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Article

Comparison of Anthocyanin and Polyphenolics in Purple Sweetpotato (*Ipomoea batatas* L.) Grown in Different Locations in Japan

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Abstract: The health benefits of purple sweetpotato, which is used as an edible food in its natural state and in processed foods and as a natural color pigment, have been recognized. In Japan, sweetpotato has been economically produced in regions below 36°4′N latitude, however, cultivation areas are beginning to expand further north. The anthocyanin and polyphenolics in purple sweetpotatoes cultivated in different locations; I (42°92′ N, 143°04′ E), II (35°99′ N, 140°01′ E), and III (31°72′ N, 131°03′ E), were compared over two years. Total anthocyanin and polyphenolic contents in purple sweetpotatoes tended to be high in location I. Their contents significantly differed over the two years in locations I and III and was dependent on temperature during cultivation. The anthocyanin and polyphenolic compositions differed between locations. The peonidin/cyanidin ratios were higher in location III compared with I and II in all varieties. The relative amount of chlorogenic acid was higher in location I, while the amount of 3,4- and 4,5-dicaffeoylquinic acids were higher in location III, suggesting that the variability of the anthocyanin and polyphenolic content and composition was dependent on cultivation conditions. This study suggested that northern areas in Japan are an alternative production area and may yield higher amounts of anthocyanin and polyphenolics.

Keywords: purple sweetpotato; anthocyanin; polyphenolics; caffeoylquinic acid; temperature

1. Introduction

Sweetpotato (*Ipomoea batatas* L.) is a tropical and subtropical root crop originally from central America or south America. It can be cultivated under a wide range of climatic conditions, between 30° and 40° latitude in both hemispheres, and in many countries, including Asia, Africa, America and Oceania [1]. In Japan, sweetpotato is grown in regions below about 36°4′N latitude to produce starch, a distilled alcoholic drink, processed foods, natural color pigment and edible food. Sweetpotato has a number of health benefits due to its content of anthocyanins, carotenoids, polyphenolics, vitamins, minerals and dietary fibers [2].

Purple sweetpotatoes, which contain anthocyanins in their flesh, have been developed over the last two decades [2]. Their production has gradually increased in the south of Japan and processed foods containing purple sweetpotato have been developed and become widely available since the first variety, Ayamusasaki, was released [3]. The major coloring constituents in purple sweetpotato have been identified as anthocyanin, peonidins and cyanidins, named as YGM-1a, YGM-1b, YGM-2, YGM-3, YGM-4b, YGM-5a, YGM-5b and YGM-6 [4]. Anthocyanin extracts, or the purified form, have been shown to have many physiological functions *in vitro* and *in vivo*: anti-oxidation [5],

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antimutagenicity [6], antihyperglycemic [7], anti-atherosclerotic [8], hepatoprotective and anti-hypertension [9] effects have been reported.

Sweetpotato storage roots contain polyphenolics, which are mainly caffeoylquinic acid derivatives, namely, caffeic acid (CA), chlorogenic acid (ChA), 3,4-di-*O*-caffeoylquinic acid (3,4-diCQA), 3,5-di-*O*-caffeoylquinic acid (3,5-diCQA) and 4,5-di-*O*-caffeoylquinic acid (4,5-diCQA) [2]. Caffeoylquinic acids have been shown to exhibit radical scavenging activity [10], antimutagenicity [11], anticancer activity [12], anti-hyperglycemic activity [13], anti-inflammatory activity [14], anti-HIV activity [15], and inhibit melanin production [16] *in vitro* or *in vivo*. Polyphenolics also play an important role in *in vivo* defense systems against insects and other pathogens: the caffeoylquinic acids in sweetpotato roots have antifungal and antiviral activities [17, 18]. The concentration of these polyphenolics has been reported to increase as a function of stress, i.e., wounding, infection, drought, and storage at low temperature [19-22].

Sweetpotato cultivation has spread to the north region of Japan where they have been shown to have a higher content of sugars [23], however, a comparison of the physiological components, such as anthocyanins and polyphenolics, in sweetpotatoes grown in different locations has not yet been performed. In this study, we evaluated the differences in anthocyanin and polyphenolics in purple sweetpotatoes cultivated in different locations from north to south in Japan over two years. The contents and components of anthocyanin and polyphenolics were compared in three varieties and three locations. Correlations between the anthocyanin and polyphenolic contents over the two years were evaluated. In addition, the relationship between climate conditions to sweetpotato contents and components, and the expected advantages of cultivation in the north region are discussed.

2. Results

2.1. Anthocyanin content and composition of purple sweetpotatoes cultivated in different locations

The anthocyanin content and composition of three purple sweetpotato varieties cultivated in three different locations were analyzed in 2015 and 2016. Eight known anthocyanins, YGM-1a, YGM-1b, YGM-2, YGM-3, YGM-4b, YGM-5a, YGM-5b and YGM-6 were detected in all three varieties cultivated in different locations. In 2015, the total anthocyanin content in the Murasakimasari (MM) sweetpotato variety was highest in location III ≥ I ≥ II (Table 1). The total content in Purple Sweet Lord (PSL) was highest in location I > III > II (Table 1). The total content in Akemurasaki (AKM) was significantly higher in location I than in III (Table 1). The anthocyanin composition showed different patterns in both the varieties and locations. Peonidin type anthocyanins (YGM-4b, 5a, 5b and 6) were dominant in MM and PSL, and the peonidin/cyanidin ratio was highest in MM among the three varieties in all locations. The peonidin/cyanidin ratio was lowest in AKM, and the proportion of cyanidin type anthocyanins (YGM-1a, 1b, 2 and 3; 57.20%) was higher than that of the peonidin type (42.79%) in AKM cultivated in location I (Table 1). The peonidin/cyanidin ratios were higher in location III than I and II in all sweetpotato varieties. In 2016, the total anthocyanin contents were significantly decreased in locations I and III (Figure 1). The reduction rate in MM, PSL and AKM was 41.4%, 42.9% and 22.3% in location I and 64.9%, 67.5% and 52.6% in location III, respectively. The anthocyanin content in MM and PSL did not significantly differ between 2015 and 2016 in location II. This resulted in a relatively higher order of location II in anthocyanin content in MM and PSL in 2016 compared to 2015. In 2016, the total anthocyanin content in MM was significantly higher in location II, followed by I then III; in PSL it was significantly higher in I, followed by II then III; in AKM it was significantly higher in location I than in III (Table 2). The anthocyanin composition in the three varieties was similar between 2016 and 2015, except for the peonidin/cyanidin ratio of PSL in location III, which was decreased from 3.1 to 1.4. The anthocyanin content of purple sweetpotato tended to be higher in location I, with a higher ratio of cyanidin type anthocyanins.

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Table 1. Anthocyanin content and composition of purple sweetpotatoes cultivated in different locations in 2015.

Variety	Location	YGM (mg/100g FW)			
		Cyanidin	Peonidin	Total	Peonidin/Cyanidin
MM	I	131.09 ± 2.72^{a} (25.05)	392.24 ± 14.72^{ab} (74.95)	523.33 ± 16.30^{ab}	3.0
	II	103.69 ± 20.51^{b} (25.27)	306.71 ± 76.41^{b} (74.73)	410.40 ± 96.92^{b}	3.0
	III	123.89 ± 8.07^{ab} (20.64)	476.39 ± 16.52^{a} (79.36)	600.28 ± 24.57^{a}	3.8
PSL	I	92.70 ± 4.93^{a} (35.42)	169.02 ± 8.65^{a} (64.58)	261.72 ± 12.91^{a}	1.8
	II	32.69 ± 2.88^{c} (34.89)	61.01 ± 8.13^{c} (65.11)	93.70 ± 11.00^{c}	1.9
	III	49.44 ± 0.71^{b} (24.19)	154.91 ± 1.69^{b} (75.81)	204.35 ± 2.25^{b}	3.1
AKM	I	516.38 ± 11.24^{a} (57.20)	386.30 ± 10.14^{a} (42.79)	902.69 ± 20.95^{a}	0.8
	II	263.97 ± 6.28^{b} (44.19)	333.38 ± 4.98^{b} (55.81)	597.35 ± 11.19^{b}	1.3

Data are presented as mean ± SD (n=4). Proportions of cyanidin and peonidin (%) in each variety cultivated in each location are shown in parentheses. MM, Murasakimasari; PSL, Purple Sweet Lord; AKM, Akemurasaki. Location I, 143°04E / 42°92'N; location II, 140°04'E / 35°99N; location III, 131°03'E / 31°72'N. Cyanidin: sum of the contents of YGM 1a, 1b, 2 and 3. Peonidin: sum of the contents of YGM 4b, 5a, 5b and 6. Comparisons of the contents of cyanidin and peonidin and total anthocyanin in each variety among locations were performed by ANOVA and multiple regression analysis was performed by Tukey's test. Different letters indicate statistically significant differences among locations in each variety at $P<0.01$.

Table 2. Anthocyanin content and composition of purple sweetpotatoes cultivated in different locations in 2016.

Variety	Location	YGM (mg/100g FW)			
		Cyanidin	Peonidin	Total	Peonidin/Cyanidin
MM	I	64.44 ± 3.47^{b} (23.30)	212.14 ± 18.53^{b} (76.70)	276.58 ± 11.97^{b}	3.3
	II	71.97 ± 1.97^{a} (21.74)	259.08 ± 5.77^{a} (78.26)	331.05± 7.71^{a}	3.6
	III	47.14 ± 2.34^{c} (22.36)	163.68 ± 8.18^{c} (77.64)	210.82 ± 10.50^{c}	3.5
PSL	I	49.03 ± 1.26^{a} (32.79)	100.48 ± 1.82^{a} (67.21)	149.51 ± 3.06^{a}	2.0
	II	30.54 ± 1.49^{b} (32.38)	63.79 ± 4.40^{b} (67.62)	94.33 ± 5.89^{b}	2.1
	III	27.63 ± 0.25^{c} (41.67)	38.68 ± 0.30^{c} (58.33)	66.31 ± 0.54^{c}	1.4
AKM	I	319.36 ± 9.62^{a} (45.51)	382.36 ± 12.79^{a} (54.49)	701.73 ± 22.41^{a}	1.2
	II	117.51 ± 8.35^{b} (41.48)	165.74 ± 7.49^{b} (58.51)	283.26 ± 15.81^{b}	1.4

Data are presented as mean ± SD (n=4). Proportions of cyanidin and peonidin (%) in each variety cultivated in each location are shown in parentheses. MM, Murasakimasari; PSL, Purple Sweet Lord; AKM, Akemurasaki. Location I, 143°04E / 42°92'N; location II, 140°04'E / 35°99N; location III, 131°03'E / 31°72'N. Cyanidin: sum of the contents of YGM 1a, 1b, 2 and 3. Peonidin: sum of the contents of YGM 4b, 5a, 5b and 6. Comparisons of the contents of cyanidin and peonidin and total anthocyanin in each variety among locations were performed by ANOVA and multiple regression analysis was performed by Tukey's test. Different letters indicate statistically significant differences among locations in each variety at $P<0.01$.

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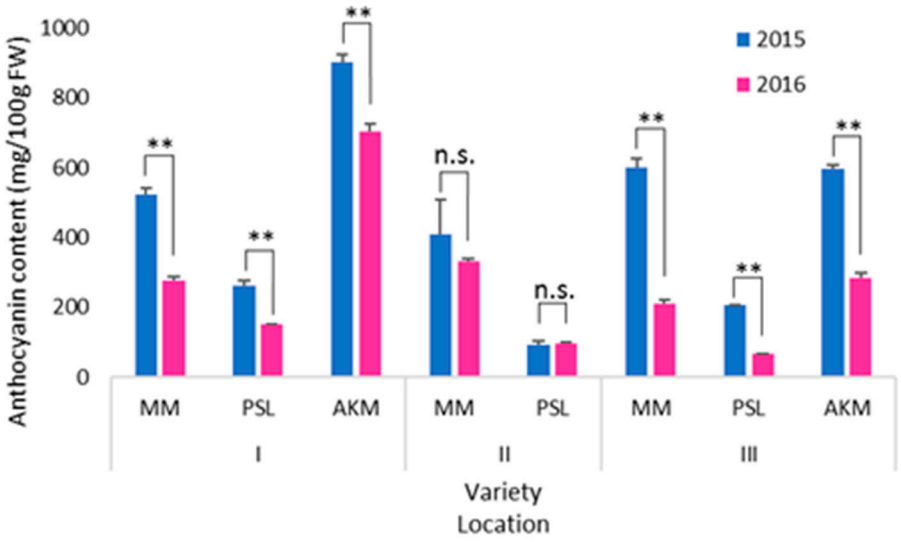

Figure 1. Total anthocyanin content in purple sweetpotatoes cultivated in different locations in 2015 and 2016. Vertical bars represent SD (n=4). MM, Murasakimasari; PSL, Purple Sweet Lord; AKM, Akemurasaki. Location I, 143°04E / 42°92'N; location II, 140°04'E / 35°99N; location III, 131°03'E / 31°72'N. Comparisons between the contents in 2015 and 2016 in each variety cultivated in each location were performed by Student's t test; *P<0.01. n.s., not significant.

2.2. Polyphenolic content and composition of purple sweetpotatoes cultivated in different locations

The polyphenolic content and composition of three purple sweetpotato varieties cultivated in three different locations were evaluated in 2015 and 2016. Five known caffeoylquinic acids, ChA, CA, 3,4-diCQA, 3,5-diCQA and 4,5-diCQA, were detected by HPLC in all the varieties cultivated in different locations. The total polyphenolic content in MM differed according to its location of growth: in 2015 the content, from high to low, was in the order of location I > II > III. In PSL, the order was location I > III > II (Table 3). The polyphenolic content in AKM was significantly higher in sweetpotatoes grown in location I compared with III (Table 3). ChA and 3,5-diCQA were the major polyphenolics in all three varieties cultivated in any location. The composition of polyphenolics differed slightly between varieties and locations. ChA was more dominant in MM and AKM compared with 3,5-diCQA, while 3,5-diCQA was more dominant in PSL. The relative amounts of ChA in MM and AKM were higher in location I, while the relative amounts of 3,4- and 4,5-diCQA in MM and AKM were higher in location III (Table 3). The relative amounts of each caffeoylquinic acid in PSL did not differ between the locations. In 2016, the total polyphenolic contents were significantly decreased in locations I and III (Figure 2). The reduction rate in MM, PSL and AKM were 37.4%, 33.6% and 29.3% in location I and 68.7%, 92.7% and 81.0% in location III, respectively. The total content of polyphenolics in MM and PSL did not significantly differ between 2015 and 2016 in location II. This resulted in a relatively higher order of polyphenolic content in location II: total polyphenolic content in MM was significantly higher in locations I and II compared with location III, and the content in PSL was significantly higher in location I, followed by location II then III (Table 4). The content in AKM was significantly higher in location I compared with III similar to 2015 (Table 4). There were no significant differences in the polyphenolic composition between locations I and II in 2015 and 2016, however, 3,4- and 4,5-CQA were more abundant in location III. The results suggest that the polyphenolic content of purple sweetpotato tended to be higher in location I, with a higher ratio of ChA.

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Table 3. Polyphenolic content and composition of purple-fleshed sweetpotatoes cultivated in different locations in 2015.

Variety	Location	Caffeoylquinic acids (mg/100g FW)					
		ChA	CA	3,4-diCQA	3,5-diCQA	4,5-diCQA	Total
MM	I	314.35 ± 12.87^{a} (54.88)	0.56 ± 0.09^{a} (0.10)	17.37 ± 1.62^{ab} (3.03)	234.60 ± 8.66^{a} (40.95)	5.96 ± 0.39^{b} (1.04)	572.84 ± 21.90^{a}
	II	177.14 ± 46.19^{b} (49.99)	0.29 ± 0.13^{b} (0.08)	13.52 ± 3.18^{b} (3.82)	159.88 ± 31.67^{b} (45.12)	3.51 ± 0.65^{c} (0.99)	354.34 ± 80.01^{b}
	III	102.46 ± 11.77^{c} (48.13)	0.37 ± 0.05^{ab} (0.17)	19.65 ± 0.56^{a} (9.23)	83.39 ± 18.21^{c} (39.17)	7.02 ± 0.40^{a} (3.30)	212.89 ± 21.56^{c}
PSL	I	124.45 ± 7.70^{a} (40.75)	0.76 ± 0.07^{a} (0.25)	2.83 ± 0.32^{a} (0.93)	173.75 ± 10.49^{a} (56.89)	3.63 ± 0.24^{a} (1.19)	305.42 ± 18.58^{a}
	II	22.60 ± 8.34^{c} (43.42)	0.22 ± 0.08^{b} (0.42)	0.96 ± 0.47^{b} (1.84)	27.68 ± 11.39^{c} (53.18)	0.59 ± 0.19^{c} (1.13)	52.04 ± 20.37^{c}
	III	87.59 ± 1.40^{b} (41.36)	0.16 ± 0.03^{b} (0.08)	2.06 ± 0.36^{a} (0.97)	119.10 ± 2.89^{b} (56.25)	2.84 ± 0.09^{b} (1.34)	211.76 ± 4.17^{b}
AKM	I	302.33 ± 13.23^{a} (56.27)	0.68 ± 0.19^{b} (0.13)	4.31 ± 1.33 (0.80)	226.02 ± 9.51^{a} (42.07)	3.91 ± 0.15^{a} (0.73)	537.25 ± 22.58^{a}
	II	44.94 ± 2.86^{b} (43.56)	1.39 ± 0.07^{a} (1.35)	3.59 ± 0.81 (3.48)	50.30 ± 5.10^{b} (48.76)	2.94 ± 0.11^{b} (2.85)	103.16 ± 8.04^{b}

Data are presented as mean ± SD (n=4). Relative amounts of individual caffeoylquinic acids (%) in each variety cultivated in each location are shown in parentheses. MM, Murasakimasari; PSL, Purple Sweet Lord; AKM, Akemurasaki. Location I, 143°04E / 42°92'N; location II, 140°04'E / 35°99N; location III, 131°03'E / 31°72'N. Comparison of the content of each caffeoylquinic acid and total content in each variety among locations were performed by ANOVA and multiple regression analysis was performed by Tukey's test. Different letters indicate statistically significant differences among locations in each variety at $P<0.01$.

Table 4. Polyphenolic content and composition of purple-fleshed sweetpotatoes cultivated in different locations in 2016.

Variety	Location	Caffeoylquinic acids (mg/100g FW)					
		ChA	CA	3,4-diCQA	3,5-diCQA	4,5-diCQA	Total
MM	I	190.78 ± 24.75^{a} (53.23)	0.39 ± 0.04^{b} (0.11)	11.58 ± 1.92^{b} (3.23)	152.10 ± 19.38^{a} (42.44)	3.53 ± 0.56^{b} (0.98)	358.38 ± 46.58^{a}
	II	125.32 ± 3.68^{b} (40.42)	0.62 ± 0.07^{a} (0.20)	36.04 ± 1.58^{a} (11.62)	143.38 ± 5.09^{a} (46.25)	4.67 ± 0.38^{a} (1.51)	310.03 ± 10.06^{a}
	III	19.29 ± 2.05^{c} (28.29)	0.43 ± 0.13^{b} (0.64)	14.00 ± 1.47^{b} (20.99)	27.87 ± 3.11^{b} (41.78)	5.11 ± 0.47^{a} (7.66)	60.70 ± 7.15^{b}
PSL	I	89.80 ± 7.78^{a} (44.26)	0.48 ± 0.04^{b} (0.24)	1.80 ± 0.16^{a} (0.89)	108.94 ± 9.27^{a} (53.70)	1.86 ± 0.18^{a} (0.92)	202.88 ± 17.35^{a}
	II	18.17 ± 2.23^{b} (36.18)	0.67 ± 0.08^{a} (1.33)	1.47 ± 0.20^{b} (2.93)	29.53 ± 6.18^{b} (58.80)	0.38 ± 0.10^{b} (0.76)	50.22 ± 6.43^{b}
	III	7.54 ± 0.31^{c} (48.80)	0.57 ± 0.04^{ab} (3.69)	0.90 ± 0.08^{c} (5.83)	6.18 ± 0.54^{c} (40.00)	0.26 ± 0.02^{b} (1.68)	15.45 ± 0.92^{c}
AKM	I	220.70 ± 11.28^{a} (58.13)	0.87 ± 0.20 (0.23)	5.02 ± 0.11^{a} (1.32)	149.92 ± 4.97^{a} (39.48)	3.18 ± 0.11^{a} (0.84)	379.67 ± 16.29^{a}
	II	7.57 ± 0.79^{b} (38.56)	0.85 ± 0.06 (4.33)	2.53 ± 0.54^{b} (12.89)	7.49 ± 0.61^{b} (38.16)	1.19 ± 0.10^{b} (6.06)	19.62 ± 1.31^{b}

Data are presented as mean ± SD (n=4). Relative amounts of individual caffeoylquinic acids (%) in each variety cultivated in each location are shown in parentheses. MM, Murasakimasari; PSL, Purple Sweet Lord; AKM, Akemurasaki. Location I, 143°04E / 42°92'N; location II, 140°04'E / 35°99N; location III, 131°03'E / 31°72'N. Comparison of the content of each caffeoylquinic acid and total content in each variety among locations were performed by ANOVA and multiple regression analysis was performed by Tukey's test. Different letters indicate statistically significant differences among locations in each variety at $P<0.01$.

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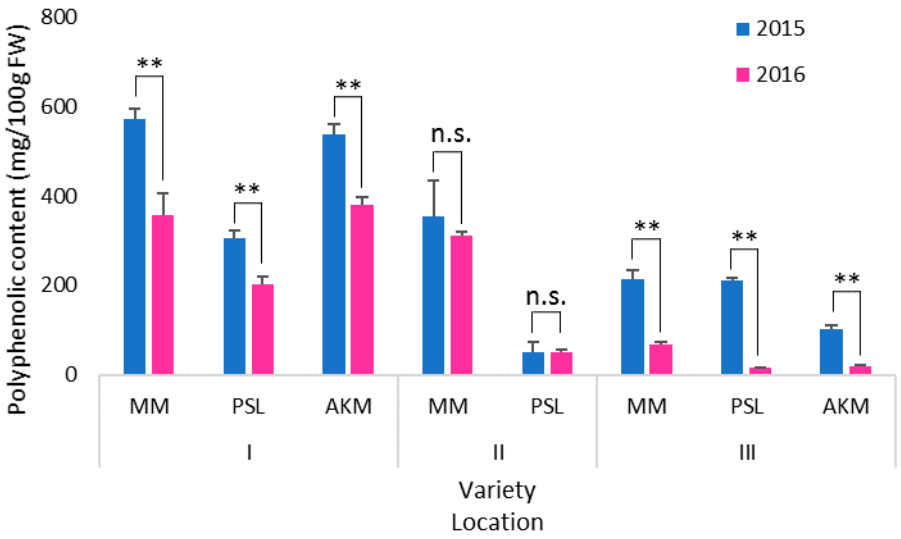

Figure 2. Total caffeoylquinic acid content of purple sweetpotatoes cultivated in different locations in 2015 and 2016. Vertical bars represent SD (n=4). MM, Murasakimasari; PSL, Purple Sweet Lord; AKM, Akemurasaki. Location I, 143°04E / 42°92'N; location II, 140°04'E / 35°99N; location III, 131°03'E / 31°72'N. Comparisons between the contents in 2015 and 2016 in each variety cultivated in each location were performed by Student's t test; *P<0.01. n.s., not significant.

2.3. Relationship between anthocyanin content, caffeoylquinic acid content and climate conditions over two years.

The total anthocyanin content positively correlated with the total caffeoylquinic acid content in 2015 (r = 0.5475, P<0.01) and 2016 (r = 0.6967, P<0.01) (Table 5). In addition, total anthocyanin content and total caffeoylquinic acid content in 2015 were highly correlated with those in 2016 (anthocyanin, r = 0.8696, P<0.01; caffeoylquinic acid, r=0.9035, P<0.01) (Table 5), although the contents were significantly different in locations I and III between the two years.

Precipitation, temperature, sunshine duration and accumulated temperature during cultivation of the purple sweetpotato varieties in the three locations in 2015 and 2016 were compared. Precipitation was, by far, the highest in location III, in both 2015 and 2016 (Table 6). Sunshine duration and accumulated temperature in 2015 and 2016 were lower in location I than II and III (Table 6). The average mean temperature, mean maximum temperature and mean minimum temperature were in the order of, lowest to highest, location I < II ≤ III in 2015 and location I < II < III in 2016 (Figure 3). The temperatures were lower in 2015 compared with 2016 in the latter stages of cultivation in location I and throughout cultivation in location III (Figure 3). The differences between mean maximum temperature and mean minimum temperature were highest in locations I and III in 2015 (Table 6). Within the climate parameters, temperature is suggested to have the most influence on the anthocyanin and polyphenolic content, because their contents tended to be higher in the location with the lowest temperature: in 2015, their contents were higher in locations I and III which were recorded to have lower temperatures than in 2016.

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Table 5. Correlation coefficient between total anthocyanin content and total caffeoylquinic acid content in 2015 and 2016.

	2015 Ant		2015 CQA		2016 Ant		2016 CQA
2015 Ant	-						
2015 CQA	0.5475		-				
2016 Ant	0.8696	**	0.6376	**	-		
2016 CQA	0.4828	**	0.9035	**	0.6967	**	-

Results are expressed as Pearson correlation coefficients with an indicated level of significance. Data with ** was significant at $P < 0.01$ (n=32). 2015 Ant, total anthocyanin content in 2015; 2015 CQA, total caffeoylquinic acid content in 2015; 2016 Ant, total anthocyanin content in 2016; 2016 CQA, total caffeoylquinic acid content in 2016.

Table 6. Weather data during cultivation in 2015 and 2016.

		Precipitation (mm)		Sunshine Duration (h)		Accumulated Temperature (°C)		Max – Min Temperature (°C)[2]	
Location	**Month**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
I	Jun	29	180	126	109	378	394	10	8
	Jul	56	135	188	91	627	558	12	8
	Aug	119	419	118	126	606	676	9	9
	Sep	112	138	155	120	471	500	11	9
	Oct	8	14	32	31	61	57	13	13
	Sum[1]	323	884	618	478	2,144	2,185	55	47
II	May	8	24	55	17	147	139	11	9
	Jun	173	106	135	141	632	644	9	8
	Jul	193	96	186	146	787	755	8	8
	Aug	101	299	146	177	795	815	7	8
	Sep	360	243	117	100	647	705	7	6
	Oct	44	51	150	108	414	430	10	9
	Sum[1]	878	818	789	689	3,420	3,488	52	48
III	May	30	99	67	113	228	160	11	10
	Jun	1095	855	61	80	644	697	6	7
	Jul	567	648	123	165	786	834	7	9
	Aug	378	117	182	223	828	860	9	10
	Sep	161	531	146	129	700	767	9	8
	Oct	12	34	64	21	158	130	13	8
	Sum[1]	2,241	2,282	641	730	3,344	3,448	56	52

The data are expressed as the mean of each month during cultivation. [1] Sum of the data of each month. [2] Max-Min Temperature is the difference between the mean maximum temperature and mean minimum temperature. Location I, 143°04E / 42°92'N; location II, 140°04'E / 35°99N; location III, 131°03'E / 31°72'N.

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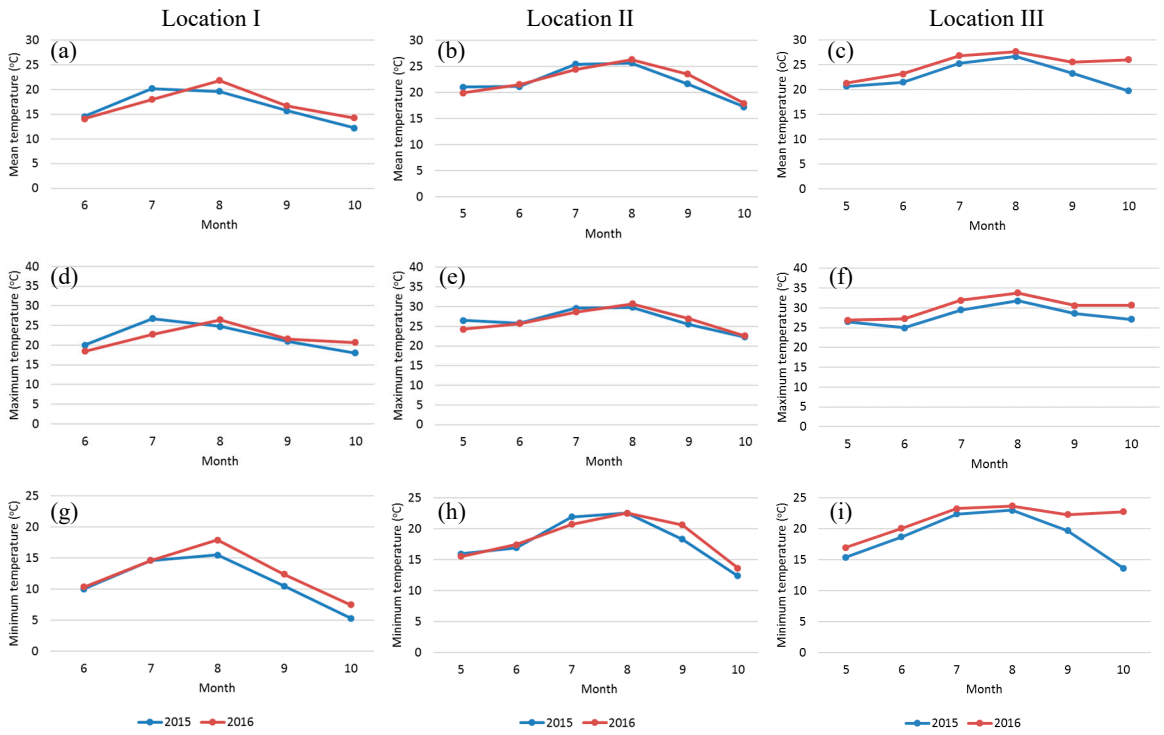

Figure 3. Mean temperature in location I (a), II (b) and III (c), mean maximum temperature in location I (d), II (e) and III (f) and mean minimum temperature in location I (g), II (h) and III (i) in 2015 (blue lines) and 2016 (red lines). Location I, 143°04E / 42°92'N; location II, 140°04'E / 35°99N; location III, 131°03'E / 31°72'N.

3. Discussion

The anthocyanin and polyphenolic contents in plants are affected by environmental conditions, such as temperature and light [24-26]. In the case of sweetpotato, temperature may be the most influential factor on their contents, because it is an underground crop. The content of anthocyanins in the periderm of sweetpotatoes was higher when grown in lower temperature growth chambers [27]. A highly negative correlation was recognized between soil temperature and anthocyanin content in the flesh of purple sweetpotato [28]. In this study, anthocyanin content tended to be higher in location I, which is in the north of Japan and had the lowest temperatures during cultivation. This suggests that the north region of Japan is a good location for sweetpotato growth to maximize anthocyanin pigment production. In 2015, the anthocyanin content was relatively high in location III as well as in location I. One possible reason for this, may be a lower soil temperature in location III since an agricultural mulching film, which is used to warm soil, was not applied. Another possible explanation is that the difference between the day and night temperatures affected accumulation of anthocyanin [29]: the difference between maximum and minimum temperatures during cultivation was relatively high in location III in 2015 (Table 6). The difference in anthocyanin content between 2015 and 2016 in location I is suggested to be due to lower temperatures and larger differences between maximum and minimum temperatures in 2015. The reduction in anthocyanin was more prominent in MM and PSL compared with AKM. MM and PSL are peonidin dominant varieties and it may be that they are more sensitive to temperature. The anthocyanin composition differed between cultivation locations. The peonidin/cyanidin ratio was higher in location III compared with I and II in 2015. Peonidin is produced by the methylation of the precursor cyanidin [30]. In sweetpotato cell culture, temperature significantly affected the peonidin/cyanidin ratio. A low temperature (15 °C) suppressed accumulation of peonidin-based pigments and a higher temperature (25-30 °C) favored

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methylation [31]. It is possible that temperature effects methyltransferase activity, which would change peonidin production, however, locational differences in the peonidin/cyanidin ratio was not clear in 2016. The effect of environmental conditions on anthocyanin composition requires further study.

The polyphenolic content also tended to be higher in location I during the two years cultivation. During the two study years, the polyphenolic content was considerably different between locations I and III where the temperatures were higher in 2016 compared with 2015. It is suggested that the temperature during cultivation has a large influence on the polyphenolic content. Caffeoylquinic acids and anthocyanins are synthesized via the phenylpropanoid pathway and the biosynthetic pathway of phenolic compounds is closely related to that of anthocyanin [32]. Accumulation of polyphenolics and anthocyanins in sweetpotato storage roots is regulated by the MYB-domain-containing transcription factor, IbMYB1 [33]. Overexpression of IbMYB1 led to higher polyphenolic acid and anthocyanin levels in storage roots. In the transgenic sweetpotato, expression of the genes involved in the anthocyanin metabolic pathway, including genes involved in the early production steps of caffeoylquinic acids, such as phenyl alanine ammonia lyase (PAL) and cinnamate 4-hydroxylase (C4H), were elevated [34]. Genes related to the phenylpropanoid pathway were highly expressed at a low temperature [25]. We found higher accumulation of caffeoylquinic acids and anthocyanin in purple sweetpotatoes cultivated in the northern location and in the year with lower temperatures, probably due to elevation of gene expression at low temperatures. ChA was more abundant in location I which is located in the north of Japan. Kojima et al. reported that ChA was enzymatically converted to 3,5-diCQA in a one-step reaction [35]. The enzyme activity required to catalyze this reaction may be lower at low temperatures. The biosynthetic pathway of caffeoylquinic acids has not been well studied. Our results will help in the understanding of the relationship between environmental conditions and polyphenolic composition.

4. Materials and Methods

4.1. Reagents and chemicals

Chlorogenic acid was obtained from Sigma-Aldrich Japan Co., LLC. (Tokyo, Japan). 3,4-diCQA, 4,5-diCQA and 3,5-diCQA, were purchased from ChemFaces (Wuhan, China). Purified YGM-6 (96 % purity) was used as a standard for anthocyanin analysis by HPLC. Other reagents were purchased from Wako Pure Chemicals Ind. (Osaka, Japan).

4.2. Sample preparation

Three purple sweetpotato varieties were grown in three different locations in Japan in 2015 and 2016. Cultivation location, variety, transplanted and harvested day, growth duration, soil type and climate in the cultivation field are summarized in Table 7. Murasakimasari (MM) is a standard variety used in food pigment production and contains a medium content of anthocyanin: it is mostly cultivated in location III. Akemurasaki (AKM) is also mostly cultivated in location III, used in food pigment production and has a high content of anthocyanin. Purple Sweet Lord (PSL) is used as an edible food, has a low content of anthocyanin and is mainly cultivated in location II. The experimental locations were as follows: location I, Memuro, Hokkaido (longitude, 143°04E; latitude, 42°92'N); location II, Tsukubamirai, Ibaraki (longitude, 140°04'E; latitude, 35°99N); location III, Miyakonojo, Miyazaki (longitude, 131°03'E; latitude, 31°72'N). The sweetpotatoes were cultivated using the standard local methods, including planting and harvesting dates, and with or without mulching film. Harvested sweetpotatoes were freeze-dried and held at -35 °C until analysis. Freeze-dried samples were used for anthocyanin and polyphenolic analyses.

4.3. Anthocyanin analyses

Anthocyanins were extracted according to the method by Oki *et al*. [36]. One g of the freeze-dried samples was vigorously mixed in 9 mL of the extraction solution (methanol/water/trifluoroacetic acid = 40/60/0.5), mixed using a vortex mixer and sonicated in a water bath at 37 °C for 5 min followed by continuous warming for 10 min. The extract was then centrifuged

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at 1,900 x g for 15 min and the supernatant was recovered. Extraction solution (8 mL) was added to the residue, and the extraction was repeated twice more. The extracts were combined and made up to 25 mL. A portion of the extract was filtered through a 0.45 µm membrane, and the anthocyanins were determined quantitatively by high-performance liquid chromatography (HPLC), as described [37]. The HPLC system consisted of two pumps (model LC-20AD; Shimadzu, Kyoto, Japan), an auto-injector (model SIL-20AC; Shimadzu, Kyoto, Japan), a column oven (model CTO-20AC; Shimadzu, Kyoto, Japan) and a photodiode array detector (model SPD-M20A; Shimadzu, Kyoto, Japan). A reversed phase column was used (250 x 4.6 mm i.d., Cadenza CD-C18, 3µm; Imtakt Corp., Kyoto, Japan). The mobile phase consisted of water containing 0.4% formic acid (A) and 50% acetonitrile containing 0.4% formic acid (B). The elution profile was as follows: a linear gradient of 25% to 53% B from 0 to 40 min, and 25% B from 41 to 60 min at a flow rate of 0.6 mL/min. The column oven was set at 30 °C. Anthocyanins, YGM-1a, YGM-1b, YGM-2, YGM-3, YGM-4b, YGM-5a, YGM-5b and YGM-6, were identified by their retention time and the UV-vis spectra. Quantification of anthocyanins was performed using a YGM-6 external standard method based on detection at 520 nm. Each anthocyanin was quantified using each specific conversion factor [37] and expressed as mg/100 g fresh weight (FW).

3.4. Polyphenolic analyses

Polyphenolics were extracted as previously reported [22]. Freeze-dried samples (100 mg) were vigorously mixed in 10 mL of 80% ethanol, and boiled at 100 °C for 5 min. A portion of the extract was filtered through a 0.45 µm membrane, and the caffeoylquinic acids were determined quantitatively by HPLC, as described, with slight modification [22]. The HPLC system was the same as for anthocyanin analysis. A reversed phase column was used (75 x 4.6 mm i.d., 3 µm, Cadenza CD-C18; Imtakt, Kyoto, Japan). The mobile phase consisted of water containing 0.2% formic acid (A) and acetonitrile (B). The elution profile was as follows: a linear gradient of 8% to 30% B from 0 to 23 min, and 8% B from 23.01 to 30 min at a flow rate of 1 mL/min. The column oven was set at 40 °C. Caffeoylquinic acids were identified by their retention time and the UV-vis spectra of standards. Quantification of caffeoylquinic acids was performed using an external standard based on detection at 326 nm. Caffeoylquinic acids were quantified as mg/100 g FW.

3.5. Climate conditions

Climate conditions in 2015 and 2016, including precipitation, mean temperature, mean maximum temperature, mean minimum temperature and sunshine duration, were cited in three cultivation locations using data provided by the Automated Meteorological Data Acquisition System (AMeDAS).

3.6. Statistical analysis

Four replications were performed for all analyses and data are shown as average ± standard deviation (SD). One way analysis of variance was performed to evaluate the differences in the contents of anthocyanin and polyphenolics among variety and location. The year differences in the contents of anthocyanin and polyphenolics were evaluated by Student's *t*-test. The Pearson correlation coefficient was used to show the correlation between the content of anthocyanin and caffeoylquinic acids using individual data from 4 replications in 2015 and 2016.

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Table 7. Location, variety, transplanted/harvested day, soil type and climate in the cultivation field in 2015 and 2016.

Location	Latitude/ Longitude	Variety	Transplant/Harvest (Growth Duration)	Soil Type	Climate[1]
I	143°04E/ 49°92N	MM, PSL, AKM	Early Jun/Early Oct (about 120 days)	Brown volcanic ash soil	Subarctic wet
II	140°01E/ 35°99N	MM, PSL	Late May/Late Oct (about 150 days)	Andosol	Warm and humid
III	131°03E/ 31°72N	MM, PSL, AKM	Mid-Late May/Early Oct (about 140-150 days)	Andosol	Warm and humid

[1]Koöppen-Geiger classification. Location I, 143°04E / 42°92′N; location II, 140°04′E / 35°99N; location III, 131°03′E / 31°72′N.

5. Conclusions

The accumulation of anthocyanin and polyphenolic acids was higher in purple sweetpotatoes cultivated in a northern location (location I), suggesting that lower temperatures enhanced their contents. The varieties used for natural pigment production, such as MM and AKM, are cultivated in southern location (location III). Our study showed that a northern location could be used as an alternative area for purple sweetpotato growth for natural pigment production. Our results suggest that the amounts of anthocyanin and polyphenolics easily fluctuates and are influenced by climate conditions, particularly temperature during cultivation. The cultivation of purple sweetpotato in a northern region could compensate for decrease in pigment production by high temperatures in southern location. The purple sweetpotato varieties used for edible food, such as PSL, are usually cultivated at lower latitudes, such as location II and III: a northern location may also be a suitable area for such varieties, because the sugar content is higher [23] and health benefits due to the higher contents of anthocyanin and polyphenolics are expected.

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Author Contributions: K.I. and K.K. conceived and designed the experiments; K.I., T.K. and Y.K. performed the experiments; K.I. analyzed the data and wrote the paper. All the co-authors reviewed and approved the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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Sample Availability: Samples of the compounds are not available from the authors.