal variation in total cultivation period (A), yields (B), yield/total cultivation periods (C), SPAD value (D), ascorbic acid (E) and nitrate contents (F). Means are based on the data in 2010 and 2011.

4. Quality

1) SPAD values

The SPAD values were highest in May or October in most of BLC (Fig. 4-2D). The SPAD values in May and October showed 32.4 and 31.5 in mean in Brassicaceae, respectively; 23.2 and 26.3 in mean in Asteraceae, respectively; 32.9 and 35.9 in mean in Amaranthaceae, respectively. By contrast, the lowest SPAD values were measured in August or November in most of BLC (25.9 or 26.7 in mean in Brassicaceae, respectively; 20.5 or 19.7 in mean in Asteraceae, respectively; 26.4 or 27.8 in mean in Amaranthaceae, respectively). However, the differences in SPAD values among the seasons were small compared to those of any other characteristics.

2) Ascorbic acid content

The ascorbic acid contents were higher in May than in August in all BLC (Fig. 4-2E). The contents of ascorbic acid in May and August showed 701 and 430 $\mu g \cdot g^{-1}$ FW in mean in Brassicaceae, respectively; 320 and 162 $\mu g \cdot g^{-1}$ FW in mean in Asteraceae, respectively; 499 and 293 $\mu g \cdot g^{-1}$ FW in mean in Amaranthaceae, respectively. GK showed more than 550 $\mu g \cdot g^{-1}$ FW ascorbic acid contents in all seasons.

3) Nitrate content

The nitrate contents were likely to be higher in June or August in most of Brassicaceae (4,818 and 5,050 $\mu g \cdot g^{-1}$ FW in mean, respectively) and Asteraceae (3,984 and 3,533 $\mu g \cdot g^{-1}$ FW in mean, respectively) (Fig. 4-2F). The smallest content was recorded in May in most of the Brassicaceae (3,454 $\mu g \cdot g^{-1}$ FW in mean) and Asteraceae (2,663$\mu g \cdot g^{-1}$ FW in mean). GK, GR, RC and RS has little seasonal differences in nitrate contents (367, 228, 211, and 269 $\mu g \cdot g^{-1}$ FW, respectively).

5. Correlations of BLC characteristics with air temperature

The total cultivation periods had significant negative correlations with air temperatures in all BLC (Table 4-3). The yield had the significant negative correlation in the 14 BLC. However, there was no significant correlations in the yield/total cultivation period. Significant correlation coefficient with SPAD value was observed only in PG.

The absolute values of correlation coefficients of ascorbic acid contents with daily minimum air temperatures during one week before harvest were likely to be larger than with daily means during total cultivation periods in most of the Brassicaceae crops (Table 4-4). On the other hand, there were 6 Asteraceae crops with significant negative correlation coefficients between ascorbic acid contents and daily minimum air temperatures during total cultivation periods. There were no significant correlations between ascorbic acid contents either air temperatures in Amaranthaceae crops.

There were 5 Brassicaceae crops with significant positive correlation coefficients between nitrate contents and daily minimum air temperatures during total cultivation periods, though no significant correlations with either air temperature in most of the Asteraceae and Amaranthaceae crops, except for GR and CD (Table 4-4).

The leaf weights had significant negative correlation coefficients with daily mean air temperatures especially in all the Amaranthaceae crops (Table 4-5). The leaf numbers had significant negative correlation coefficients with daily mean air temperatures in 5 BLC. There were no significant correlation coefficients in the leaf length, leaf width, leaf area and dry matter ratio with daily mean air temperatures during total cultivation periods in most of BLC (Table 4-5). There were 10 BLC with significant negative correlation coefficients between the leaf weight/leaf areas and daily minimum air temperatures during total cultivation periods. There were no significant correlations between dry matter ratio and daily mean air temperatures during total cultivation periods in most of BLC.

Discussion

1. Seasonal variation in cultivation period

Table 4-3. Pearson product-moment correlation coefficient of cultivation period, yield and SPAD value with daily mean air temperature during total cultivation period.

Abbreviation[z]	Total cultivation period	Yield	Yield/total cultivation period	SPAD value
Brassicaceae				
WM	-0.972 ***[y]	-0.753 *	-0.133	-0.316
GKM	-0.978 ***	-0.729	-0.006	-0.127
PG	-0.972 ***	-0.829 *	-0.173	-0.866 **
TS	-0.978 ***	-0.715 *	-0.133	-0.388
GK	-0.928 ***	-0.399	0.233	-0.503
GM	-0.961 ***	-0.525	0.094	-0.063
RC	-0.967 ***	-0.666	0.087	-0.224
RK	-0.948 ***	-0.156	0.570	-0.677
RKM	-0.951 ***	-0.755 *	-0.234	-0.148
RM	-0.972 ***	-0.865 *	-0.470	-0.262
Asteraceae				
GR	-0.946 ***	-0.875 **	0.371	-0.654
LG	-0.958 ***	-0.798 *	-0.092	-0.303
EN	-0.911 **	-0.787 *	-0.140	0.218
CD	-0.933 **	-0.880 *	-0.563	-0.402
GO	-0.937 ***	-0.721 *	-0.072	-0.074
RR	-0.927 **	-0.544	0.062	-0.404
LR	-0.981 ***	-0.842 **	-0.320	-0.220
RO	-0.934 ***	-0.433	0.044	-0.148
IR	-0.945 ***	-0.769 *	-0.062	-0.055
Amaranthaceae				
GS	-0.882 **	-0.606	0.020	-0.605
RS	-0.898 **	-0.840 **	-0.438	-0.020
DT	-0.918 **	-0.939 ***	-0.073	-0.281

[z] See Table 2-4 for abbreviations.

[y] *, **, *** Significant at $P < 0.05$, 0.01, or 0.001, respectively ($n = 6$-8).

Table 4-4. Pearson product-moment correlation coefficient of ascorbic acid and nitrate contents with air temperatures during cultivation period.

Abbreviation[z]	Ascorbic acid				Nitrate			
	Total cultivation period		One week before harvest		Total cultivation period		One week before harvest	
	Daily mean	Daily minimum	Daily mean	Daily minimum	Daily mean	Daily minimum	Daily mean	Daily minimum
Brassicaceae								
WM	-0.678	-0.718 *[y]	-0.682	-0.752 *	0.265	0.250	0.016	0.089
GKM	-0.662	-0.690	-0.662	-0.703	0.891 **	0.903 **	0.841 *	0.848 *
PG	-0.678	-0.719	-0.671	-0.783 *	0.684	0.749	0.626	0.759 *
TS	-0.447	-0.459	-0.545	-0.605	0.290	0.287	0.208	0.226
GK	-0.263	-0.271	-0.304	-0.315	0.355	0.411	0.444	0.452
GM	-0.710 *	-0.745 *	-0.736 *	-0.808 *	0.772 *	0.836 **	0.698	0.800 *
RC	-0.534	-0.526	-0.548	-0.593	0.647	0.719 *	0.570	0.637
RK	-0.640	-0.685	-0.675	-0.745 *	0.696	0.664	0.629	0.645
RKM	-0.768 *	-0.803 *	-0.796 *	-0.836 *	0.907 **	0.895 **	0.814 *	0.800 *
RM	-0.514	-0.540	-0.522	-0.629	0.860 *	0.911 **	0.820 *	0.893 **
Asteraceae								
GR	-0.832 *	-0.851 **	-0.741 *	-0.828 *	0.720 *	0.753 *	0.598	0.669
LG	-0.692	-0.709 *	-0.544	-0.491	0.555	0.561	0.589	0.615
EN	-0.749 *	-0.742 *	-0.674	-0.698	0.402	0.378	0.528	0.406
CD	-0.774	-0.803	-0.577	-0.550	0.867 *	0.884 *	0.876 *	0.891 *
GO	-0.860 **	-0.872 **	-0.852 **	-0.862 **	0.387	0.373	0.498	0.488
RR	-0.816 *	-0.830 *	-0.790 *	-0.871 *	0.499	0.509	0.543	0.566
LR	0.250	0.289	0.367	0.329	0.051	0.058	-0.196	-0.124
RO	-0.751 *	-0.741 *	-0.755 *	-0.752 *	0.457	0.458	0.497	0.469
IR	-0.668	-0.692	-0.610	-0.634	-0.065	-0.106	0.031	-0.019
Amaranthaceae								
GS	-0.340	-0.337	-0.433	-0.465	0.112	0.121	0.093	0.137
RS	-0.277	-0.314	-0.401	-0.470	0.370	0.403	0.184	0.253
DT	-0.552	-0.533	-0.465	-0.533	-0.201	-0.196	-0.260	-0.256

[z] See Table 2-4 for abbreviations.

[y] *, ** Significant at $P < 0.05$, or 0.01, respectively (n = 6-8).

Table 4-5. Pearson product-moment correlation coefficient of leaf characteristics with daily mean air temperature during total cultivation period.

Abbreviation[z]	Leaf weight	Leaf number	Leaf length	Leaf width	Leaf area	Leaf weight/ leaf area	Dry matter ratio
Brassicaceae							
WM	-0.285	-0.818 *[y]	0.035	0.602	0.131	-0.494	0.364
GKM	-0.679	-0.748	-0.035	0.298	0.049	-0.822 *	-0.302
PG	-0.876 **	-0.481	-0.308	0.081	-0.257	-0.924 **	-0.052
TS	-0.409	-0.683	0.700	-0.513	-0.772 *	-0.285	0.854 *
GK	-0.593	-0.127	0.053	-0.206	-0.090	-0.619	0.027
GM	-0.324	-0.595	0.288	-0.062	-0.406	-0.215	0.099
RC	-0.470	-0.434	0.386	-0.310	-0.237	-0.476	0.214
RK	-0.470	0.619	0.594	0.010	0.189	-0.708 *	-0.616
RKM	-0.764 *	-0.732	-0.391	-0.016	-0.809 *	0.270	0.159
RM	-0.547	-0.874 *	-0.127	-0.110	-0.241	-0.747	0.088
Asteraceae							
GR	-0.701	-0.097	0.568	-0.329	-0.362	-0.970 ***	-0.363
LG	-0.511	-0.762 *	0.558	-0.208	-0.409	-0.503	-0.119
EN	-0.398	0.298	0.422	0.464	-0.077	-0.584	-0.460
CD	-0.903 *	-0.817 *	0.007	-0.409	-0.788	-0.869 *	0.028
GO	-0.453	-0.563	0.307	-0.015	-0.127	-0.648	-0.652
RR	-0.543	-0.237	0.433	-0.248	-0.165	-0.871 *	-0.477
LR	-0.826 *	-0.405	0.113	-0.734 *	-0.550	-0.878 **	0.417
RO	-0.451	-0.220	0.283	-0.215	-0.284	-0.708 *	-0.616
IR	-0.349	-0.828 *	0.102	-0.312	-0.593	-0.061	0.002
Amaranthaceae							
GS	-0.733 *	0.021	-0.115	-0.548	-0.409	-0.388	-0.559
RS	-0.871 **	-0.608	0.502	-0.657	-0.678	-0.783 *	-0.039
DT	-0.888 **	-0.631	0.614	-0.562	0.318	-0.861 **	0.150

[z] See Table 2-4 for abbreviations.

[y] *, **, *** Significant at $P < 0.05$, 0.01, or 0.001, respectively ($n = 6\text{-}8$).

The total cultivation periods were different depending on examined seasons in all BLC; they showed longer total cultivation periods in the spring and shorter ones in the summer (Fig. 4-2A). This result was in agreement with the previous report (Grahn et al., 2015b; Hall et al., 2012). Furthermore, strong correlations between each period and daily mean air temperature emerged for all BLC (Table 4-3). This result will be a useful information for planning of sowing and harvest date for stable marketing supply of the BLV.

2. Seasonal variation in yield and its factor

There were seasonal variations in yields in most of BLC; they tended to have more than twice yields in spring than in summer (Fig. 4-2B). This result was in agreement with the report that yields of rocket was larger in winter than in spring in Australia, southern semisphere (Hall et al., 2012). These seasonal variations could be suggested that most of BLC tended to increase yields according to the decrease of daily mean air temperature during total cultivation periods (Table 4-3), as reported by Grahn et al. (2015b).

The leaf weights and leaf weight/leaf areas also increased according to decreasing daily mean air temperature in Amaranthaceae crops (Table 4-5). This result suggests that low temperatures induced leaf thicknesses and furthermore caused increased leaf weights and yields. The causes of increased yields by low temperatures in most of the Brassicaceae and Asteraceae crops were not so clear as in Amaranthaceae crops. However, the leaf thickness was induced by low temperatures were observed in some Brassicaceae and Asteraceae crops, which implies the relations between leaf weights and low temperatures. By contrast, there were no significant correlations in yield/total cultivation periods in most of BLC (Fig. 4-2C); that is to say, less seasonal differences in growth rate. However, seasonal variations in yields in IR and, RK were smaller than any other BLC. Because RK had largest yield and higher growth rate in summer than in spring and RK could be a suitable crop in summer.

3. Seasonal variation factor in SPAD value

The SPAD values varied according to the seasons (Fig. 4-2D). However, there were also different tendencies depending on crops. Therefore, the variations in SPAD values could not result from seasonal environment. The significant negative correlation between SPAD value and cold temperature stress were reported in watermelon (Korkmaz and Dufault, 2001), in spite of less correlation with air temperatures in most of BLC (Table 4-3). A lack of light caused a reduction of the SPAD value in rice (Junjun et al., 2014). Moreover, the SPAD values and nitrogen contents presented strong linear relationship in green leafy vegetables (Limantara et al., 2015). However, the SPAD values seem not to change so much as any other characteristics among various seasons in most of BLC.

4. Difference of factor affecting ascorbic acid contents among BLC

There were differences in ascorbic acid contents among seasons especially in Brassicaceae and Asteraceae crops; they showed more ascorbic acid contents in spring and less in summer (Fig. 4-2E). Vitamin C contents of spinach and komatsuna can be increased by controlling in the minimum air temperature at 5° C for 10 days before harvesting (Tamura, 2004). There were slightly larger correlation coefficients in ascorbic acid contents with daily minimum air temperatures for one week before harvests than daily means for total cultivation periods in most of the Brassicaceae crops (Table 4-4). On the other hand, more than half of Asteraceae crops showed the significant coefficients between ascorbic acid contents and air temperatures, especially daily minimum temperatures during total cultivation periods. This result could be useful for improvement of ascorbic acid contents in some Brassicaceae and Asteraceae crops.

The Amaranthaceae crops such as spinach showed no correlations between ascorbic acid contents and air temperatures (Table 4-4), contrary to previous reports (Tamura, 2004). Another previous research suggests that the ascorbic acid content in spinach was strongly affected by total amount of solar radiation received by the plants one day before harvest (Yoshida and Hamamoto, 2010). Therefore, the environmental conditions just before harvest such as air temperatures or solar radiations could have influences on ascorbic acid contents in Amaranthaceae crops rather than those during the total cultivation period.

5. Seasonal variation in nitrate content and environmental factor

There were seasonal variations in nitrate contents in some of BLC; they tended to contain higher nitrates in summer (Fig. 4-2F). Such result was similar to the result obtained in commercial komatsuna and spinach (Yorifuji et al., 2005). The significant correlations between nitrate contents and daily minimum air temperatures for total cultivation periods were recognized especially in PG, mustard crops (GKM, GM, RKM and RM), RC, GR and CD (Table 4-4). Above all, the nitrate level of mustard crops will be able to estimate by air temperatures. By contrast, other 14 BLC showed less relations between nitrate contents air temperatures. Other environmental conditions such as light intensity, watering, or application of nitrogen or manure could affect the behaviors of nitrates (Ikeba et al., 2005; Nakamoto et al., 1995; Ohashi-Kaneko et al., 2007; Takebe et al., 1995). Brassicaceae and Asteraceae crops had higher nitrate contents especially in June with less sunshine duration and in July, August, October, in which plastic house were covered with shading. Therefore, the nitrate contents in these two families could affect light conditions besides air temperatures.

Chapter 5

Productivity and internal quality in winter

Introduction

Hokkaido is located in the northern subarctic region with heavy snow in Japan (Dfa or Dfb, according to the Köppen classification; 1936). Therefore, it has been said that the production of vegetables is difficult in cold and snowy winter in Hokkaido and a main obstacle in year-round BLV production. On the other hand, Borrelli et al. (2013) showed that it was possible to produce cold-tolerant crops during winter months by using heat insulation structures such as high tunnels. Tamura (2000) evaluated the freezing tolerance in spinach and komatsuna. Tamura et al. (2003) also revealed the increases of sugars and vitamin C contents in spinach and komatsuna under cold condition. These reports suggested the positive possibility of BLV production in severe cold season. The yield and internal qualities were investigated to reveal the possibility of BLV production in winter in this chapter.

Materials and Methods

1. Baby-leaf crops and experimental design

The experiments were carried out 3 times from 2013 to 2016 (Table 5-1). All experiments were done in the same unheated single-layer plastic house in the Donan Agricultural Experiment Station in Chapter 3. Experimental designs generally followed those explained in Chapter 3. Almost the same crops were used as in Chapter 3. Sowing date was determined considering the results of the total cultivation period of each BLC in Chapter 3.

2. Determination of the cultivation period

The measurements were carried out same as Chapter 3. The day when more than 50% of the seeds had emerged was recognized as the emergence day for each crop. The emergence period

Table 5-1. Sowing date for the cultivation in winter.

Abbreviation[z]	Examination year		
	2013 - 2014	2014 - 2015	2015 - 2016
Brassicaceae			
WM	22 Oct	29 Oct	1 Nov
GKM	22 Oct	29 Oct	29 Oct
PG	22 Oct	29 Oct	1 Nov
TS	22 Oct	29 Oct	29 Oct
GK	22 Oct	27 Oct	26 Oct
GM	22 Oct	29 Oct	29 Oct
RC	22 Oct	29 Oct	29 Oct
RK	22 Oct	27 Oct	26 Oct
RKM	22 Oct	29 Oct	29 Oct
RM	22 Oct	29 Oct	29 Oct
Asteraceae			
GR	18 Oct	27 Oct	26 Oct
LG	18 Oct	27 Oct	26 Oct
EN	18 Oct	24 Oct	23 Oct
CD	18 Oct	24 Oct	23 Oct
GO	18 Oct	27 Oct	26 Oct
RR	18 Oct	27 Oct	26 Oct
LR	18 Oct	24 Oct	23 Oct
RO	18 Oct	27 Oct	26 Oct
IR	18 Oct	24 Oct	23 Oct
Amaranthaceae			
GS	22 Oct	27 Oct	29 Oct
RS	22 Oct	27 Oct	29 Oct
DT	18 Oct	24 Oct	23 Oct

[z] See Table 2-4 for abbreviations.

[y] *, **, *** Significant at $P < 0.05$, 0.01, or 0.001, respectively ($n = 6\text{-}8$).

was calculated as the period from the sowing date to the emergence date. The growing period was estimated as the difference between the harvest date, as mentioned below, and the emergence date. The total cultivation period was computed as the sum of the days necessary for emergence and the growing period.

3. Harvest method

Every crop was harvested for three times in each experiment. The first harvest was carried out in each crop in around December, in which BLC growth reached the harvest criteria, same as Chapter 3. The true leaves were harvested using scissors above cotyledons between 11 a.m. and 2 p.m. The fresh weight (FW) per 0.1 m^2 of a whole 1 m^2 area was measured to determine the yield ($g \cdot m^{-2}$). The second and third harvest were performed from the rest of December-harvest in January and February. The yields in January and February were defined as adjusted one, that is to say, removed withered or too large leaves from intact harvest. The yield/total cultivation period was calculated a growth rate.

4. Ascorbic acid contents

The measurements were carried out same as Chapter 3. Five g FW of leaves from each plot was 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). Aliquot of each extract was analyzed with an RQflex plus reflectometer (Merck, Millipore, MA, USA) and by means of a Reflectoquant Ascorbic Acid Test (Merck, Millipore, MA, USA).

5. Nitrate content

The measurements were carried out same as Chapter 3. Five g FW of leaves from each plot was 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 with 4 ml of distilled water and analyzed with an RQflex plus reflectometer and by means of a Reflectoquant Nitrate Test (Merck, Millipore, MA, USA).

6. Brix

Brix is sometimes used as an indicator of quality evaluation in winter sweet spinach (Aoki, 2007), and was investigated in these experiments. Five g FW of leaves from each plot was crashed by using garlic press. Squeezed juice was analyzed with PAL-1 refractometer (Atago, Tokyo, Japan).

7. Leaf characteristics

The measurements were carried out same as Chapters 3. Aliquots of 20 well-developed leaves were prepared for each crop at adjusted 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, whose 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 calculated as an indicator of leaf thickness. The number of leaves were obtained by dividing the yield by the mean leaf weight. More than 30 g of harvested leaves was dried at 60 °C, using a drying oven, for one week, in order to calculate the dry matter ratio.

8. Correlations of yield, internal quality and leaf characteristics with air temperature s

Correlation coefficients of ascorbic acid and nitrate contents, Brix and dry matter ratio were calculated using the daily minimum air temperature during one week before harvest in each crop. Statistical analysis was performed with the above-mentioned EZR.

9. Correlation of internal quality with dry matter ratio

Correlation coefficients of ascorbic acid and nitrate contents, Brix and dry matter ratio was calculated using the daily mean air temperature during total cultivation period in each crop. The coefficients of ascorbic acid and nitrate contents were calculated using the daily mean, daily maximum and daily minimum air temperature during total cultivation period and one week before harvest in each crop. Statistical analysis was performed with the above-mentioned EZR.

Results

1. Temperatures and sunshine durations during the cultivation period

The plastic house environmental conditions in winter were compared (Fig. 5-1). The daily mean air temperatures between sowing date to third harvest date in February in WM was 4.2, 3.9, and 3.6°C in 2013 - 2014, 2014 - 2015, and 2015 – 2016, respectively. The lowest air temperature was recorded -10.1, -8.5, and -10.5°C in 2013 - 2014, 2014 - 2015, and 2015 - 2016, respectively. The total sunshine durations from sowing to third harvest date in WM was 306, 299, and 278 hours in 2013 - 2014, 2014 - 2015, and 2015 – 2016, respectively.

2. Cultivation periods

The total cultivation periods in December-harvest (40.4, 52.1, and 44.7 days mean in Brassicaceae, Asteraceae, and Amaranthaceae crops, respectively) were longer than in May (Fig 5-2). The differences in growing periods among 3 years in most of the Asteraceae crops were larger than Brassicaceae and Amaranthaceae crops. CD and IR had largest differences (23.4 and 20.5 in standard deviation, respectively) in growing periods among tested years and could not reach harvest criteria within December in 2014.

3. Yields and productivities

Most of BLC could be harvested until early February, though withered or large and aged

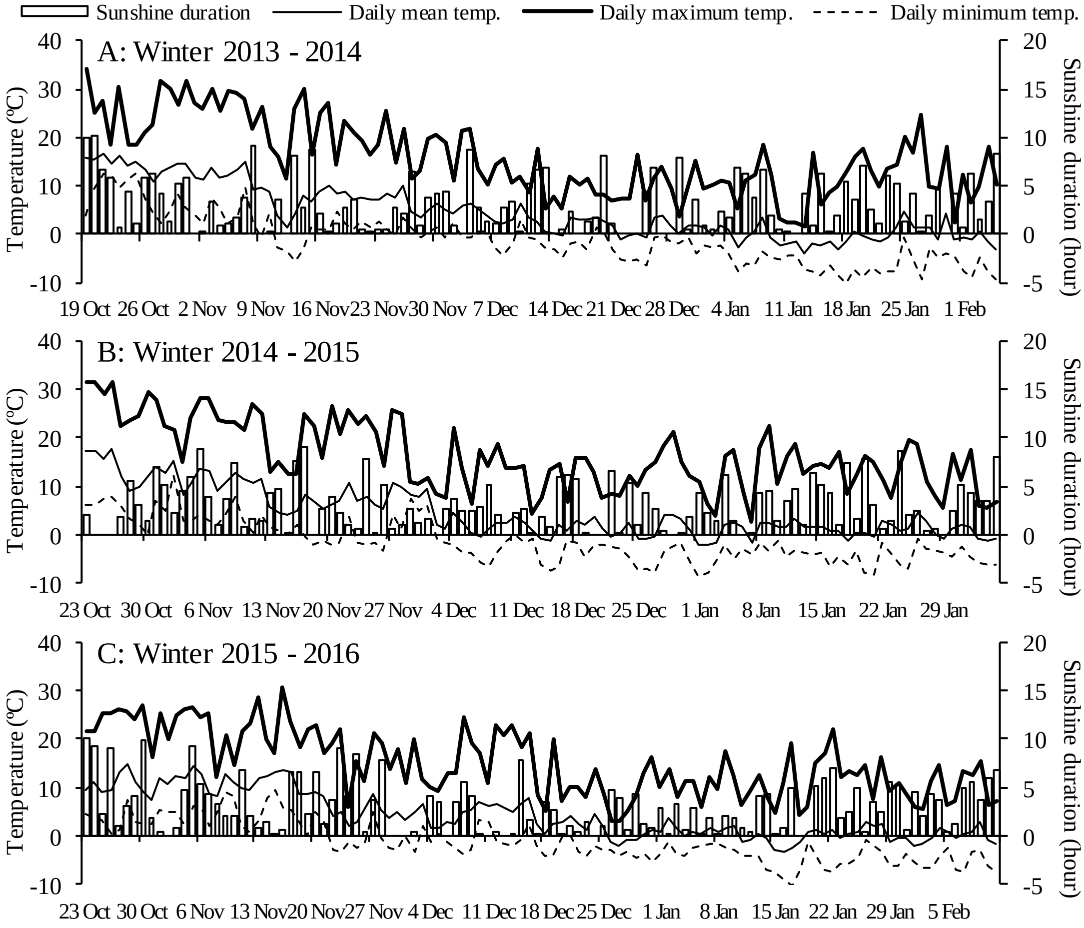


Fig. 5-1. Temperature lines and sunshine duration bars during the cultivations in winter. The sunshine duration was obtained from AMeDAS (Automated Meteorological Data Acquisition System) in Hokuto, Hokkaido, Japan.

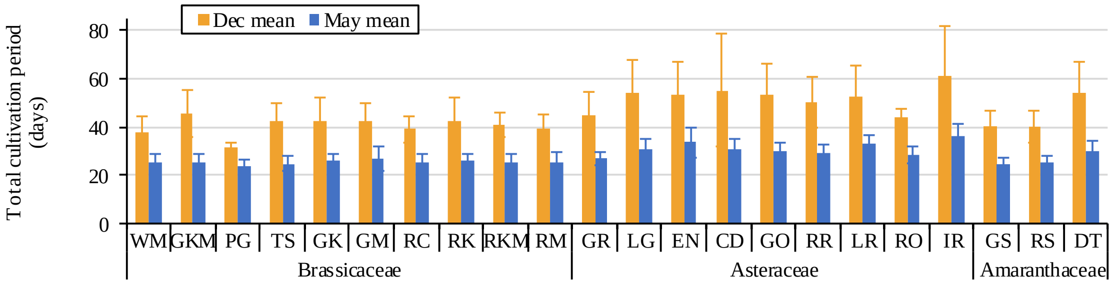


Fig. 5-2. Total cultivation periods in December-harvest. December mean is based on the data in 2013-2014, 2014-2015, and 2015-2016 with standard deviation bars. May mean as reference is based on the data in 2010 and 2011 with standard deviation bars.

leaves were observed in January and February (Figs. 5-3 and 5-4). Most of BLC were wilted in the morning and were recovered in afternoon (Fig. 5-5). The yields tended to increase from December to February in most of BLC. The yield and those in February (824, 788, and 1,144 g·m^{-2} in mean in Brassicaceae, Asteraceae, and Amaranthaceae crops, respectively) were equal to or larger than in May (682, 691, and 772 g·m^{-2} in mean in Brassicaceae, Asteraceae, and Amaranthaceae crops, respectively) (Fig. 5-6A). By contrast, yield/total cultivation periods tended to decrease from December to February and were likely to lower in February (8.0, 7.4, and 10.9 g·m^{-2}·day^{-1} in mean in Brassicaceae, Asteraceae, and Amaranthaceae crops, respectively) than in August (21.7, 18.2, and 22.0 g·m^{-2}·day^{-1} in mean in Brassicaceae, Asteraceae, and Amaranthaceae crops, respectively) (Table 5-6B). WM, TS, GK, RK, LG, EN, GS and RS tended to have more yield in winter than May. TS had largest yield and highest yield/total cultivation period in winter (1042 - 1892 g·m^{-2} and 18.4 - 24.6 g·m^{-2}·day^{-1} , respectively) PG had smaller yield and lower yield/total cultivation period in winter (527-727 g·m^{-2} and 5.2-22.2 g·m^{-2}·day^{-1}, respectively) than in May (812 g·m^{-2} and 35.1 g·m^{-2}·day^{-1}, respectively).

4. Quality

1) Ascorbic acid contents

The ascorbic acid contents in February were higher (995, 449, and 937 μg·g^{-1} FW in mean in Brassicaceae, Asteraceae, and Amaranthaceae crops, respectively) than in May (701, 320, and 499 μg·g^{-1} FW in mean in Brassicaceae, Asteraceae, and Amaranthaceae crops, respectively) in most of BLC (Table 5-6C). The significant increases from December to February were observed in most of the Brassicaceae and Amaranthaceae crops. Each ascorbic acid content in GKM, GK, RC, RK, RM and RS was more than 1,000 μg·g^{-1} FW in February.

2) Nitrate content

The nitrate contents in February were lower (1,204, 971, and 1,142 μg·g^{-1} FW in mean in Brassicaceae, Asteraceae, and Amaranthaceae crops, respectively) than in May (3,454, 2,663,

Fig. 5-3. Baby-leaf vegetables cultivation in unheated plastic house in winter (11 Jan 2014, Donan Agricultural Experiment Station).

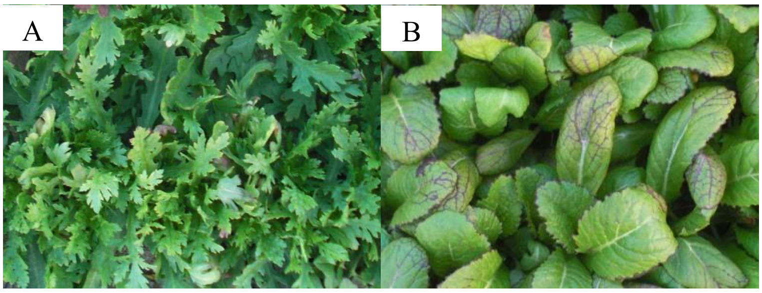


Fig. 5-4. Withered leaves ’Kiwame Chuba Shungiku’ (CD, A), and large and aged leaves in ’Red Mustard’ (RM, B) in unheated plastic house in winter (15 Feb 2015).

Fig. 5-5. ‘Wase Mizuna’ (WM) in unheated plastic house in winter at 7 AM (A) and at 14 PM (B) (11 Jan 2014).

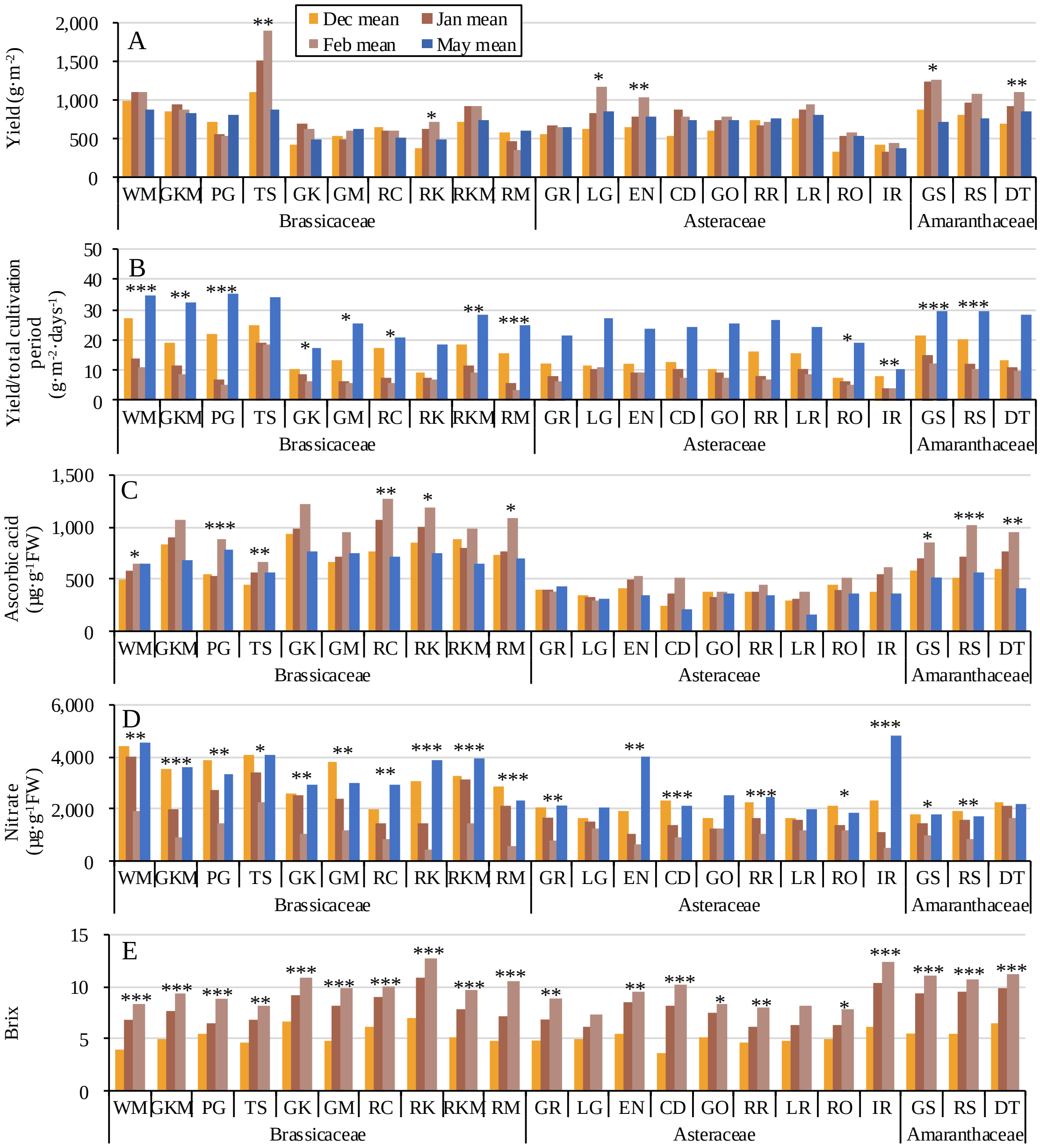


Fig. 5-6. Yields (A), growth rate (B), ascorbic acid (C) and nitrate contents (D), and Brix (E) in winter. May mean is based on the data in 2010 and 2011. December mean, January mean, and February mean are based on the data in 2013-2014, 2014-2015, and 2015-2016. *, **, *** Significant at $P < 0.05$, 0.01, and 0.001 by ANOVA among December, January, and February.

and 1,900 $\mu g \cdot g^{-1}$ FW in mean in Brassicaceae, Asteraceae, and Amaranthaceae crops, respectively) in most of BLC (Table 5-6D). The significant decreases from December to February were observed in most of BLC. On the other hand, the differences in nitrate contents were smaller among harvest months in LG, GO, LR and DT than any other BLC.

3) Brix

The significant increases in Brix from December to February were observed in most of BLC (Table 5-6E). Each Brix in GK, RC, RK, RM, CD, IR, GS, RS and DT was more than 10 in February.

4) Leaf characteristics

The leaf weights in GKM, RK, RKM, LG, GO, RR and LR increased significantly from December to February, and were larger in February (763, 629, 680, 726, 589, 491, and 631 μg/leaf, respectively) than in May (534, 463, 373, 526, 383, 356, and 548 μg/leaf, respectively) (Table 5-7A). The leaf numbers in TS, GS and RS increased from December to February, and were larger in February (2,146, 2,213, and 1,980 leaves$\cdot m^{-2}$, respectively) than in May (1,414, 1,141, and 1,369 μg/leaf, respectively) (Table 5-7B). In contrast, PG and RM had smaller leaf number in February (789 and 630 leaves$\cdot m^{-2}$, respectively) than in May (1,213 and 1,132 leaves$\cdot m^{-2}$, respectively). The leaf areas in GKM, RK, RKM, and RR were larger in February (1,925, 1,483, 1,736, and 1,620 m^2, respectively; data not shown) than in May (1,301, 1,184, 1,460, and 1,297 m^2, respectively; data not shown) due to increased leaf lengths or leaf widths.

The leaf shapes somewhat changed from December to February in RK (Fig. 5-8). The leaf weight/leaf areas in PG, RK, GR, LG, GO, RR, LR, IR and DT increased significantly from December to February and were higher in February (0.43, 0.43, 0.35, 0.33, 0.38, 0.30, 0.30, 0.31, and 0.54 $\mu g \cdot mm^{-2}$, respectively; data not shown) than in May (0.39, 0.39, 0.29, 0.26, 0.23, 0.27, 0.27, 0.26, and 0.47 $\mu g \cdot mm^{-2}$, respectively; data not shown). The dry matter ratios in December were almost same as May or August in most of BLC. However, the dry matter ratio increased significantly from December to February (Fig. 5-7C).

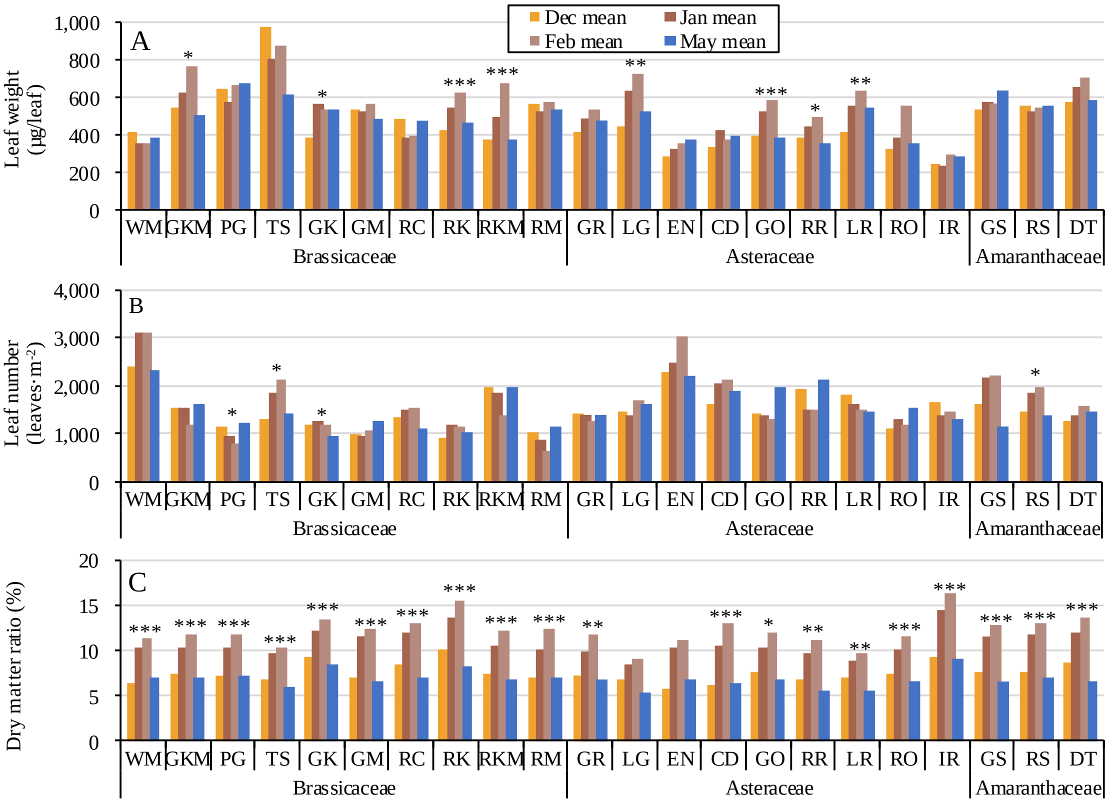


Fig. 5-7. Leaf weight (A), leaf number (B), and dry matter ratio (C) in winter. May mean is based on the data in 2010 and 2011. December mean, January mean, and February mean are based on the data in 2013-2014, 2014-2015, and 2015-2016. *, **, *** Significant at $P < 0.05$, 0.01, and 0.001 by ANOVA among December, January, and February.

Fig. 5-8. Leaf shapes observed in December (A) and February (B), in ‘Red Kale’ (RK).

5. Correlations of BLC characteristics with air temperatures

The correlation coefficients between BLC characteristics with daily minimum air temperature during one week before harvest were shown in Table 5-2. There were no significant correlation coefficients between ascorbic acid content and daily minimum air temperatures in Asteraceae crops. The significantly positive correlation coefficients between nitrate content and daily minimum air temperature during one week before harvest were observed in the 16 BLC. In addition, the significantly negative ones between Brix and daily minimum air temperature during one week before harvest were observed in all BLC except for LR. There were significant negative correlation coefficients between dry matter ratio and daily minimum air temperature during one week before harvest in all BLC.

6. Correlations of BLC internal qualities with dry matter ratio

The correlation coefficients between BLC internal qualities with dry matter ratio were shown in Table 5-3. The significantly positive correlation coefficients with ascorbic acid contents were observed in TS, RK, RM, RS and DT. There were significantly negative correlation coefficients with nitrate contents in the 15 BLC and significantly positive ones with Brix in the 19 BLC.

Discussion

1. Growth characteristics of BLC in winter cultivation

The total cultivation periods in December-harvest shows the same tendencies as those in spring to autumn (Fig. 5-2). BLC grow slowly in mid-winter as it got cold. In consequence, total cultivation periods became longer in winter than any other season, especially in Asteraceae crops. Therefore, Asteraceae crops hand the risk of harvest impossibility due to the growth delay in winter. By contrast, Brassicaceae or Amaranthaceae crops, especially spinach, grew earlier than

Table 5-2. Correlation coefficient of ascorbic acid and nitrate contents, Brix and dry matter ratio with daily mean air temperature during one week before harvest in winter cultivation.

Abbreviation[z]	Ascorbic acid	Nitrate	Brix	Dry matter ratio
Brassicaceae				
WM	-0.367	0.497	-0.830 **	-0.972 ***
GKM	-0.382	0.909 ***[y]	-0.841 **	-0.950 ***
PG	-0.561	0.668 *	-0.789 *	-0.948 ***
TS	-0.345	0.625	-0.895 **	-0.903 ***
GK	-0.251	0.352	-0.853 **	-0.887 **
GM	-0.560	0.774 *	-0.916 ***	-0.945 ***
RC	-0.248	0.769 *	-0.795 *	-0.939 ***
RK	-0.553	0.922 ***	-0.868 **	-0.907 ***
RKM	-0.261	0.839 **	-0.799 **	-0.932 ***
RM	-0.619	0.741 *	-0.806 **	-0.922 ***
Asteraceae				
GR	0.133	0.734 *	-0.727 *	-0.888 **
LG	0.473	0.723 *	-0.695 *	-0.710 *
EN	-0.267	0.836 **	-0.684 *	-0.675 *
CD	-0.147	0.698	-0.801 *	-0.761 *
GO	0.593	0.422	-0.723 *	-0.847 **
RR	-0.319	0.773 *	-0.704 *	-0.913 ***
LR	-0.163	0.666 *	-0.629	-0.897 **
RO	-0.377	0.661	-0.669 *	-0.916 ***
IR	0.053	0.900 **	-0.853 **	-0.885 **
Amaranthaceae				
GS	-0.774 *	0.717 *	-0.961 ***	-0.956 ***
RS	-0.601	0.703 *	-0.911 ***	-0.985 ***
DT	-0.599	0.771 *	-0.877 **	-0.893 **

[z] See Table 2-4 for abbreviations.

[y] *, **, *** Significant at $P < 0.05$, 0.01, or 0.001, respectively (n = 7-9).

Table 5-3. Correlation coefficient between dry matter ratio and internal qualities of the baby-leaf crops in winter cultivation.

Abbreviation[z]	Ascorbic acid		Nitrate		Brix	
Brassicaceae						
WM	0.583		-0.531		0.860	**
GKM	0.504		-0.962	***	0.954	***
PG	0.649		-0.773	*	0.911	***
TS	0.728	*[y]	-0.745	*	0.926	***
GK	0.368		-0.563		0.927	***
GM	0.645		-0.883	**	0.943	***
RC	0.627		-0.841	**	0.928	***
RK	0.796	*	-0.982	***	0.986	***
RKM	0.355		-0.950	***	0.887	**
RM	0.727	*	-0.900	***	0.925	***
Asteraceae						
GR	-0.063		-0.796	*	0.952	***
LG	-0.479		-0.824	**	0.833	**
EN	-0.153		-0.404		0.589	
CD	0.676		-0.886	**	0.977	***
GO	-0.266		-0.587		0.928	***
RR	0.436		-0.851	**	0.634	
LR	0.335		-0.606		0.451	
RO	0.125		-0.653		0.870	**
IR	0.601		-0.921	**	0.894	**
Amaranthaceae						
GS	0.539		-0.633		0.968	***
RS	0.753	*	-0.779	*	0.930	***
DT	0.737	*	-0.730	*	0.959	***

[z] See Table 2-4 for abbreviations.

[y] *, **, *** Significant at $P < 0.05$, 0.01, or 0.001, respectively ($n = 7\text{-}9$).

Asteraceae crops and could be more suitable for the winter cultivation.

2. Yield and growth rate during winter

These experiments revealed that BLC could survive even in the -10ºC of air temperatures in a plastic house (Figs. 5-1 and 5-3). This fact coincides with another report on the winter production of BLV (Borrelli et al., 2013), and suggested the possibility of winter production in Hokkaido, which has been told to be difficult before. However, BLC grew so slowly during winter due to colder temperature and smaller sunshine duration. Therefore, the yield/total cultivation period were smaller in winter than in spring to autumn. Furthermore, an extra work for removing process of withered or large leaves from intact harvest will be required in January and February (Fig. 5-4). Yields in January or February were equal to or greater than the yields in May, the best yield season, in most of BLC (Fig. 5-6A). The leaf weights and leaf thickness increased in TS, RK and LG, and led the yield increase (Fig. 5-7A). Furthermore, increase of the leaf area by change of leaf shape in February was observed in RK (Fig. 5-8). This morphological change could be caused by the transition from juvenile to adult phase as the result of long-term cultivation (Chuck et al., 2007; Huijser and Schmid, 2011; Wang et al., 2011; Wang et al., 2014). On the other hand, WM, TS, GK, EN, GS and RS increased leaf number (Fig. 5-7B). TS showed large yield and growth rate as the results of increasing leaf weight and leaf number even in winter. Therefore, TS could be suitable crop for winter cultivation. By contrast, PG and RM had less leaves by adjusting harvests. It had been also observed that larger leaves and less leaf emergences during long-term cultivation, and removal work for large leaves need according to harvest criteria in these crops compared with any other BLC in marketing. Therefore, the winter cultivation of PG and RM should be avoided from the view point of their growth characteristics.

3. Quality change in winter cultivation

Low air temperature treatments tended to increase ascorbic acid contents and Brix and decrease nitrate contents in spinach and komatsuna (Aoki, 2007; Tamura et al., 2003; Watanabe and Ayugase, 2015). And numerous studies have demonstrated quantitative and qualitative changes in the free saccharide content of plants exposed to low temperature (Guy et al, 1992; Jitsuyama et al, 2002; Kaurin et al, 1981; Leborgne et al, 1995). These tendencies might also hold true for other BLC (Figs. 5-6C, 5-6D, 5-6E and Table 5-2). Furthermore, the dry matter ratios increased in winter cultivation in all BLC contrary to the case of spring to autumn cultivations (Fig. 5-7C). Higher dry matter ratio increases turgor and induces crispness (Martin-Diana et al., 2006) and could make BLV taste stronger. Therefore, higher dry matter ratio could affect taste such as texture in BLC.

On the other hand, the nitrate contents and Brix had negative and positive correlations with dry matter ratios in the most BLC, respectively (Table 5-3). This result could suggest following hypothesis; 1) the Brix and dry matter ratio increase according to the inhibition of water absorption from root by lower temperature; 2) the nitrate is inhibited the absorption from soil along with water by lower temperature (Aoki, 2007). Accordingly, these hypotheses should have explained the increases in biosynthesized ascorbic acid as well as Brix. However, ascorbic acid contents had neither significant correlation with lower air temperatures nor dry matter ratios in the most of BLC (Table 5-2). These contradictions could be caused by low solar radiations, as mentioned in Chapter 4. A previous report mentioned that ascorbic acid content decreased in the low light intensity conditions by shading treatment in lettuce (Shinohara and Suzuki, 1981). Winter, especially December, is the lowest solar radiation season of the year. Furthermore, much snow piles up on and around plastic house, which could show the same effect as shading. For these reasons, the effect of solar radiation on the ascorbic acid content might be more severe in winter than any other seasons. However, GK, RC, RK, RM and RS had more than 1,000 $\mu g \cdot g^{-1}$ FW of ascorbic acid contents and more than 10 of Brix and were suitable crops in winter cultivation from the view point of internal qualities (Fig 5-6C).

Chapter 6
Effect of internal quality by salinity application in hot season

Introduction

Seasonal fluctuations in BLV yields and qualities were observed in the Chapter 4. The depression of yield and ascorbic acid contents in summer could be a commercial weak point from the view of a stable year-round production. On the other hand, previous researches have suggested that the rises of the beneficial components (e.g. antioxidants) and the reductions of harmful ones (e.g. nitrate) in the plants can be obtained by exposing plants to stress, such as salinity stress (Fernández et al., 2016; Pérez-López et al., 2015). However, the reaction of the plants in terms of yield and of components to the salinity exposure is different depending on vegetables (Fernández et al., 2016). The yield reduction by high salinity treatment in baby lettuce has been reported (Neocleous et al., 2014). The aims of this chapter were to evaluate the influence of salinity application in BLV soilless culture production and to clarify the factors affecting the improvement of yields and qualities.

Materials and Methods

1. Baby-leaf crops and experimental design

The experiments were carried out in plastic and glass houses in the Experimental Center of the Department of Agricultural, Forest and Food Science, University of Turin (44°53’11.67” N; 7°41’7.00” E–231 m a.s.l.) in Tetti Frati, Carmagnola (TO), Italy (Fig. 6-1). The same 22 leafy crops of the other chapters were used (Table 2-4 and Fig. 2-4). These crops were sown in 30-cell stayrofoam trays (0.25 m × 0.3 m) and filled with peat-based medium (Neuhaus Huminsubstrat N17; Klasmann-Deilmann® GmbH, Geeste, Niedersachsen, Germany), from 9 to 11 April 2018 for each replicate. The sown trays were placed into the nursery plastic house, and irrigated by spraying foggy mist for 3-5 minutes, twice a day. Seedlings were thinned out to 4 plants per cell (ca. 1,500 plants per m^2) on 17 and 18 April. Styrofoam trays were transferred into the floating growing system (FGS) in the glass house that has an automatically controlled opening system (Nicola et al., 2016) on 20 April. FGS consisted of 3 benches, each one split into 4 separate water beds (2.50 m × 1.40 m; 0.15 m depth) equipped with an aeration system. Each water bed was filled with 160 L of hydroponic nutrient solution (HNS) containing 12 N, 60/40 $N\text{-}NO_3^-/N\text{-}NH_4^+$, 2 P, 6 K, 2 Mg and 2.5 Ca $mM \cdot L^{-1}$. HNS was adjusted to electrical conductivity (EC) 2.5 $dS \cdot m^{-1}$ and pH 5.5 - 6.5 using NaCl and sulfuric acid, respectively. The air temperature was recorded every hour during at about 100 cm above the ground, near the expanding plant leaves on the FGS.

Four salinity treatments were prepared (Fig. 6-2): (1) maintained EC 2.5 $dS \cdot m^{-1}$ (0 mM NaCl) for about two weeks from 20 April, the date of starting FGS, to 4 May, the date of harvest mentioned below (two-weeks/EC 2.5; control); (2) added NaCl for 4 days up to EC 5.0 $dS \cdot m^{-1}$ (40.6 mM NaCl) gradually from 20 April and maintained until 4 May (two-weeks/EC 5.0); (3) added NaCl for 4 days up to EC 7.5 $dS \cdot m^{-1}$ (59.9 mM NaCl) gradually from 27 April, the 7th day after starting FGS, and maintained until 4 May (one-week/EC 7.5); (4) added NaCl for 4 days up to EC 5.0 $dS \cdot m^{-1}$ (28.6 mM NaCl) gradually from 27 April and maintained until 4 May (one-week/EC 5.0). The experiment followed a randomized complete block design with 3 replicates (2 replicates in 3 Amaranthaceae crops) per treatment.

2. Harvest method

Crops were harvested between 2 May and 4 May in the early-grown order, not subjected to the harvest criteria (Table 2-4), different from experiments in other chapters. Yields were calculated based on the total fresh weights per 30-cell tray.

3. Na^+, K^+, and Ca^{2+} elements

Fifty g fresh leaves for each unit were freeze-dried and ground in powder. Extraction was carried out by adding 1.3M of HNO_3 (25mL) to 100 mg of each sample and digesting at 60°C for

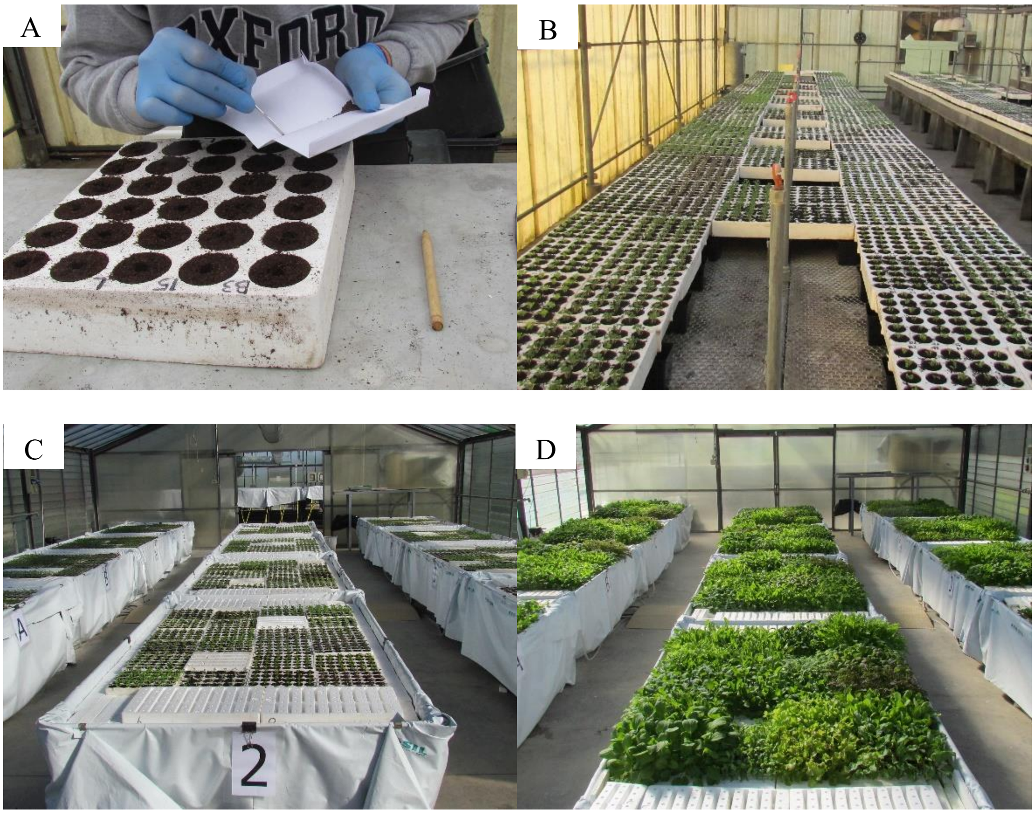


Fig. 6-1. Soilless culture of BLV using FGS in this experiment. Baby-leaf crops were sown in stayrofoam tray (A). The trays were placed in plastic house for about 10 days (B). The trays were transferred to FGS after thinning and salinity treatments were started (C). And baby-leaf crops were harvested about two-weeks after transferring to FGS (D).

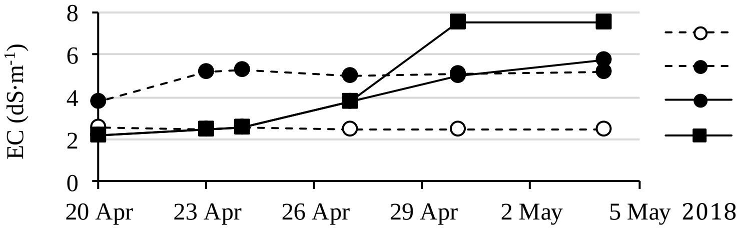


Fig. 6-2. Transition of EC by each salinity treatment.

4 h. Obtained solutions were filtered and diluted with 0.1 M HNO_3, and mineral element concentrations were analyzed by using ICP–AES (ICPE-9000, Shimazu, Kyoto, Japan) (Kumano and Araki, 2017).

4. Ascorbic acid contents

Extraction was carried out by adding to 5% metaphosphoric acid (1 mL) to 50 mg of each freeze-dried sample and shaking at room temperature for 3 hours. Supernatant obtained after 10,000 rpm centrifugal separation for 10 min were diluted to 10 times with 5% metaphosphoric acid and analyzed by the 2, 4-dinitrophenylhydrazine (DNP) method using Microplate reader (Synergy HT, Biotek, Winooski, VT).

5. Nitrate content

Extraction was carried out by adding distilled water (3 mL) to 20 mg of each freeze-dried sample and shaking at room temperature for 6 h. Supernatant obtained after 10,000 rpm centrifugal separation for 10 min were analyzed by using Auto-analyzer (SWAAT, HACH, Tokyo, Japan).

6. Total polyphenolics content

Extraction was carried out by adding 80% ethanol (10mL) to 20 mg of each freeze-dried samples and shaking at room temperature for 6 hours. Supernatant obtained after 10,000 rpm centrifugal separation for 10 min were analyzed and calculated as gallic acid equivalents (GAE) by the Folin-Ciocalteu colorimetric method.

7. Leaf characteristics

Ca. 20 randomly sampled fresh leaves in each crop were measured for leaf weights then dried at 60°C for more than 3 days using a drying oven to measure dry weights and calculate the dry matter ratio.

8. Correlations among yield, internal contents and leaf biomass

Correlation coefficients of yield, internal contents and leaf characteristics were calculated using the before-mentioned EZR.

Results

1. Environmental conditions during the cultivation period

Temperatures in the plastic house from April to May were shown in Fig. 6-3. The daily mean, maximum and minimum air temperature during the experiment in FGS was 21.7, 27.8 and 17.0°C, respectively. The climate during this experiment is equivalent to one in July or August in Hokkaido in Chapter 4 (Table 4-1).

2. Cation contents by salinity treatments

Significant increases in the Na^+ contents due to salinity treatments occurred in all 22 crops (Fig. 6-4A). The highest absorptions were observed in two-weeks/EC 5.0 salinity treatments in all crops. Contents in EC 7.5 salinity treatments (one-week/EC 7.5) tended to be higher than those in EC 5.0 treatments (one-week/EC 5.0) in the same treatment period. There were little differences in Na^+ contents by salinity treatment in DT, IR and EN. The K^+ contents tended to decrease due to two-weeks/EC 5.0 salinity treatment in most of the Brassicaceae crops (Fig. 6-4B).

The Na^+: K^+ ratios, an indicator of plant resistance to salinity (Juan et al., 2005), showed lower values in all salinity treatments in DT, IR and EN (Fig. 6-4C). The Ca^{2+} contents were not clearly different among salinity treatments in most of the crops (data not shown). The sum of Na^+, K^+ and Ca^{2+} (three-cations) contents due to two-weeks of salinity treatment were the same level as those due to one-week treatments in most of the Amaranthaceae and Asteraceae crops. In contrast, the three-cations contents due to two-weeks of salinity treatments were lower than those

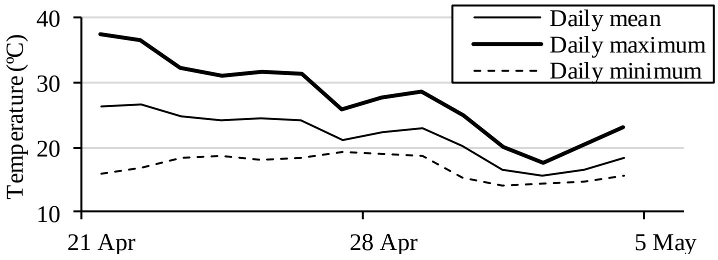


Fig. 6-3. Temperature during the FGS cultivations from 21 Apr to 4 May.

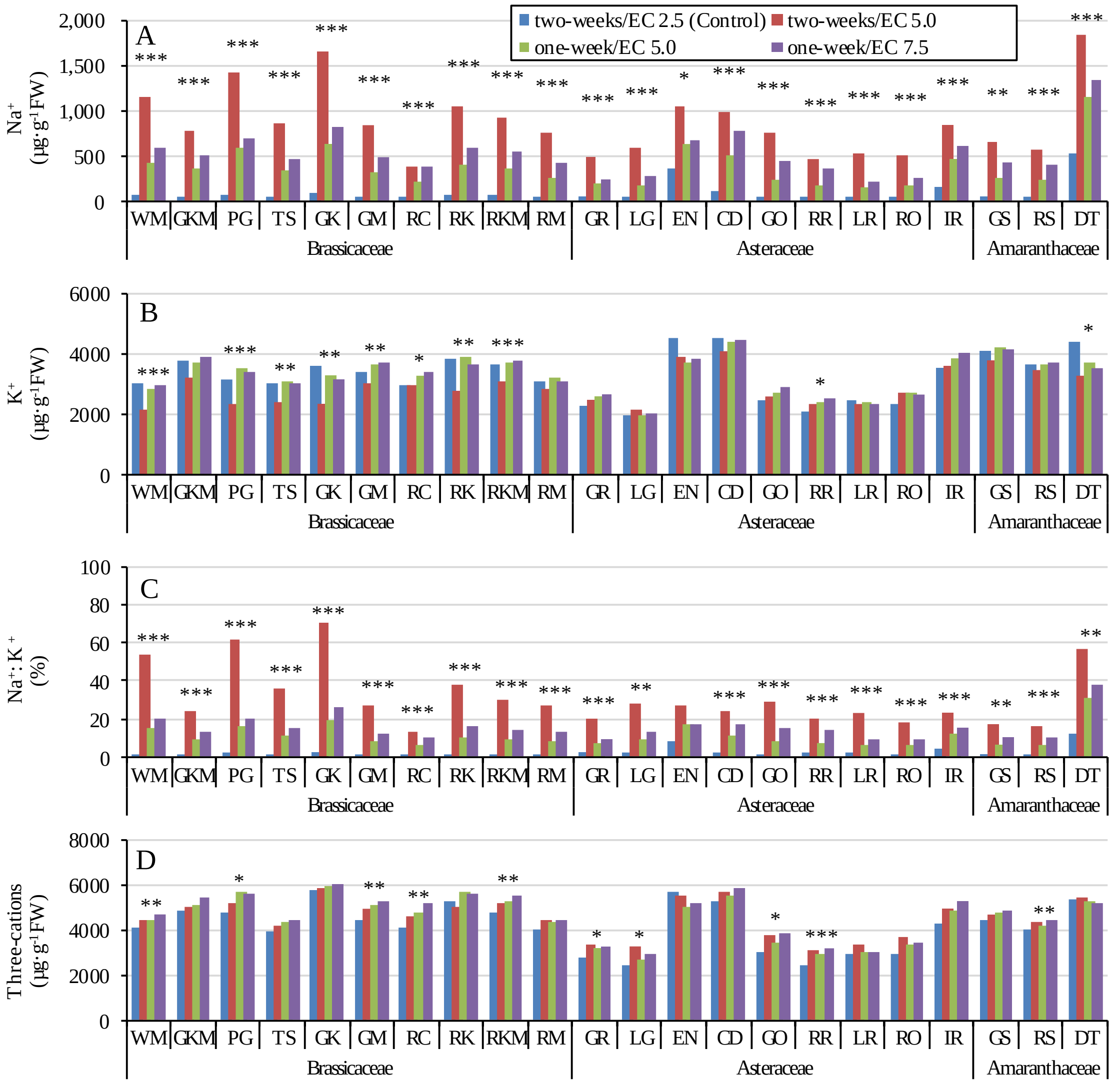


Fig. 6-4. Effects of salinity treatments on Na$^+$ content (A), K$^+$ content (B), Na$^+$: K$^+$ ratio (C) and three-cations (Na$^+$, K$^+$ and Ca^{2+}) content (D) in 22 crops. See Table 2-4 for abbreviation. *, **, *** Significant at $P < 0.05$, 0.01 or 0.001 by ANOVA, respectively (n = 2-3).

due to one-week of salinity treatments in most of the Brassicaceae crops (Fig. 6-4D).

3. Yields in response to salinity treatment

There were no significant differences in the yields among salinity treatments in all crops. However, RK, CD, GS, RS, and DT tended to increase yields due to some of the treatments (At most 82, 52, 27, and 31% increase compared to two-weeks/EC 2.5 salinity treatment, respectively) (Fig. 6-5A).

On the other hand, GR could be an unsuitable crop for salinity treatments because of its slightly decreased yield.

4. Qualities in response to salinity treatment

1) Ascorbic acid contents

There were no significant differences in the ascorbic acid contents among salinity treatments in all crops. However, TS, GM, RC, RM, GS, and RS tended to increase ascorbic acid contents due to all the salinity treatments (At most 31, 42, 82, 13, 93, and 84% increase compared to two-weeks/EC 2.5 salinity treatment, respectively) (Fig. 6-5B).

2) Total phenolics contents

PG, RC, RKM, LG, EN, GO, 'Red Oak' (RO), and RS tended to increase total phenolics contents due to all the salinity treatments (At most 12, 40, 16, 22, 71, 18, and 6% increase compared to two-weeks/EC 2.5 salinity treatment, respectively) (Fig. 6-5C). Especially in GO and RC, significant differences are observed among salinity treatments (P = 0.007 and 0.024, respectively).

3) Nitrate contents in response to salinity treatment

All salinity treatments decreased the nitrate contents in all the Asteraceae crops (Fig. 6-5D). The significant decreases in the nitrate contents were also observed in 7 Brassicaceae crops. However, one-week of salinity treatments were less effective for decreasing nitrate contents

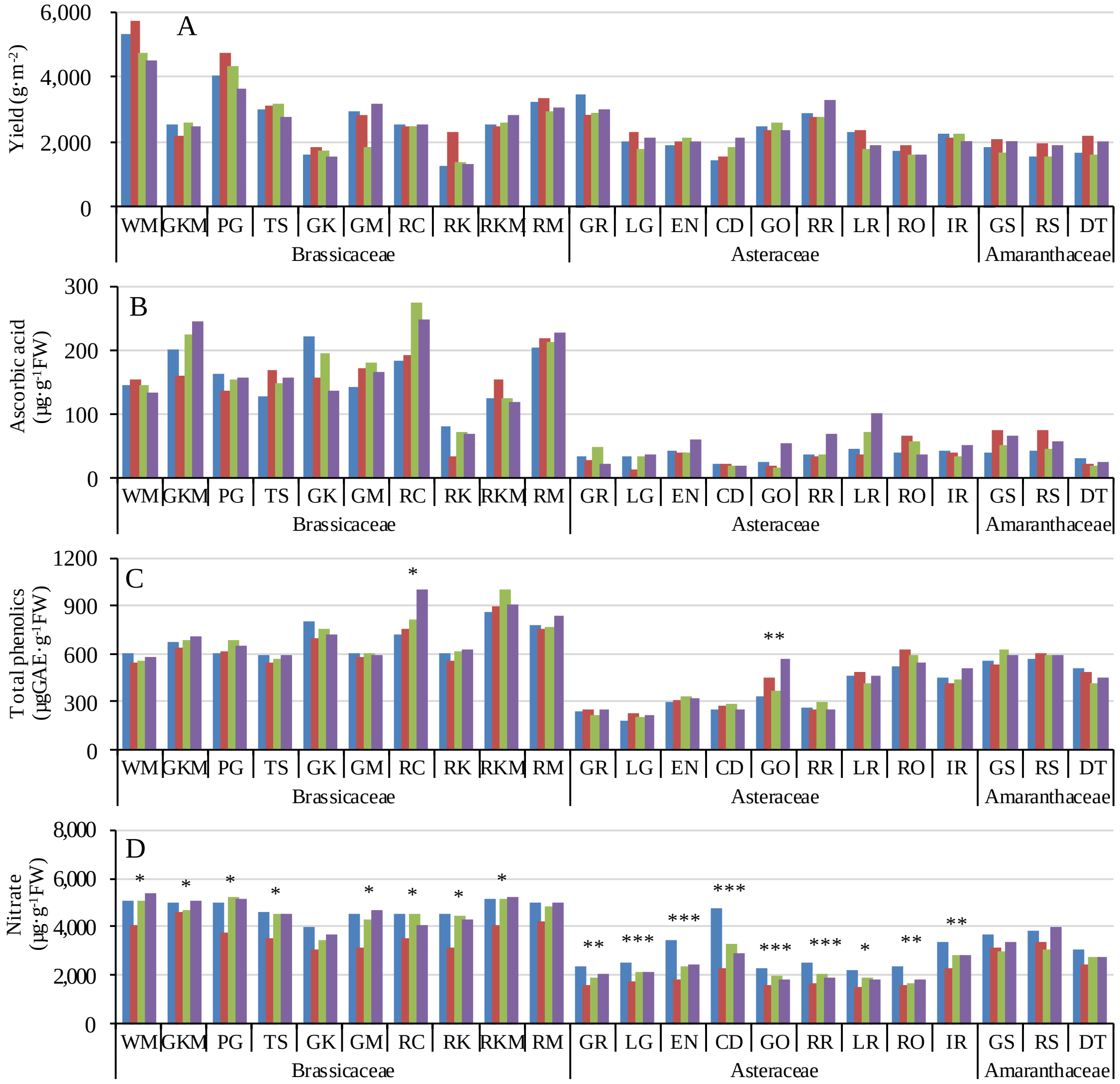


Fig. 6-5. Effects of salinity treatments on yields (A), and ascorbic acid (B), total phenolics (C) and nitrate contents (D) in 22 crops. See Table 2-4 for abbreviation. The value below abbreviation is the yields (kg·m^{-2}), ascorbic acid, total phenolic (GAE) and nitrate content (μg·g^{-1}FW) of each crop in two-weeks/EC 2.5 (control) treatment. *, **, *** Significant at $P < 0.05$, 0.01 or 0.001 by ANOVA, respectively ($n = 2\text{-}3$).

compared with two-weeks of salinity treatment in most of the Brassicaceae crops. The nitrate contents also tended to decrease due to some of salinity treatments in the Amaranthaceae crops, though not significantly.

5. Correlation coefficient between characteristics and cation contents

As to the BLC whose characteristics were improved due to salinity treatment, correlation coefficients with cation contents were calculated. There were no significant correlation coefficients between yields and Na^+ contents in RK, CD, GS, RS, and DT. However, Na^+ content had strong positive correlation with leaf weight and negative one with dry matter ratio in DT (Fig. 6-6).

The significantly positive coefficients between ascorbic acid content and the Na^+ contents were observed in GS and RS ($r = 0.912$ and 0.885, respectively) (Fig. 6-7). TS, GM, RC, and RM had no significant correlation coefficient with Na^+ content (r = 0.360, 0.198, 0.165, and 0.431, respectively).

The significantly positive coefficients between total phenolics content and the three-cations contents were observed in GO and RC ($r = 0.716$ and 0.736, respectively) (Fig. 6-8), contrary to low coefficients between total phenolics content and Na+ contents ($r = 0.551$ and 0.485 in GO and RC, respectively). Significantly positive coefficients with three-cations contents were also observed in DT, LG, RK, PG and GKM ($r = 0.743$, 0.669, 0.640, 0.838, and 0.802, respectively).

There were significant correlations between nitrate and Na^+ contents rather than K^+ contents in 18 BLC. In contrast, 6 BLC had significant correlations between nitrate and K^+ contents referred to another report (Wang et al., 2001).

Discussions

1. Evaluation of salt tolerance based on cation contents

As the EC in salinity treatment was higher, or as salinity treatment period was longer, Na^+

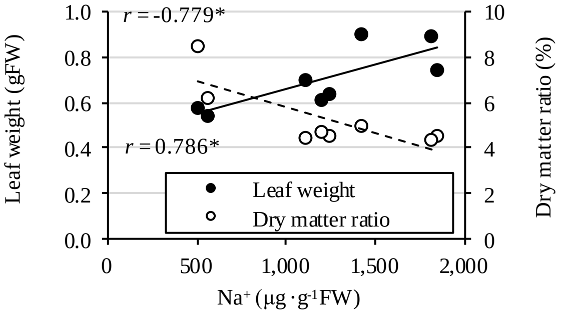


Fig. 6-6. Relationship between Na^+ content and leaf weight or dry matter ratio in 'Detroit' (DT). ** Significance at $P < 0.01$ ($n = 8$).

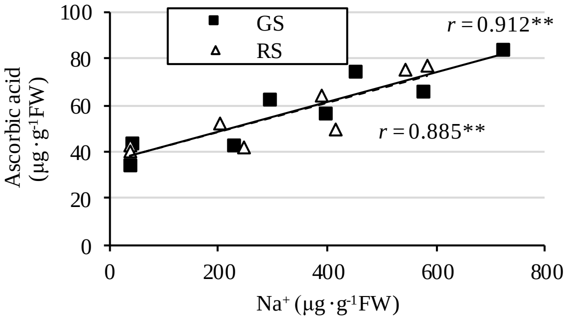


Fig. 6-7. Relationship between Na^+ content and ascorbic acid content in 'Green Spinach' (GS) and 'Red Spinach' (RS). ** Significance at $P < 0.01$ ($n = 8$).

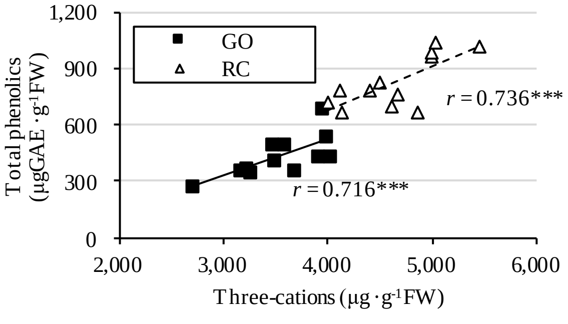


Fig. 6-8. Relationship between total phenolics content and three-cations content (Na^+, K^+ and Ca^{2+}) in 'Green Oak' (GO) and 'Rucola' (RC). *** Significance at $P < 0.001$ ($n = 12$).

Table 6-1. Correlation coefficients between the nitrate and Na$^+$, K$^+$ contents in the baby-leaf crops.

Abbreviation[z]	Na$^+$		K$^+$	
Brassicaceae				
WM	-0.573		0.744	**
GKM	-0.400		0.052	
PG	-0.652	*[y]	0.831	***
TS	-0.755	**	0.589	*
GK	-0.649	*	0.278	
GM	-0.604	*	0.503	
RC	-0.681	*	-0.056	
RK	-0.712	**	0.587	*
RKM	-0.610	*	0.707	*
RM	-0.612	*	0.202	
Asteraceae				
GR	-0.814	**	-0.155	
LG	-0.902	***	-0.093	
EN	-0.870	***	0.306	
CD	-0.962	***	0.490	
GO	-0.907	***	-0.109	
RR	-0.888	***	-0.495	
LR	-0.809	**	0.351	
RO	-0.619	*	-0.114	
IR	-0.781	**	0.200	
Amaranthaceae				
GS	-0.486		-0.042	
RS	-0.115		0.452	
DT	-0.736	*	0.855	**

[z] See Table 2-4 for abbreviations.

[y] *, **, *** Significant at $P < 0.05$, 0.01, or 0.001, respectively (n = 8-12).

contents in the BLC leaves rose. By contrast, K^+ contents by salinity treatments were different depending on BLC. The Na^+: K^+ ratios are known as an indicator of plant resistance to salinity (Juan et al., 2005), Na^+: K^+ ratio increase in most non- halophytic species under saline conditions (Ashraf, M. and T. McNeilly. 1990). In the experiment DT, EN, and IR had higher Na^+ contents even in control and Na^+: K^+ ratios did not rise by salinity treatment as much as any other BLC. Table beet such as DT is known as moderately salt-tolerant crop (Shannon and Grieve, 1999). In addition, *Cichorium* crops such as IR and EN could have salt-tolerance. These reports could be supported by the Na^+: K^+ ratios in these crops.

On the other hand, K^+ contents depressed due to two-weeks salinity treatment and three-cation contents due to two-weeks of salinity treatments were lower than those due to one-week of salinity treatments in most of the Brassicaceae crops unlike most of the Asteraceae and Amaranthaceae crops (Fig. 6-4D). In general, the disproportionate presence of Na^+ impacts on the acquisition of K^+ and Ca^{2+} and excess Na^+ tend to substitute K^+ (Maathuis, 2006). The temporal three-cations contents increase by the salinity treatment could return to the usual level more quickly in Brassicaceae crops than in others. However, Na^+: K^+ ratios increased due to most impactful salinity treatment (two-weeks/EC 5.0) in most of Brassicaceae crops compared to Asteraceae and Amaranthaceae crops. This result could suggest that Brassicaceae crops are more sensitive to salinity treatment than Asteraceae and Amarantahceae crops.

2. The relation among yield, leaf characteristics, and salinity treatment

The yield decreased due to the EC 10.0 $dS \cdot m^{-1}$ salinity treatment in watercress in a previous report (Fernández et al., 2016). However, in the present study there were no significant differences in the yields among EC 2.5 and 7.5 $dS \cdot m^{-1}$ salinity treatment in any crops. This result suggests that salinity treatment should be carried out in at most EC 7.5 $dS \cdot m^{-1}$ or less. However, Amaranthaceae crops tended to increase yields due to salinity treatments (Fig. 6-5A). Beet and spinach are potentially tolerant to salinity (Shannon and Grieve, 1999), and there was a case that salinity treatment increased fresh weight of leafy part of beet (da Silva and Klar, 2016). The results coincided with these reports. As Na^+ content increased, dry matter ratio decreased and leaf weight increased in DT (Fig. 6-6). It was inferred from the results that salinity treatments affect water content in DT for maintaining turgor pressure and increase leaf weight and yield. Hence, Amaranthaceae crops, especially DT, could be suitable for salinity treatment (two-weeks/EC 5.0 or one-week/EC 7.5) in the view of its productivity.

The yields in CD and RK increased salinity treatments. However, the Na^+ content did not have a significant correlation with the yield in either crop. Another factor could increase the yields in these crops.

3. Ascorbic acid contents in response to salinity treatment

The ascorbic acid contents due to the salinity treatments stably in some BLC (Fig. 6-5B). Especially in GS and RS, there were significant positive correlations between Na^+ and ascorbic acid contents (Fig. 6-7). Previous report described a reduction of ascorbic acid contents in lettuce (Pérez-López et al., 2015), watercress (Kaddour et al., 2013), and wheat (Athar et al., 2008) due to salinity treatment. On the other hand, another report described that exogenously applied ascorbic acid could alleviate the salt-induced stress on the growth in wheat (Athar et al., 2008), which suggest relations among salinity, ascorbic acid and yield. This result could lead to the conclusion that the endogenous ascorbic acid promoted to alleviate the stress by salinity application stress and enhanced growth and yield in spinach such as GS and RS. Further studies need to verify our hypothesis. However, spinach may be suitable crop for salinity treatment in the view of the productivity and internal quality.

4. Total phenolics contents in response to salinity treatment

There were significant differences in the total phenolics contents among salinity treatments only in GO and RC (Fig. 6-5C). The increase of phenolics by salinity treatment has already reported in lettuce (Fernández, et al., 2016; Kaddour et al., 2013). Additionally, the result of this experiment showed that RC could be a suitable crop for salinity application, especially by one-week/EC 7.5, in the view of its phenolics content. The significantly positive coefficients between total phenolics content and the three-cations contents were observed in these 2 crops (Fig. 6-8), contrary to low coefficients between total phenolics content and Na^+ contents ($r = 0.551$ and 0.485 in GO and RC, respectively; data not shown). Significantly positive coefficients with three-cations contents were also observed in DT, LG, RK, PG and GKM (data not shown). This result could lead to the conclusion that the absorption of excessive cations content, including Na^+, by salinity treatment as the stress factor caused the increase of total phenolics content. The three-cations contents tended to rise due to one-week of salinity treatment and then fall into the same as the level of control salinity treatment by two-weeks of salinity treatment in most of the Brassicaceae crops (Fig. 6-4D). The timing of salinity application to maintain the higher level of three-cations content at harvest could be important to improve the total phenolics contents in the Brassicaceae crops.

5. Influence of nitrate contents due to different salinity treatment

Nitrate contents changed more remarkably due to salinity treatments than any other characteristics in most of BLC. This result was coincided with a previous report (Scuderi et al., 2009). Therefore, the decreasing nitrate contents due to salinity treatment could be expected in various BLC. However, suitable salinity application could be different depending on the crops and Brassicaceae crops should be applied stronger treatment than Asteraceae crops to depress the nitrate contents. Previous report has suggested that nitrate provision and uptake is associate with K^+ influx as the major charge-balancing cation (Wang et al., 2001). Nevertheless, this experiment suggested that there were more significant correlations between nitrate and Na^+ contents rather than K^+ contents in most of the crops, contrary to the report (Table 6-1). However, there was limited report on the relation between nitrate and Na^+ contents. Further investigation is needed to clarify the roles of Na^+ in nitrate absorption. On the other hand, salinity treatment depressed nitrate uptake, which has been attributed to antagonism between nitrate and Cl^- (Cerezo et al., 1999), or competition between nitrate and Cl^- for high affinity transport systems (Cerezo et al., 1997). These reports suggest the importance of Cl^- absorption level by salinity treatment. It remains a challenge for future research on the relationship between nitrate and Cl^- content.

Chapter 7

General Discussion

“Baby-leaf vegetables” is one of the new categories in leafy vegetables and are used as the mixture. The similar salad mixtures have used in Europe for a few hundred years. However, BLV had been taken attention as ready-to-eat salad mixtures such as cut lettuces and distributed recently also in Japan. Ready-to-eat stuffing is expected to be available all year round (Richardson and Stevens, 2003), and the expectation is true of BLV. That is to say, 1) Baby-leaf vegetables (BLV) should be supplied all year around. Furthermore, 2) keeping stable quality throughout the year is also important for year-round supply.

On the other hand, features of BLV different from any other vegetables or ready-to-eat salad mixtures are: 1) Baby-leaf vegetables consist of some kinds of crops and 2) BLV use small and juvenile leaves and therefore, 3) Baby-leaf vegetables are produced in short periods.

For year-round production and improvement of quality in BLV, the author pointed out to solve the following problems.

1) Unraveling the characteristics of BLC
2) Investigating the seasonal variation of characteristics
3) Establishing a BLV production in winter
4) Improving BLV qualities in summer production

Obtaining of these informations will support to the further increase of the BLV market in Japan.

1. Estimation of total cultivation periods in BLC

Continuous cultivation system in a plastic house is important for year-round production. Therefore, the total cultivation period in each crop should be unraveled to determine the crops to be mixed in each season for the efficient continuous cultivation.

The difference of total cultivation periods among 22 BLC was discussed in Chapter 3: Brassicaceae crops had shorter cultivation periods, Asteraceae had longer one and Amaranthaceae had represented a mean between them. Therefore, Brassicaceae and Amaranthaceae crops were suitable for efficient cultivations. By contrast, some of Asteraceae crops, especially IR, stands in disadvantage position. Seasonal variation of them was examined in the Chapters 4 and 5. There was a strong negative correlation between total cultivation period and air temperature: the longer cultivation period in December and the shorter cultivation period in August.

There were missing values in some crops and seasons, e.g. GKM in July. The author estimated these missing values by using quantification I group model, a kind of multiple regression analysis. Estimated values of total cultivation periods in BLC were approximately corresponding to the observed values from May to November (Fig. 7-1). Thus, it might be possible to estimate the missing values during spring to autumn. Category scores of BLC were shown in Fig. 7-2. In the actual BLV production, it is not reasonable to manage a homogenous cultivation suitable for every crop. Then the author proposes the grouping of crops with similar growth properties to cultivate some of various BLC under the same management. Twenty-two BLC were classified to fall into four groups based on order of the score: Group A is composed of BLC with shortest total cultivation period; Group B is composed of ones with slightly shorter period; Group C is ones is composed of ones with slightly longer period; Group D is composed of ones with longest total cultivation period. Category scores of seasons are shown in Fig. 7-3. Category score of September is missing in the serial month from May to November. The author estimated the score of September provisionally by secondary regression formula. The total cultivation period in each season from May to November was arranged in Table 7-1.

On the other hand, there were large margin of errors between estimated values and observed ones in most of BLC in December. Some of lettuce and broccoli crops stopped their growth at 5ºC air temperature (Hatfield and Prueger, 2015; Komatsu et al., 2004). There were some days in which daily mean air temperature within the plastic house was felling around 0ºC and BLC could

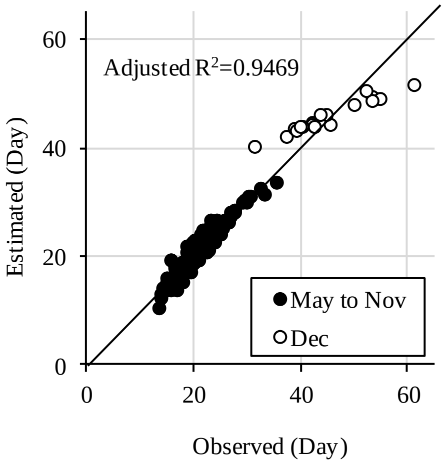


Fig. 7-1. Correlation between observed and estimated total cultivation period. Calculated by using quantification I Group.

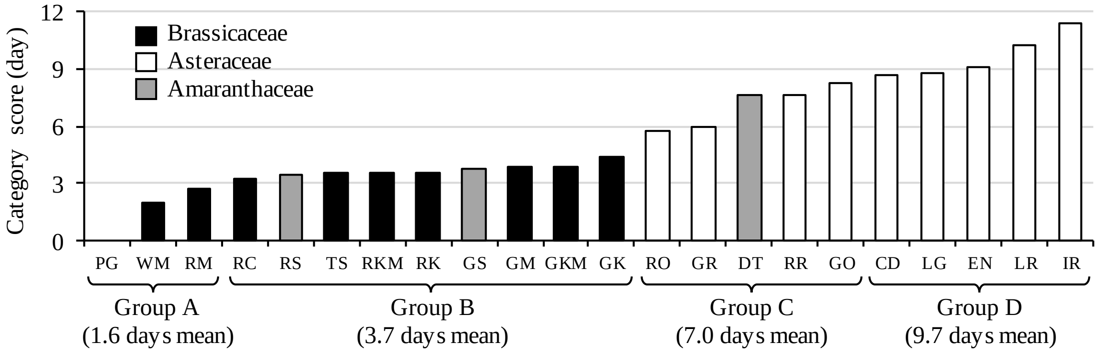


Fig. 7-2. Category scores of baby-leaf crops calculated by using quantification I Group.

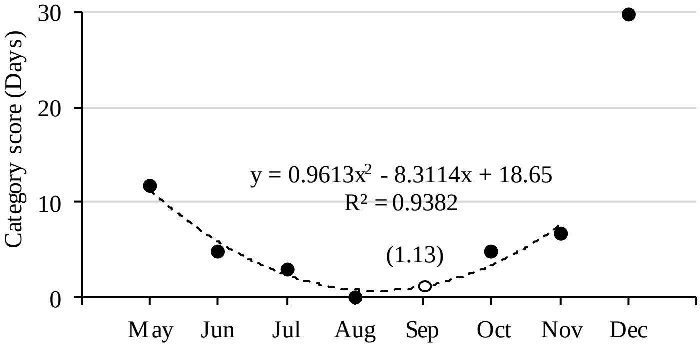


Fig. 7-3. Category scores of the seasons. Calculated by quantification I Group.

Table 7-1. Estimation of total cultivation period in each crop group and season.

Harvest season	Estimated total cultivation period[z]			
	Group A[y]	Group B	Group C	Group D
May	24	26	29	32
Jun	17	19	22	25
Jul	15	17	20	23
Aug	12	14	17	20
Sep	13	15	18	21
Oct	17	19	22	25
Nov	19	21	24	27
Dec	39(PG: 32)[x]	42	53 (RO,GR:44, IR: 61)	

[z] Based on Figs. 7-2 and 7-3 for category score and constant term (10.33 days) except for December.

[y] See Fig. 7-2 for Group A to D.

[x] Based on observed data (Fig. 5-2) for estimated total cultivation period in December.

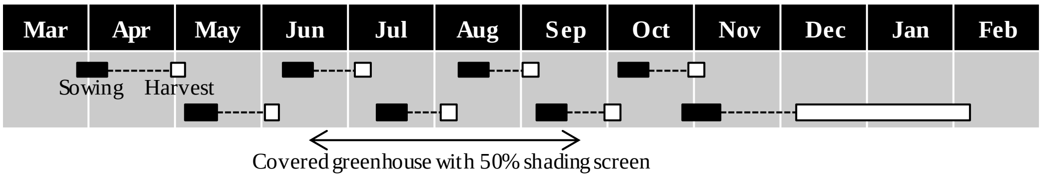


Fig. 7-4. Year-round production model of baby-leaf vegetables in an unheated plastic house in Hokkaido.

not grow in December. For this reason, wide dispersion among the years in total cultivation period and discrepancy between observed values and estimated by quantification I group model could occur in winter (Figs. 5-2 and 7-1). Therefore, the total cultivation period of December in Table 7-1 was arranged based on observed value.

Meanwhile, the stops of BLC growths in cold environmental condition contribute to the elongation of harvest period. Furthermore, BLC didn't die completely even though air temperature temporally fell to -10ºC, though some leaves were withered (Fig. 5-4). It was revealed that BLV could be harvested from December to early February in winter cultivation, though it is necessary to adjust the harvests, which might enable to determine the BLV shipping time freely in accordance with producer's intention. Specifically, a producer can avoid shipping when BLV are distributed excessively, and can ship actively when BLV products are hardly sold and are required by consumers. This sales strategy is applied only in cold product region such as Hokkaido in Japan.

By assembling the information of cultivation period, BLV can be harvested from May to February by cultivating 8 times in an unheated plastic house (Fig. 7-4). This annual BLV production plan suggests almost year-round production in Hokkaido.

2. Improvement of BLV quality

Most consumers have "tasty" and "healthy" images to BLV (Fig. 2-3). That is to say, they are expected higher qualities to BLV. The author investigated mainly the SPAD values as external appearance and ascorbic acid and nitrate contents as internal qualities. Differences of BLC in qualities were mentioned in the Chapter 3, and seasonal variations in qualities are done in the Chapters 4 and 5. Furthermore, the improvement of BLC qualities by salinity treatment in soilless culture was tested in the Chapter 6. Based on these results, the author proposed the assortment of BLC in each season.

1) Baby-leaf crops suitable for spring season production

BLC evaluations in spring season were carried out in Chapter 3. In general, Brassicaceae crops had higher ascorbic acid contents, Asteraceae had lower ascorbic acid contents, and Amaranthaceae had lower nitrate contents. Based on the PCA, some crops located in 1st or 4th quadrant of Fig. 3-2 could be considered suitable for BLV from the view point of yield. In addition, the ones located in 1st or 2nd quadrant of Fig. 3-2 could show high ascorbic acid contents. On the contrary, ones located in 3rd quadrant of Fig. 3-2, such as IR, could not be suitable for BLV from the view point of internal quality.

Some crops had lower SPAD value, which will bring out the color tone of BLV. It takes a longer time from sowing and harvest in spring season, and BLC with shorter total cultivation period should be selected. Based on the above discussion, a possible assortment of BLC in spring season could results as below listed.

PG (shortest total cultivation period, larger yield and higher ascorbic acid content), RM (shortest total cultivation period, and higher ascorbic acid content), WM (shortest total cultivation period, and higher nitrate content), GO (higher yield and paler green lyrate leaves), and RS (larger yield, lower nitrate content and red vein leaves) (Table 7-2).

2) Baby-leaf crops suitable for summer production

In general, the ascorbic acid contents decreased in all BLC in summer (Fig. 4-2E). Therefore, it is most important to select BLC with high ascorbic acid contents. By contrast, the nitrate contents increased in most of BLC in summer. It is not recognized that nitrate is not a harmful component necessarily according to Food Safety Commission of Japan (https://www.fsc.go.jp/sonota/factsheets/f04_nitrate.pdf) at present. However, the maximum levels of nitrates in foodstuffs have been determined in the European Union or European countries (Santamaria, 2006). Therefore, BLC with excessive nitrate contents such as EN or IR could be unsuitable (Fig. 4-2F). On the other hand, total cultivation period is not so important because all BLC grow faster in summer than in any other season (Fig. 4-2A). The SPAD value was almost the same in most of BLC from spring throughout autumn (Fig. 4-2D). Based on these seasonal variations, the following BLC should be cultivated in summer; GKM (larger yield), GK (higher ascorbic acid content), RK (larger yield and higher ascorbic acid), LG (paler green spathulate undulated leaves) (Table 7-3).

Shading treatment was carried out to plastic house in the summer cultivation for the purpose of depressing the air temperature inside the plastic house. Depression of air temperature by shading is useful for improvement of yield in some BLC (Table 4-3). However, depression of solar radiation lead to decrease of ascorbic acid content (Yoshida and Hamamoto, 2010). The choice of shading materials and the timing of shading need to be examined further.

Salinity treatment was examined to improve yield and qualities of BLV in hot season. The following effects were confirmed by altering EC of hydroponic solution from 2.5 to 5.0 or 7.5 $dS \cdot m^{-1}$ with NaCl; increase of yield in DT, GS, and RS; increase of ascorbic acid contents in GM, RC, TS, GS and RS; increase of total phenolics in GO and RC (Figs. 6-5A, 6-5B, and 6-5C). These BLC may suit salinity application in hot season cultivation (Table 7-3). Furthermore, the nitrate contents decrease in all the Brassicaceae and Asteraceae crops by salinity treatment. The excessive nitrate contents in EN or IR could diminish by salinity application. Salinity treatment is usually applied in hydroponic culture system and may be a promising technique in Hokkaido in which most of the BLV production companies include hydroponic soil culture or soilless culture.

3) Baby-leaf crops suitable for winter production

Most of BLC increased ascorbic acid contents and Brix, and decreased nitrate contents from December throughout February (Figs. 5-6C, 5-6D and 5-6E). Above all, the ascorbic acid contents and Brix in February are higher than those in any other experimental data on spinach and komatsuna cultivated in winter (Citak and Sonmez, 2010; Martínez-Sánchez et al, 2008; Tamura and Taguchi, 2001). Furthermore, the dry matter ratio of BLC remarkably increased and their texture changed in January and February (Fig. 5-7C). Some of these quality changes could be correlated with low temperature, given that BLV with such characteristic qualities are produced only in cold region. Therefore, BLV produced in Hokkaido in winter are the unique

Table 7-2. Recommended baby-leaf crops in spring season.

Abbreviation[z]	Total cultivation period	Yield	Ascorbic acid	Remarks
WM	+ [y]	+	+	
PG	+ +	+	+ +	
RM	+	−	+ +	Red-colored leaf
GO	−		− −	Paler-colored leaf
RS				Red-colored leaf

[z]See Table 2-4 for abbreviations.

[y]+ + shorter total cultivation period, larger yield, or higher ascorbic acid contents, + slightly shorter, larger or higher, (Blank) middle, − slightly longer, smaller or lower, − − longer, smaller, or lower.

Table 7-3. Recommended baby-leaf crops in summer.

Abbreviation[z]	Yield	Ascorbic acid	Nitrate	Remarks
GKM	+ [y]	+ +	+	
GK	−	+ +	−	
RK	+	+ +	+ +	Red-colored leaf
LG		− −	− −	Paler-colored leaf
GS	+ + (+) [x]	− (+ +)	− − (−)	
DT	+ (+ +)	− (−)	− − (−)	Red-colored leaf

[z]See Table 2-4 for abbreviations.

[y]+ + larger yield, higher SPAD value, ascorbic acid, or nitrate contents, + slightly larger or higher, (Blank) middle, − slightly smaller or lower, − − smaller or lower.

[x]Within the parenthesis is the evaluation by salinity application.

Table 7-4. Recommended baby-leaf crops in winter.

Abbreviation[z]	Yield	Ascorbic acid	Brix	Remarks
TS	+ + [yx]	−		Higher productivity
RC	−	+ +	+	
RK	−	+ +	+ +	Red-colored leaf
RKM	+	+ +		Red-colored leaf
LG	+	− −		Paler-colored leaf
GS	+ +		+	
RS	+	+	+	Red-colored leaf

[z]See Table 2-4 for abbreviations.

[y]+ + larger yield, higher ascorbic acid contents or Brix, + slightly larger or higher, (Blank) middle, − slightly smaller or lower, − − smaller or lower.

salad mixtures reflected the climate environment and are expected not only to meet consumption within Hokkaido but shipping outside of Hokkaido as specialty valuable salad mixtures.

Every crop has higher qualities in winter than in any other season generally. The recommended BLC are RC (higher ascorbic acid content and Brix), RK (higher ascorbic acid content and Brix), and RS (higher ascorbic acid content and Brix) (Table 7-4). Yield is also important. GS, LG, RKM, TS, and WM showed larger yield in January and February. TS had especially larger yield and growth rate in winter cultivation than any other BLC, and could be suitable for winter cultivation (Figs. 5-6A and 5-6B).

This experiment was carried out at the unheated single-layer plastic house in the Donan Agricultural Experiment Station which was located in south Hokkaido. Therefore, winter BLV production could not be applied under the same heat insulation equipment in north Hokkaido which was the coldest region in Japan. However, the minimum temperature inside unheated plastic house equipped with plastic film multiple-layer insulation showed almost as same as ones observed in the experiment in the Donan Agricultural Experiment Station (data not shown). Actually, winter BLV are produced at the unheated plastic house with heat insulation equipment in east Hokkaido where it is colder than south Hokkaido. Furthermore, the costs of some heat insulation materials such as plastic films are much lower than fuel cost for heating plastic house. Hence, unheated winter BLV production will be applied over wide range of Hokkaido.

4) Attention on BLC choice

The author selected the recommended BLC in each season. However, it is not appropriate to produce only recommended BLC. The questionnaire shows consumers had “colorful” or “fashionable” impression to BLV as well as “healthy” and “tasty” (Fig. 2-3). The mixture of different shaped and colored leaves is also important as commercial value from the view point of external appearance (Table 2-2). While BLV consisting the similar kind of crops can reduce commercial attractiveness. Furthermore, mustard (GKM, GM, RKM and RM) and rucola (RC) have unique flavor. Therefore, producers should select crops according to not only characteristics of their growth and internal quality, but also flavor and external appearance as well as SPAD value.

3. Prospects and problems of BLV in Hokkaido

Based on results obtained from the present research, year-round BLV production system in Hokkaido was established and screened suitable BLC for each season. These results will contribute to the enhancement of BLV production in Hokkaido and the improved recognition of BLV produced in Hokkaido.

However, the author points out two key factors affect the future BLV production in Hokkaido, which are ‘soilless culture system’ and ‘global warming’.

It is not too much to say that soilless culture system is one of the innovative cultivation systems. The system has some merits compared to traditional soil culture system: high productivity, reduction of pesticide, water, and fertilizer use, improvement of quality, prolongation of shelf life (Nicola et al, 2007; Nicola et al, 2016). Above all, shelf life and microbial safety are important for ready-to-eat salad mix such as BLV. Furthermore, high-valued salad mix products are highly appreciated by consumers (Nicola et al, 2007). Soilless culture systems are essential to improve qualities by salinity treatment. Many soilless cultivation systems have already been introduced and will increase in the future in Hokkaido. However, the author used soil culture system in most of the experiments. Hence, the differences between soil culture system and soilless one in BLV characteristics and seasonal variations should be investigated.

Global warming is one of the important world problems. The global mean temperature rose at a rate of 0.74°C over the 20th century, and the mean temperature in Japan also rouse at a rate of 1.06°C per century (Sugiura et al, 2012). The impacts on agricultural productions by global warming have been reported. Zhao et al, (2017) reported that temperature increase would reduce global yields in wheat, rice, maize, and soybean. In Japan, warming increases in fruit coloring disorders and incidences of chalky rice kernels, alterations in the type of disease and pest, and reductions in yield of vegetables in summer (Sugiura et al, 2012). Therefore, summer productions including BLV will increasingly shift from south regions to north ones such as Hokkaido. However, this study revealed that BLV yields and qualities in summer depressed even in Hokkaido. To solve the low productivity and quality reduction in summer, salinity treatment may be effective technique in some BLC. It will be necessary to establish the more suitable treatment technique in each crop.

On the other hand, global warming will prolong the harvest season. This study does not refer to March and April BLV production. Global warming may allow to harvest BLV in early spring. March to April harvest will have to be researched in the far future.

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Summary

‘Baby-leaf vegetables’ (BLV) is a new category in leafy vegetables and they are used as the mixture of juvenile stage of different kinds of leafy vegetables. They include Brassicaceae, Asteraceae, Amaranthaceae, and other vegetable leaves. These crops show wide ranges of leaf shapes, colors, textures and flavors. Baby-leaf vegetables production began in early 1980s. mainly in Europe and the USA as kinds of ready-to-eat salad mixtures, and has increased gradually also in Japan. In such a consumption trend, Hokkaido has been expected as a new production region. Ready-to-eat salad mixtures such as BLV are expected to be available in stable qualities all year-round. However, BLV contains many kinds of crops (baby leaf crops; BLC) and production and quality characteristics are different among BLC. Furthermore, the characteristics in each crop vary among growing season. Therefore, the following studies were examined for the establishment of year-round production of BLV with higher quality.

In Chapter 2, questionnaire survey was carried out to grasp consumer needs unique to BLV. More than half of consumers had “tasty” and “healthy” image for BLV more strongly and “colorful” and “fashionable” impression next to them. Based on the result of survey, the measurement of ascorbic acid and nitrate content as most interesting internal qualities and SPAD value as external appearance was determined. Commercial BLV from each production group had 4 to 10 crops and contained green-colored crops and red one. Leaf length and leaf area in most of BLC in commercial BLV products are around 80 mm and 1,000 - 1,500 mm^2 except for spatulate incised leaf blade crop (Mizuna etc., leaf length is below 120 mm) and some leaf lettuce (leaf area is below 2,000 mm^2). Based on the survey results, harvest criteria were established as to BLC.

In Chapter 3, the emergence periods, growing periods, total cultivation periods, from sowing to harvest, as well as the yields, SPAD values, ascorbic acid and nitrate contents, and leaf characteristics of 22 BLC were investigated in spring. A principal component analysis, based on the total cultivation periods, the yields, the SPAD values, as well as the ascorbic acid and nitrate

contents, showed 3 main groups: Brassicaceae crops, which had short total cultivation periods and high ascorbic acid contents; Asteraceae crops (except for ‘Italian Red’ chicory), which had long total cultivation periods and low ascorbic acid contents; Amaranthaceae crops, which had a comparatively large yields and low nitrate contents. ‘Italian Red’ chicory did not fall into any of these 3 groups. The yield had very limited effects on the grouping. The larger-yield crops tended to have more leaves and less dry matter ratios, and the crops with higher SPAD values tended to have thicker leaves.

In Chapter 4, the total cultivation periods, yields, SPAD values, ascorbic acid and nitrate contents and leaf characteristics of 22 BLC were investigated from spring to autumn. Most of BLC had longer total cultivation period, larger yield, higher ascorbic acid and lower nitrate contents in May and showed shorter total cultivation period, smaller yield, lower ascorbic acid and higher nitrate contents in August. The lower daily mean air temperature during total cultivation period is, the longer total cultivation period, the larger yield, and lower nitrate contents most of BLC had. The leaf weight and leaf thicknesses increased by low temperatures in some BLC. The ascorbic acid contents were affected more strongly by daily minimum temperature immediately before harvest in some Brassicaceae and Asteraceae crops. There were less relations between SPAD value and air temperature in most of BLC.

In Chapter 5, the total cultivation periods, yields, ascorbic acid and nitrate contents, Brix and leaf characteristics of 22 BLC were investigated in an unheated plastic house in winter. All BLC could survive though the lowest air temperature during the total cultivation period was shown about -10°C within plastic house and could be harvested from December to February, though the adjustment of intact harvest were required. The yields, ascorbic acid contents, and Brix increased and the nitrate contents decreased from December throughout February in almost all BLC. The dry matter ratio increased in January and February, unlike any other seasons from spring to autumn. The increase of Brix and the decrease of nitrate content showed the strong relations with the increase of dry matter ratio caused by low air temperature. Consequently, the possibility of BLV production in severe cold condition such as winter in Hokkaido was shown.

In Chapter 6, the influences of salinity treatment to BLC in soilless culture which ranged EC 2.5 to 7.5 $dS \cdot m^{-1}$ of hydroponic solutions by adding NaCl for one to two weeks before harvest were evaluated in hot season in which BLV yield and qualities depress easily. The yield was likely to increase due to rise of water content in leaves of ‘Detroit’ table beet by the salinity treatment. The ascorbic acid contents of spinach crops increased according to increase of the Na^+ absorption. The total phenolics increased in ‘Green Romaine’ lettuce and rucola when the crops contained more Na^+, K^+, and Ca^{2+}. The nitrate contents decreased according to the increasing Na^+ absorption, especially in Asteraceae crops.

Based on the studies so far, year-round BLV production system in Hokkaido was established and screened BLC suitable for each growing season. These results on the studies will contribute to the enhancement of BLV production in Hokkaido and the improved publicity of BLV produced in Hokkaido.

Ackowledgements

I would like to extend my deep gratitude to Professor Hajime ARAKI, Field Science Center for Northern Biosphere, Hokkaido University, my supervisor, for all of his guidance, patience, kindness, encouragement and great help. I wish to sincerely thank Professor Toshihiko YAMADA, Field Science Center for Northern Biosphere, Hokkaido University, Associate Professor Yoichiro HOSHINO, Field Science Center for Northern Biosphere, Hokkaido University, Associate Professor Takashi SUZUKI, Graduate School of Agriculture, Hokkaido University, Professor Toshihiko HARA, Institute of Low Temperature Science, Hokkaido University and Professor Silvana NICOLA, Department of Agricultural, Forest and Food Sciences, University of Turin, for their valuable advice and critical review of this thesis.

My deep thanks go to Assistant Professor Toshiyuki HIRATA, Field Science Center for Northern Biosphere, Hokkaido University, for his useful advice concerning the statistical analysis.

I am greateful to Dr. Takahiro KUMANO, Bejo Japan K. K., for his great help with componential analyses.

Thanks are due to all the staff in Donan Agricultural Experimental Station, especially Dr. Haruhiko NAKAZUMI, Dr. Harukuni HORITA, Mr. Toru MIZUKOSHI, Dr. Koji KAWAGISHI, Mr. Hiroto OZAKI, Mr. Yuichi OTOBE, Dr. Tetsuo HAYASHI, Mr. Shinichi OKUBO, Mr. Reiichiro UENO, Mr. Shinya MUNEKATA, Mr. Kazuya ARAKI, Mr. Akito SUGAWARA, for encouragements and help, and Mr. Masaki TAMURA, Mr. Tatsuya TAKEUCHI and Mr. Morihisa SASAKI for dedicated support for plant and field managements in many experiments.

Because of International cooperative research agreement* between Torin University and Hokkaido University, some researches in the thesis could be conducted. I am indebted to all the staff in Department of Agricultural, Forest and Food Sciences, University of Turin, especially, Mr. Walter GAINO, Mr. Pierre Anice Tonye GWODOG, Mr. Francesco MATTALIA, Ms. Francesca FAGIANO, Mr. Simone DIMINO, Mr. Michel CONTINI for their encouragement and support in my experiment and my stay in Turin.

*Framework Agreement for Educational and Scientific Cooperation between Department of Agriculture, Forest and Food Science, University Turin (Italy) and Faculty of Environmental Earth Science and Graduate school of Environmental Science, Hokkaido University (Japan)

ベビーリーフの周年生産と品質改善に関する研究

髙濱　雅幹

要約

ベビーリーフは新規のカテゴリーの葉菜類であり、様々な葉菜類の若葉をミックスしたものである。アブラナ科、キク科、ヒユ科その他の葉菜類がベビーリーフとして利用される。これらの品目は葉の形状、葉色、食感や香りが異なっている。ベビーリーフ生産はそのまま食べられるサラダミックスのひとつとして、1980年代に主にヨーロッパや米国で始まり、日本でも広がりつつある。そのような消費動向の中で、北海道は新たなベビーリーフ生産地として期待されている。　ベビーリーフのようにそのまま食べられるサラダミックスは周年安定供給が求められる。しかしベビーリーフには様々な品目が含まれており、生産性や品質は品目により異なる。さらに、作型により特性が変化する。そのため、高品質ベビーリーフを周年生産確立のため、以下の試験研究を実施した。

第2章では、ベビーリーフに特異的な消費者ニーズを把握するためアンケート調査を行った。消費者の半数以上はベビーリーフに対し「おいしい」「健康的」なイメージを強く持っており、次いで「色鮮やか」「おしゃれ」なイメージが続いた。この結果を基に、最も関心の高い内部品質としてアスコルビン酸と硝酸を、外観形質として SPAD 値を調査することとした。各地で生産された市販ベビーリーフには 4～10 品目含まれており、緑色の葉の品目と赤色の葉の品目が含まれていた。葉長および葉面積はそれぞれ80mm、1,000～1,500mm^{2}であったが、水菜のような篦型鋭浅裂要は葉長120mm未満、または一部レタスで葉面積2,000mm^{2}以下であった。これらの調査結果をもとにベビーリーフ品目の収穫基準を作成した。

第3章では、春季に22品目のベビーリーフについて播種から出芽までの期間、出芽か

ら収穫までの生育期間、播種から収穫までの総栽培期間、収量、SPAD 値、アスコルビン酸濃度、硝酸イオン濃度，葉の特性について調査した。総栽培期間、収量、SPAD 値、アスコルビン酸および硝酸イオン濃度について主成分分析を実施したところ，①総栽培期間が短くアスコルビン酸濃度が高いアブラナ科品目、②総栽培期間が長くアスコルビン酸濃度の低いキク科品目(チコリー‘イタリアンレッド’を除く)、③収量が比較的高く硝酸イオン濃度が低いヒユ科品目の 3 群に分類された。しかしチコリー‘イタリアンレッド’はこれら 3 群のいずれにも属さなかった。収量は 3 群の分類ではその傾向が判然としなかった。多収性の品目は葉数が多く乾物率が低い傾向があり、SPAD 値の高い作物は葉が厚い傾向を示した。

第 4 章では、春から秋までの作型について、22 品目の総栽培期間、収量、SPAD 値、アスコルビン酸濃度、硝酸イオン濃度および葉の特性を調査した。ほとんどの品目において、5 月では総栽培期間が長く、収量およびアスコルビン酸濃度が高く、硝酸イオン濃度が低かった。一方、8 月には総栽培期間は短いものの、収量およびアスコルビン酸濃度は低く、硝酸イオン濃度は高かった。総栽培期間中の日平均気温が低いほど、総栽培期間が長く、収量が高く、硝酸イオンが低い品目が多かった。また、低温条件で葉重および葉の厚さが増す品目が見られた。アブラナ科やキク科品目の中には、収穫直前の日最低気温がアスコルビン酸濃度に強い影響を及ぼしていた。一方 SPAD 値については気温条件との関連性はほとんどの品目で見られなかった。

第 5 章では冬季無加温ハウスでの総栽培期間、収量、アスコルビン酸濃度、硝酸イオン濃度、Brix および葉の特性について調査した。総栽培期間中のハウス内最低気温は約−10℃であったが、全 22 品目いずれも枯死せず、12 月から 2 月にかけて、一部低温障害を受けた葉や規格外の葉を除去する調整作業は必要となるが、収穫可能であった。ほとんどの品目で、12 月から 2 月にかけて調整後収量、アスコルビン酸および Brix が増加し、硝酸イオンは減少した。また、春季から秋季までの作型とは異なり、1 月および 2 月には乾物率が増加した。Brix

の増加と硝酸イオンの減少には、低温の影響による乾物率上昇と強い関係が認められた。以上より、冬季の北海道のような厳寒条件下でのベビーリーフ栽培の可能性が示された。

第 6 章では、ベビーリーフの収量および品質が低下しやすい夏季において、栽培期間中の 1～2 週間に養液栽培の養液に NaCl を添加し EC を 5.0～7.5 $dS \cdot m^{-1}$ で管理した際の影響について評価した。テーブルビート「デトロイト」では塩ストレス処理により葉の水分量が高まり収量が増加した。ホウレンソウ品目では、Na^+吸収量の増加に伴いアスコルビン酸が増加した。「グリーンロメイン」レタスやルッコラでは Na^+、K^+、Ca^{2+}の総カチオン濃度が高いとポリフェノールが増加した。硝酸イオン濃度は、Na^+吸収量の増加に伴い減少した。特にキク科品目でその傾向が顕著であった。

これまでの研究成果をもとに、北海道におけるベビーリーフ周年生産体系を確立し、各作型に適した品目を選定した。これらの成果により北海道におけるベビーリーフ生産の促進が図られるとともに、北海道産ベビーリーフの知名度向上に貢献することが期待される。