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‘Sunrouge’, a New Tea Cultivar with High Anthocyanin

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Abstract

‘Sunrouge’ is an anthocyanin-rich tea cultivar, which was selected from the population derived from the natural crossing of ‘Cha Chuukanbohon Nou 6’ (*C. taliensis* X *C. sinensis*). The anthocyanin content of ‘Sunrouge’ exceeded that of ‘Cha Chuukanbohon Nou 6’, which was bred as an anthocyanin-rich tea parental line. However the anthocyanin content of ‘Cha Chuukanbohon Nou 6’ depends on the plucking period, growth stage and the part of the new shoot. The emergence of the ‘Sunrouge’ crop is moderately early, as with the Japanese leading cultivar ‘Yabukita’ (*C.sinensis var. sinensis*). The ‘Sunrouge’ tree is of the medium type and has many branches in comparison to typical cultivars. In terms of resistance, it is slightly resistant to anthracnose (*Discula theae-sinensis* (I. Miyake) Moriwaki & Toy. Sato comb. nov.), highly resistant to gray blight (*Pestalotiopsis longiseta* (Spegazzini) Dai & Kobayashi) and has relatively low resistance to brown blight (*Glomerella cingulata* (Stoneman) Spaulding & Schrenk). The survival rate of ‘Sunrouge’ is lower than ‘Yabukita’ and the other control cultivars in the cutting propagation. We try to propagate using the photoautotrophic culture method and the propagation of ‘Sunrouge’ was incident-free. We anticipate its use as a functional food and the material of the natural pigment.

Discipline: Plant breeding
Additional key words: *camellia sinensis, camellia taliensis,* cha chuukanbohon nou 6

Introduction

Anthocyanin is well known as a natural pigment in plants such as berries[22] and can be expected to have antioxidant[2,6] and anti-mutagenic[26] actions. Efforts to develop an anthocyanin-rich cultivar have advanced with some crops such as the sweet potato[7,21,24], potato[12,13,25], and rice[4,8,17,23] to date in Japan. The anthocyanin-rich potato and sweet potato have been already used as raw materials for food, drinks and brewage[3,5,11,14]. There is also a native tea variety ‘Benibana-cha’ (*C.sinensis var. sinensis*), which contains anthocyanin in new shoots, flower and roots. However ‘Benibana-cha’ is not suitable for cultivation due to its fragility and low yield. The cultivation characteristics must be enhanced to use the anthocyanin in tea.

Catechin in tea is known as a highly functional component[1]. Both anthocyanin and catechin could be used simultaneously if we could develop a new tea cultivar with high anthocyanin. Accordingly, we bred an anthocyanin-rich parental line ‘Cha Chuukanbohon Nou 6’ in 2004[18]. However the anthocyanin content of this cultivar rapidly declines and its growth and cultivation characteristics were inferior as a tea plant. Therefore we aimed to breed a tea cultivar with high anthocyanin content and ease of cultivation.

A new anthocyanin-rich tea cultivar ‘Sunrouge’ was bred and released in 2009. In this literature, we introduce its characteristics.

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Breeding process

The seeds derived from natural crossings of 'Cha Chukanbohon Nou 6' were seeded in the green house at the Makurazaki Tea Research Station of the NARO (National Agriculture and Food Research Organization) institute of vegetable and tea science (NIVTS) in 2001. Seedlings were planted in the field, and their growth and disease resistance were investigated from 2002 to 2004. The anthocyanin content and adaptability for photoautotrophic culture in individuals were determined in 2006 and 2007, and 2 individuals, 'Makura-Ko 02-712' (Beni-Kou27 (progeny of 'Benibana-cha) X Makura F1-95180 (*C.taliensis* X *C.sinensis*)) and 'Makura-Ko 03-1384' (natural crossings of 'Cha Chuukanbohon Nou 6' (*C.taliensis* X *C.sinensis*)), were selected in 2008 and investigated in clonal evaluation. The plants made by photoautotrophic culture (cut stem with a bud and a leaf of cuttings were cultivated in the growth room, under conditions of optimal CO_2, light and moisture) were used for the clonal test. We found that 'Makura-Ko 03-1384' was superior to 'Makura-Ko 02-712' as an anthocyanin-rich tea cultivar, whereupon we applied to the Ministry of Agriculture, Forestry and Fisheries (MAFF) as 'Sunrouge' on June 3, 2009. This application was published on August 18 (Application Number: 23800) by the MAFF (Table 1).

Morphological and cultivation characteristics

The shoot length of 'Sunrouge' was about the same as 'Yabukita', and smaller than 'Cha Chuukanbohon Nou 6'. The mature leaf was smaller than 'Yabukita' and 'Cha Chuukanbohon Nou 6'. The gloss of the mature leaves was the same as 'Yabukita'. The edge of the mature leaves was slightly wavy (Fig. 1). The 'Sunrouge' plant had an intermediate type shape, even though 'Yabukita' and 'Cha Chuukanbohon Nou 6' were of the erect type. Its flower was white in color, like 'Yabukita' and 'Cha Chuukanbohon Nou 6'.

The bud opened before 'Yabukita', but the plucking took place at about the same time as the latter in the first plucking period, while the plucking period of 'Sunrouge' and 'Cha Chuukanbohon Nou 6' tended to be delayed, hence the plucking date was late (Table 2).

The growth of 'Sunrouge' and other comparative cultivars and lines in the first year is shown in table 3. The growth of 'Cha Chuukanbohon Nou 6' was vigorous and its tree form was seemingly of the longitudinal type like *C.taliensis*. The height and width of 'Sunrouge' exceeded those of 'Yabukita', 'Makura-Ko 02-712' and

Table 1. 'Sunrouge' breeding process

Breeding process	Year
Collection of seedlings from 'Cha chuukanbohon nou 6'	2001
Raising seedlings in the greenhouse	2001
Planting in the field & individual selection	2002
Screening by anthocyanin content	2006
Adaptability to photoautotrophic culture	2007
Nursery test	2008
Clonal test	2008
Apply for variety registration	2009

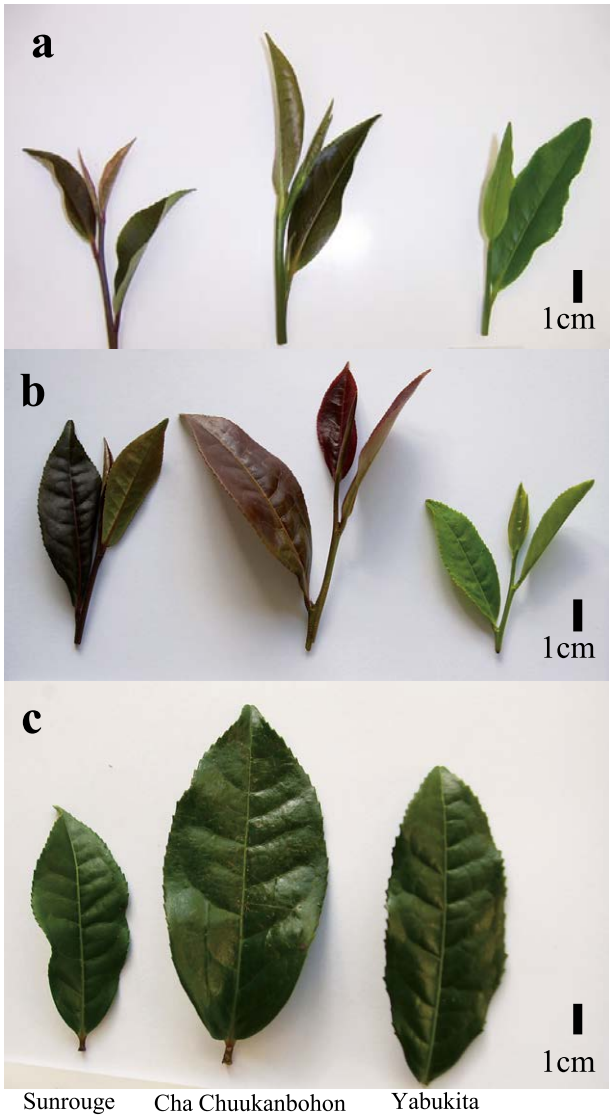

Fig. 1. Characteristics of new shoot and mature leaf
a: New shoot of first crop in April.
b: New shoot of fourth crop in September.
c: Mature leaf in September.

'Benibana-cha'. Moreover, the plant was of the crosswise type compared with 'Yabukita' and 'Makura-Ko 02-712'. As with 'Yabukita', very few 'Sunrouge' trees died after being planted in the field, nor were any symptoms of anthracnose and gray blight seen there. However, brown blight was commonplace (Table 3). An inoculation test of resistance to anthracnose and gray blight was performed on 'Sunrouge' and 'Yabukita'. Based on the results, we judged the disease resistance of 'Sunrouge' as follows. Resistance to anthracnose is slightly high, gray blight is high and brown blight is slightly low.

The yield of fresh leaves in the first, second and third plucking periods were investigated in 2011. Consequently, the yield of 'Sunrouge' seemed to exceed that of 'Yabukita' but be less than 'Cha Chuukanbohon Nou 6' (Table 4). The yield of fresh leaves in the first, second and third plucking periods of 'Sunrouge' was 5038 kg/ha, hence the annual yield of dry matter weight was anticipated to exceed 1000 kg/ha.

The length of the new 'Sunrouge' shoot in the first and second plucking periods showed no significant difference compared with 'Yabukita' and 'Cha Chuukanbohon Nou 6'. However, during the third plucking period, 'Sunrouge' was shorter (Table 5). There were numerous leaves per bud of 'Sunrouge' during the first and second plucking periods, but few in the third plucking period (Table 6).

Component characteristics

The contents of anthocyanins, catechins and caffeine in tea leaves were analyzed by high-performance liquid chromatography (HPLC) in 2010 and 2011. The anthocyanin content of 'Sunrouge' was found to exceed that of the other cultivar in both years and throughout all plucking periods. However, the content ratio differed de-

Table 2. Bud opening and plucked dates in 2010 and 2011

Cultivar	Year	Time bud opened in the first crop period	Plucked date		
			First	Second	Third
Sunrouge	2010	-	20-Apr	14-Jun	20-Jul
	2011	18-Mar	22-Apr	23-Jun	2-Aug
Cha Cuukanbohon Nou 6	2010	-	13-Apr	14-Jun	20-Jul
	2011	12-Mar	12-Apr	22-Jun	1-Aug
Yabukita	2010	-	19-Apr	7-Jun	16-Jul
	2011	22-Mar	21-Apr	16-Jun	22-Jul

The date of upper column was investigated in 2010 and the sub-column was investigated in 2011.
The plants were planted in March, 2008.

Table 3. Growth and disease resistance to anthracnose and Brown blight in young tea plants

Cultivar / line	Height (cm)	Width (cm)	Rate of dead plants (%)	Shape of plant	Vigor of plant	Disease resistance		
						Anthracnose	Gray blight	Brown blight
Sunrouge	47.8±4.2 a	52.2±4.1 a	1.7±0.6 a	5.0	7.0	8.0 (6.0mm)	7.0 (3.5mm)	4.7
Cha Chuukanbohon Nou 6	54.1±1.7 a	41.6±2.1 b	10.0±2.0 a	4.0	6.7	8.0 ( )	7.0 ( )	4.0
Yabukita	43.9±3.3 b	35.1±2.0 bc	0 a	3.0	5.0	3.7 (18.4mm)	3.0 (7.1mm)	6.0
Makura-Ko 02-712	46.1±0.8 ab	34.4±2.5 c	36.7±3.2 b	4.7	4.3	8.0	-	3.3
Benibana-cha	36.1±1.0 c	23.7±1.3 d	11.7±0.6 a	4.0	3.0	6.7	-	4.0

The investigation was carried out on October 27, 2008, one year after the plant had been planted in the field.
Average of three replications (mean±SD).
Values followed by the same letters do not differ significantly (Turkey, $P<0.05$).
Value of shape and vigor of plants are based on 'Shubyo Tokusei Bunrui Chosa Kijun'. Shape : 2 (very erect) - 5 (medium) - 8 (very spread). Vigor : 2 (very weak) - 5 (medium) - 8 (very strong). Disease resistance : 2 (very low) -5 (medium) - 8 (very high).
Resistance to Anthracnose and Gray blight : The value in ( ) shows the length of lesion by inoculation test.

pending on the plucking period, and the first plucking period was lower than that of the second and third plucking periods (Table 7). Although the catechin content in 'Sunrouge' was less than that in 'Yabukita', it exceeded that in 'Cha Chuukanbohon Nou 6' in both years and throughout all plucking periods (Table 7). The caffeine content in 'Sunrouge' and 'Cha Chuukanbohon Nou 6' tended to exceed that of 'Yabukita' (Table 7).

The chemical ingredients of fresh tea leaves may change when they are stored for an extended period till parching after being plucked. The influence of the storage time of fresh leaves on the chemical components (anthocyanin, catechin and caffeine) was investigated in the first plucking period in 2010. The anthocyanin and catechin contents of fresh leaves in 'Sunrouge' were unchanged when kept at ambient temperature for 14 hours after 8 hours' cooling in the refrigerator, while the caffeine content increased after treatment (Table 8). The changes in the anthocyanin content of fresh leaves in 'Sunrouge' left for 8 hours in the room (at an ambient temperature of about 25˚C) were investigated in the third plucking period in 2010. Consequently, the anthocyanin content in 'Sunrouge' showed almost no change (Fig. 2).

Anthocyanins, catechins and caffeine contents in each leaf order of 'Sunrouge' were measured for the first crop in 2009 and the second in 2011. The anthocyanin content of the upper-order exceeded that of the lower-order, and this tendency was more remarkable in the second crop rather than the first. Moreover, the anthocyanin content in the stem exceeded that of the third leaf in the first crop (Fig. 3). The catechin content of the upper-order exceeded that of the lower-order, and likewise for the anthocyanin content, but terminal buds showed high content, which peaked in the second crop. The content in the second crop clearly exceeded that of the first crop (Fig. 4). The caffeine content, as with catechin, was dependent on the leaf-order, even though no clear difference emerged between the first and second crops (Fig. 5).

Japanese green tea is usually manufactured using a tea manufacturing machine, which incorporates some drying process. The influence of the drying process on the content of anthocyanin of the first crop in 'Sunrouge' was investigated in 2010. Consequently, anthocyanin

Table 5. Length of new shoots during each crop season

Cultivar	Length (cm)		
	First	Second	Third
Sunrouge	4.36±0.21	5.27±0.87	4.15±0.35 a
Cha Chuukanbohon Nou 6	4.22±0.53	4.72±0.52	5.98±0.86 b
Yabukita	5.44±0.91	5.80±0.48	5.76±0.56 b

The investigation was carried out in 2011.
An average of three replications (mean±SD).
Values followed by the same letters do not differ significantly (Tukey, P<0.05).
Sampling of new shoots was carried out by shear plucking.

Table 6. The leaf totals of a new shoot in each crop season

Cultivar	Number of leaves/shoot		
	First	Second	Third
Sunrouge	3.1±0.2 a	3.4±0.4 a	3.0±0.1 a
Cha Chuukanbohon Nou 6	2.7±0.1 b	2.7±0.1 b	3.4±0.1 b
Yabukita	3.1±0.2 b	3.3±0.1 b	3.5±0.1 b

The investigation was carried out in 2011.
An average of three replications (mean±SD).
Values followed by the same letters do not differ significantly (Tukey, P<0.05).
Sampling of new shoots was carried out by shear plucking.

Table 4. Yield in first, second and third plucking periods

Cultivar	Yield of plucked fresh leaves (kg/ha)			Amount
	First	Second	Third	
Sunrouge	1031±360	2170±791	1837±997 a	5038
Cha Chuukanbohon Nou 6	1063±485	2797±770	3333±545 a	7193
Yabukita	1210±208	2266±275	1489± 80 b	4965

The investigation was carried out in 2011.
The tea trees were 4 years old.
An average of three replications (mean±SD) was conducted.
Values followed by the same letters do not differ significantly (Tukey, P<0.05).
Harvesting was carried out by the plucking machine.

content in ‘Sunrouge’ was not changed by the drying process (Fig. 6).

The level of caffeine in tea leaves is known to have been reduced by hot water treatment[9]. We also investigated the influence of the hot water treatment on the content of anthocyanins, catechins and caffeine of ‘Sunrouge’ in the first crop in 2010. Consequently, anthocyanin and catechin in ‘Sunrouge’ were found to be unchanged, regard-

Table 7. Anthocyanin, catechin and caffeine contents in 2010 and 2011

Components	Cha Chuukanbohon Nou 6	Year	Content rate (%DB)		
			First	Second	Third
Anthocyanin	Sunrouge	2010	0.086±0.006 a	0.133 ±0.003 a	0.217±0.109 a
	Cha Chuukanbohon Nou 6		0.075±0.006 b	0.097 ±0.007 b	0.109±0.009 b
	Yabukita		0.009±0.001 c	0.010 ±0.002 c	0.016±0.003 c
	Sunrouge	2011	0.089±0.001 a	0.207 ±0.047 a	-
	Cha Chuukanbohon Nou 6		0.081±0.008 a	0.144 ±0.016 a	-
	Yabukita		0.000±0.000 b	0.002 ±0.001 b	-
Catechin	Sunrouge	2010	12.2±0.4 c	12.0 ±0.0 b	14.9±0.9 c
	Cha Chuukanbohon Nou 6		10.4±0.1 b	11.1 ±0.1 b	12.7±0.3 c
	Yabukita		13.7±0.3 a	17.5 ±0.8 a	18.5±0.8 a
	Sunrouge	2011	12.1±0.1 a	12.6 ±0.7 a	-
	Cha Chuukanbohon Nou 6		9.7±0.5 b	11.2 ±0.3 b	-
	Yabukita		14.9±0.2 c	18.6 ±0.4 c	-
Caffeine	Sunrouge	2010	3.02±0.05 a	2.40 ±0.07 ab	2.99±0.27
	Cha Chuukanbohon Nou 6		2.93±0.02 a	2.14 ±0.07 c	2.79±0.11
	Yabukita		2.50±0.07 b	2.25 ±0.07 bc	2.63±0.14
	Sunrouge	2011	3.39±0.04 a	2.74 ±0.08 a	-
	Cha Chuukanbohon Nou 6		3.49±0.11 a	2.49 ±0.15 a	-
	Yabukita		3.10±0.03 b	2.20 ±0.07 b	-

The investigation of anthocyanin, catechin and caffeine was carried out in 2010 and 2011.
The compound value was analyzed by high-performance liquid chromatography (HPLC)
An average of three replications (mean±SD).
Values followed by the same letters do not differ significantly (Tukey, $P<0.05$).
Sampling of new shoots was carried by the shear plucking.

Table 8. Influence of the period from plucking to parching to the content of anthocyanin, catechin and caffeine in ‘Sunrouge’

Treatment	content rate (%DB)		
	Anthocyanin	Catechin	Caffeine
Cont.	0.081±0.009	11.731±0.618	2.952±0.095
Left in the room	0.079±0.016	12.104±1.109	3.154±0.217
	n.s.	n.s.	*

* means differing significantly (Tukey, $P<0.05$).
Average of eight replications (mean±SD).
Sampling of the new shoots was carried out by the tea plucker.
The investigation of anthocyanin, catechin and caffeine was carried out on April 20, 2010.
The compound values were analyzed by high-performance liquid chromatography (HPLC) .
Treatment samples were left in the room for 14 hours after cooling for 8 hours in the refrigerator (7˚C).
Control samples were parched soon after plucking.

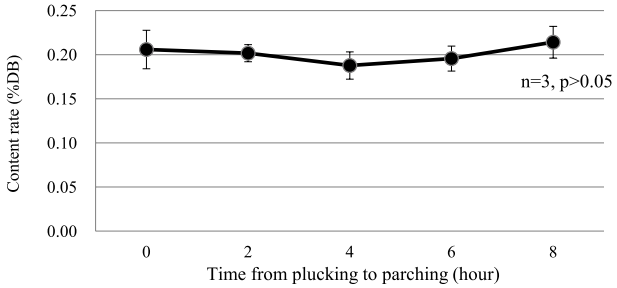

Fig. 2. Influence of the keeping time of fresh leaves on anthocyanin content in 'Sunrouge'
—●— : content rate of anthocyanin (%DB).

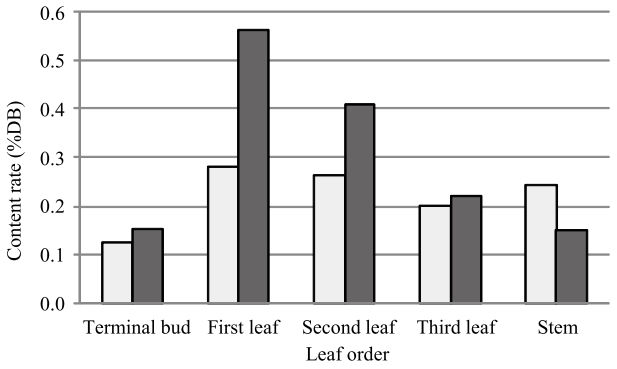

Fig. 3. Anthocyanin contnent according to the leaf order
□ : First crop in 2009, ■ : Second crop in 2011.

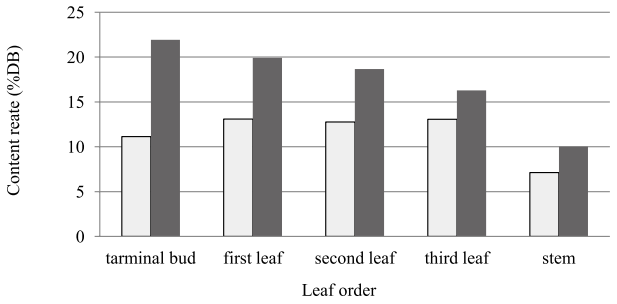

Fig. 4. Catechin contnent according to the leaf order in 'Sunrouge'
□ : First crop in 2009, ■ : Second crop in 2011.

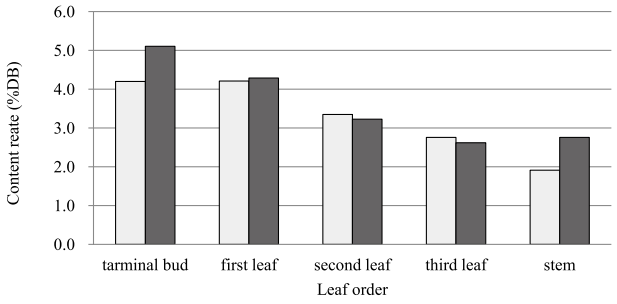

Fig. 5. Caffeine contnent according to the leaf order in 'Sunrouge'
□ : First crop in 2009, ■ : Second crop in 2011.

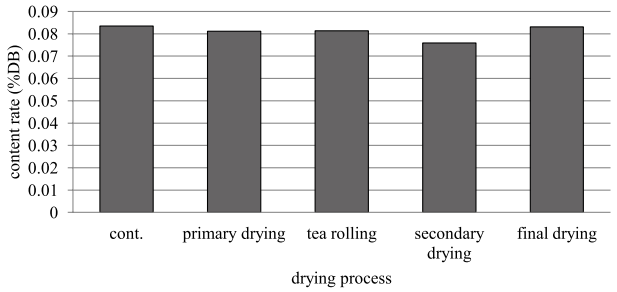

Fig. 6. Influence of the drying process on the anthocyanin content in 'Sunrouge'
A tea manufacturing machine of the 2kg type was used.
The control sample was dried after parching.

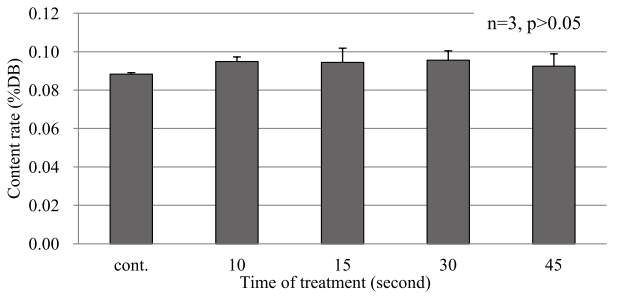

Fig. 7. Influence of hot water treatment on anthocyanin content in 'Sunrouge'
The control sample was dried after parching.

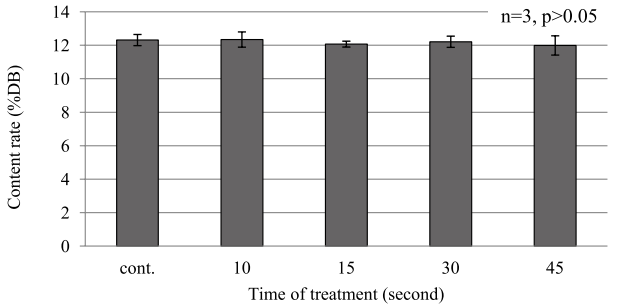

Fig. 8. Influence of hot water treatment on catechin content in 'Sunrouge'
The control sample was dried after parching.

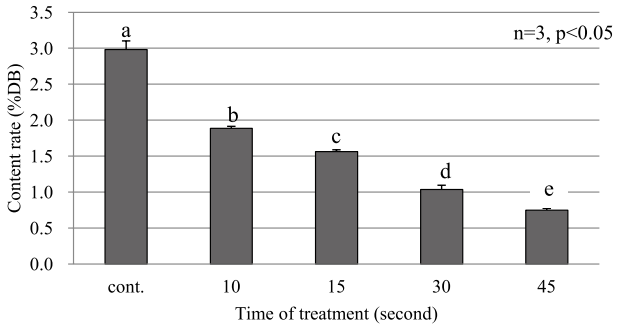

Fig. 9. Influence of hot water treatment on caffeine content in 'Saunrouge'
Values followed by different letters differ significantly.

less of treatment time, but the caffeine content declined with increasing treatment time (Figs. 7, 8, 9).

Discussion

We commenced the development of high anthocyanin tea in 1993[18]. Subsequently, 'Cha Chuukanbohon Nou 6' was bred as a high anthocyanin tea cultivar in 2004, and registered to protect the rights of the breeder based on the Seed and Seedlings Law in 2008. The anthocyanin content in 'Cha Chuukanbohon Nou 6' exceeded that of the native red leaf tea variety 'Benibana-cha', but 'Cha Chuukanbohon Nou 6' was not suitable for Japanese tea cultivation, because some of its characteristics resembled those of the mother parent *C.taliensis.* For example, the arboreal tree shape, large leaf size, strong apical dominance and scarcity of shoots. Therefore, we selected individuals resembling Japanese tea cultivars from open pollinated seedlings of 'Cha Chuukanbohon Nou 6' planted in the tea field and targeted a tea breeding cultivar with both anthocyanin and catechin.

We selected some individuals with high anthocyanin from the progeny of 'Cha Chuukanbohon Nou 6' or 'Benibana-cha'. However, the growth of these cuttings in the cutting bed was poor and the photoautotrophic culture method is known to be effective in plants having difficulty in cuttings[15]. Accordingly, we used rooted cuttings made using the photoautotrophic culture method for the clonal test.

'Sunrouge' was bred as a tea cultivar for use as a functional ingredient rather than a green tea drink. The color of the resulting tea was blackish, its taste bitter, and the color of the liquor blackish with a neutral pH and pinkish or reddish when in an acidic pH condition. When used as a pigment extraction material, the anthocyanin must be extracted under acidic conditions.

Caffeine is known as a tea plant ingredient with considerable functionality, including its ability to promote wakefulness and cardiotonic action. Conversely, low-caffeine tea is demanded by many sensitive to caffeine, including babies and sick people. No caffeine-free tea cultivar has yet been raised in Japan, although a study on the breeding of reduced-caffeine tea has been carried forward in the NARO institute of vegetable and tea science[19]. 'Sunrouge' contains caffeine equivalent to other tea cultivars. However, in a product used by so many people, the level of caffeine must be reduced, hence a low caffeine processing machine has already been developed[10]. The result of this study suggested that we could produce low-caffeine 'Sunrouge' using a low caffeine processing machine.

The anthocyanin content varied from about 0.08 to 0.22%DB with the plucking period (Table 7), and 0.15 to 0.56%DB with the leaf order in the second crop (Fig. 3). This result shows that the plucking period and method are key for the anthocyanin yield. It is necessary to develop a method to judge the optimum plucking time.

Tea plants may flourish for dozens of years after being planted in the field and almost all the work involved in cultivation and processing can be mechanized in Japan. Therefore, 'Sunrouge' is considered suitable as a crop using anthocyanins. In addition, 'Sunrouge' also contains catechins like other tea cultivars. The anthocyanins in 'Sunrouge' have already been isolated[20] and their functional activities are under investigation. We expect that 'Sunrouge' will make a contribution to human health.

We developed a parental line of high catechin tea entitled 'Cha Chuukanbohon Nou 3' in 1998[16]. Breeding a new cultivar with high anthocyanins, high catechins and low caffeine is our future objective.

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