

Capsaicinoid Content in Fruits of Parental Cultivars and Intervarietal Hybrids of *Capsicum annuum* L.

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Abstract

The capsaicinoid content of F₁ hybrids between *Capsicum annuum* L. 'California Wonder', four native Japanese cultivars, and three Korean F₁ cultivars were evaluated and the results compared with parental cultivars.

The capsaicinoid content was higher in the placenta than in fruit flesh. The placenta of 'Yatsufusa', 'Taka no Tsume', 'Cheongyang', 'Sinhong', and 'Geoseong' were the most pungent cultivars, with capsaicinoid content of more than 1000mg/100g DW, while 'California Wonder' and 'Fushimi Amanaga' contained trace amounts. Capsaicinoid content in F₁ hybrids varied with reciprocal crossing. Capsaicinoid content also varied with fruit development, rapidly increasing in the fourth or fifth week after flowering, and thereafter slowly declined.

Key words : *Capsicum annuum*, capsaicinoid content, intervarietal hybrids, HPLC, pungency

Introduction

The domesticated peppers are used around the world in various forms, such as dried powders, paprika, Tabasco sauce, pungent chili peppers, and the sweet bell peppers. An important genetic characteristic of peppers is pungency. The pungency is attributed to capsaicin, a vanillyl amide of isodexlyanic acid, contained in pepper fruit. The expression of capsaicin is hereditarily controlled by a single dominant allele¹⁾.

Accurate determination of capsaicinoid levels(indicator of pungency) is required not only for consumers who eat southwestern foods, but also for pharmaceuticals²⁾.

Currently, analysis of capsaicinoids is conducted by spectrophotometric^{3,4,5)}, gas chromatographic⁶⁾, and high-performance liquid chromatographic(HPLC) procedures^{7,8,9,10,11,12,13,14)}. Techniques using HPLC provide accurate

and efficient analysis of content and type of capsaicinoids present in a chile sample.

The objective of this study was to determine the amount of capsaicinoids in twelve F₁ hybrids obtained by crossing the bell pepper cultivar 'California Wonder' and four native Japanese cultivars, three Korean F₁ cultivars. Changes in capsaicinoid content during fruit development were also determined.

Materials and Methods

Plant material and growth conditions

Capsicum annuum cultivars 'California Wonder', 'Fushimi Amanaga', 'Shishitou', 'Yatsufusa', 'Taka no Tsume', twelve F₁ hybrids obtained by crossing among these cultivars in 1993, and three Korean F₁ cultivars, 'Cheongyang', 'Sinhong' and 'Geoseong' were used for

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the experiments.

Seeds were sown in polyester container (50.5cm long×36.0cm wide×10.0cm high) packed with vermiculite, and placed in a glasshouse at Okayama University, Japan on April 5, 1994. After germination, seedlings with 1 to 2 true leaves were transplanted into 9cm-diameter black vinyl pots containing a mixture of compost : sand (2 : 1, v/v) on April 26, 1994. Five seedlings per cultivar with 8 to 12 true leaves were planted in the field on May 29, 1994. The soil in the field had been fertilized with 'Sumika Ace' (18N-10P-14K) to provide N, P, and K at the rate of 240, 200, and 230 kg/ha, respectively. Plots were 8.0m long with 1.2m between beds, on a raised bed (75cm×20cm). Within row spacings was 40cm. Twenty plants per plot were planted in single rows. Plots of each cultivar were arranged in a completely randomized design with five replications.

Immature fruits (3~4 weeks after flowering) were harvested August, and red mature fruits (6~7 weeks after flowering) were harvested September in 1994.

The fruits used for analysis of capsaicinoid content during fruit development were two Japanese cultivars, 'Shishitou', 'Taka no Tsume', and two Korean F₁ cultivars, 'Cheongyang', 'Sinhong'. Flowers were tagged at anthesis in 1994. All fruits was harvested on September 14, 1994.

Apparatus

High-performance liquid chromatography (HPLC) (LC-10AD; Shimadzu Seisakusho, Kyoto, Japan), employing a CTO-10A column oven fitted with STR ODS-II column (4.6mm i. d., 5µm pore size; Shimadzu Techno-Research, Inc., Kyoto, Japan) was used for analysis of capsaicinoids. Peaks were detected using a SPD-10A UV-vis detector set at 280nm.

Reagents

HPLC-grade methanol and acetonitrile were used. Water was purified by Organo Puric pure water distillation

units (model-S; Organo Co., Ltd., Japan). All solvents were filtered and degassed.

Capsaicinoid analysis

Sliced pepper fruits were dried at room temperature, ground using a coffee mill (CM-602; Hitachi Co. Ltd., Japan), and stored at 25°C in a desiccator containing silica gel until use. Capsaicin (2.5mg) (Tokyokaseikogyo Co. Ltd., Tokyo, Japan) was diluted with 100ml of acetonitrile, and used for preparation of standard solutions. Capsaicinoid extraction was performed as follows: Dried powder of flesh and placenta was extracted with methanol in Erlenmeyer flask or glass sample bottle using Bransonic ultrasonic cleaner (Yamato 5210; Emerson Co. Ltd., Japan). The extracts were filtered through Advantec PTFE 0.20µm DISMIC-13_{HP} filter unit on a 3ml disposable syringe (Toyo Roshi Kaisha, Ltd., Japan) for refinement, and stored at 4°C until analyses.

For the determination of capsaicinoid concentration, an aliquot (25µl) was used for HPLC.

Results

Capsaicinoid content of the flesh and placenta of immature and red mature fruits

The HPLC chromatogram of capsaicinoids is shown in Fig. 1. Retention time for the 4 major constituents was 10.7min for nordihydrocapsaicin, 11.7min for capsaicin, 17.3min for dihydrocapsaicin, and 18.9min for n-vanillyl-n-decanamide.

The capsaicinoid content of immature and red mature fruits of each cultivar in 1994 is shown in Table 1. In immature pepper, the capsaicinoid content of the placenta of 'Taka no Tsume', 'Cheongyang', 'Sinhong' and 'Geoseong' were more than 3000mg per 100g dry weight. The placenta of 'Yatsufusa' contained more than 1000mg per 100g dry weight. 'California Wonder' and 'Fushimi Amanaga' contained trace amount of capsaicinoids. 'Shishitou' contained a small amount of capsaicinoids. The capsaicinoid content of F₁ hybrids

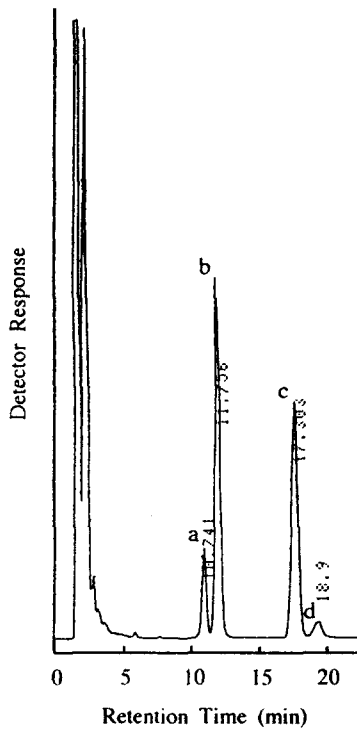


Fig. 1. HPLC chromatogram of capsaicinoids in *Capsicum annuum* cv. 'Taka no Tsume' showing baseline separation.

Illustrated chromatogram is the separation of (a) nordihydrocapsaicin, (b) capsaicin, (c) dihydrocapsaicin, and (d) n-vanillyl-n-decanamide.

were also little. The capsaicinoid content of the flesh was lower than that of the placenta.

In red mature fruits, the placenta of 'Yatsufusa', 'Cheongyang' and 'Sinhong' contained more than 3000 mg per 100g dry weight. 'California Wonder' and 'Fushimi Amanaga' contained trace amount of capsaicinoids, 'Shishitou' contained a little amount of capsaicinoids.

The capsaicinoid content of F₁ hybrids was more than that of parental cultivars. In F₁ hybrids, the placenta of 'Nos. 8, 9, 15, 16, and 17' contained more than 1000 mg of capsaicinoids per 100g dry weight.

In F₁ hybrids, the placenta of 'Nos. 8 and 9' contained

more than 1000mg of capsaicinoids per 100g dry weight. Among the Japanese cultivars, reciprocal crossing between 'California Wonder' and 'Yatsufusa', between 'California Wonder' and 'Taka no Tsume', and between 'Fushimi Amanaga' and 'Yatsufusa' resulted in increased amounts of capsaicinoids. Among the F₁ hybrids, crossing between 'California Wonder' and 'Taka no Tsume' gave the highest capsaicinoid content in red mature fruits (Table 1, Fig. 2).



Fig. 2. Fruit of the F₁ hybrid (middle) obtained by crossing between 'California Wonder' (lower, ♀) and 'Taka no Tsume' (upper, ♂).

Capsaicinoid content during fruit development

The capsaicinoid content of flesh and placenta varied with fruit development in 1994 (Table 2).

In 'Shishitou', the capsaicinoid content increased to 182mg per 100g dry weight 4 weeks after flowering, and thereafter decreased to reach 56mg per 100g dry weight by 7 weeks later. However, the capsaicinoid content increased to 105mg per 100g dry weight at 8 weeks later.

In 'Taka no Tsume', the capsaicinoid content increased up to 1069mg per 100g dry weight 4 weeks after flowering, then it gradually decreased.

In 'Cheongyang', on the other hand, the capsaicinoid content increased rapidly 2 weeks after flowering, after which, it remained relatively stable. The capsaicinoid content trends during fruit development of 'Sinhong' was

Table 1. Content of capsaicinoids in immature and red mature fruits from August to September, 1994

Group	No. Cultivar	Content of capsaicinoids (mg/100g DW) ²									
		Immature fruit					Red mature fruit				
		NDHC	C	DHC	VD	Total	NDHC	C	DHC	VD	Total
Bell pepper	1. California Wonder	- ^y	-	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-	-
Japanese cultivar	2. Fushimi Amanaga	-	-	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	1	2	-	3
	3. Shishitou	1 ^x	5	8	1	15	6	17	13	1	37
		13 ^w	49	86	8	156	95	378	369	12	854
	4. Yatsufusa	4	32	38	4	78	11	40	42	3	96
		79	847	835	58	1819	431	1682	1654	99	3866
5. Taka no Tsume	5	66	73	2	146	14	54	51	4	123	
	169	1921	2010	56	4156	224	931	799	61	2015	
F ₁ hybrid	♀ ♂	-	-	-	-	-	-	-	-	-	-
	6. 1×2	-	6	7	-	13	1	5	5	-	11
	7. 1×3	-	2	2	-	4	-	-	-	-	-
	8. 1×4	4	42	51	4	101	5	25	25	2	57
		-	5	5	-	10	2	16	13	1	32
	9. 1×5	19	194	214	16	443	49	621	466	23	1159
		-	1	-	-	1	1	23	15	1	40
	10. 2×1	4	64	54	7	129	97	1535	1011	51	2694
		-	2	1	-	3	-	-	-	-	-
	11. 2×3	-	-	-	-	-	-	1	1	-	2
		1	2	1	-	4	1	2	2	-	5
	12. 2×4	-	1	1	-	2	25	50	64	7	146
		1	1	1	-	3	3	10	9	1	23
	13. 3×1	10	11	18	5	44	45	151	135	14	345
		-	1	1	-	2	-	3	3	-	6
	14. 3×2	8	38	45	2	93	35	219	248	11	513
		2	2	3	1	8	-	2	2	-	4
15. 4×1	7	8	15	2	32	12	27	31	3	73	
	2	8	8	1	19	1	14	9	1	25	
16. 4×2	72	319	333	28	752	90	1329	946	38	2403	
	2	4	4	-	10	5	14	16	2	37	
17. 5×1	53	116	122	12	303	176	432	504	59	1171	
	1	3	2	-	6	1	10	7	1	19	
22	127	115	7	271	64	584	485	25	1158		
	10	101	47	2	160	6	58	29	1	94	
Korean F ₁ cultivar	18. Cheongyang	250	2265	1151	83	3749	230	2315	1098	31	3674
		11	145	63	4	223	11	186	68	4	269
19. Sinhong	321	3792	1683	114	5910	293	5203	1834	102	7432	
	8	30	49	5	92	2	14	20	1	37	
20. Geoseong	277	1019	1810	164	3270	100	627	851	12	1590	

²NDHC, C, DHC and VD mean nordihydrocapsaicin, capsaicin, dihydrocapsaicin and n-vanillyl-n-decanamide or isomer of dihydrocapsaicin, respectively.

^yTrace amount.

^xUpper row shows content in fruit flesh.

^wLower row shows content in placenta.

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Table 2. Weekly changes in capsaicinoid content of fruits, 1994

Weeks after flowering	Content of capsaicinoids(mg/100g DW) ^{a,y}					Total
	NDHC	C	DHC	VD		
			'Shishitou'			
1	1	1	— ^x	—	2	
2	1	2	1	—	4	
3	9	24	16	1	50	
4	20	85	73	4	182	
5	20	65	55	3	143	
6	9	28	28	2	67	
7	9	18	27	2	56	
8	13	38	49	5	105	
			'Taka no Tsume'			
1	2	5	—	—	7	
2	26	63	35	11	135	
3	61	395	239	27	722	
4	67	623	347	32	1069	
5	82	473	359	46	960	
6	54	500	312	28	894	
7	65	558	467	29	1119	
8	41	281	248	20	590	
			'Cheongyang'			
1	—	—	—	—	—	
2	33	357	118	14	522	
3	26	371	139	9	545	
4	24	409	170	5	608	
5	17	281	117	4	419	
6	26	317	164	6	513	
7	19	275	137	4	435	
8	15	185	96	3	299	
			'Sinhong'			
1	1	1	1	—	3	
2	28	106	40	11	185	
3	75	635	243	26	979	
4	41	567	200	17	825	
5	27	446	155	12	640	
6	16	286	99	7	408	
7	34	330	179	11	554	
8	23	380	171	10	584	

^aContent of capsaicinoids in fruit flesh and placenta.^yNDHC, C, DHC and VD mean nordihydrocapsaicin, capsaicin, dihydrocapsaicin and n-vanillyl-n-decanamide or isomer of dihydrocapsaicin, respectively.^xTrace amount.

similar to that of 'Cheongyang'.

Discussion

The pungency of peppers is attributed to capsaicinoids, which are produced by secretory cells of the interocular septum of the pepper fruit^{15,16}. Content of capsaicinoids in placenta was 10~130 times higher than that in flesh. Capsaicinoids of peppers constituted 4 main compounds present, in most cultivars and F₁ hybrids. Capsaicin constituted the highest concentration, followed by dihydrocapsaicin, nordihydrocapsaicin and n-vanillyl-n-decanamide, in that order. 'Yatsufusa', 'Taka no Tsume', 'Cheongyang', 'Sinhong', and 'Geoseong' were the most pungent cultivars, with capsaicinoid content of more than 1000mg/100g DW, while 'California Wonder' and 'Fushimi Amanaga' contained trace amounts. 'Shishitou' contained a small amount of capsaicinoids. Capsaicinoid content in F₁ hybrids varied with reciprocal crossing.

Heiser¹⁾ reported that pungency is hereditarily controlled by a single dominant allele. Therefore, there is variation in pungency. The capsaicinoid content of F₁ hybrids were much lower than that of the pungent parents and the progenies from intraspecific crosses between 'California Wonder' and pungent varieties of *C. annuum*. Capsaicinoids were not detected in cases of crosses between 'California Wonder' and sweet varieties¹⁷⁾. Capsaicin content has also been found to increase rapidly until 4 or 5 weeks after flowering, and then decrease gradually, followed by a moderate increase at the fully ripened stage¹⁸⁾.

Intervarietal F₁ hybrids obtained from crosses between the bell pepper 'California Wonder' and 'Fushimi Amanaga' contained a small amount of capsaicinoids. There was difference in the amount of capsaicinoids of intervariatal F₁ hybrids between sweet and pungent cultivars. In the case of using 'California Wonder' as female parent, F₁ hybrids contained high amount of capsaicinoids, and in the opposite case of using 'California Wo-

nder' as male parent, they contained low amount. On the other hand, F₁ hybrids between 'Fushimi Amanaga' and 'Yatsufusa' showed the reverse trend.

Generally, the capsaicinoid content of Korean F₁ cultivars were higher than that of intervariatal F₁ hybrids. Changes in the capsaicinoid content of pepper fruit during development was investigated with four cultivars in 1994. The capsaicinoid content increased rapidly until fourth or fifth week after flowering, thereafter slowly declined. Previous reports showed an inverse correlation between the fresh weight and the capsaicin content of fruit in both the parental cultivars and the F₁ hybrids¹⁸⁾. In the present study, however, an inverse correlation was not found between the fresh weight and the capsaicin content of fruit in both the parental cultivars and the F₁ hybrids.

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초록 : 고추의 친품종 및 품종간 잡종의 과실에 있어서 캡사이시노이드 함량

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고수량·고품질의 고추 신품종을 육성할 목적으로 피만의 'California Wonder'와 일본재래종 4품종, 이들의 F₁잡종 12조합 그리고 한국의 F₁ 3품종을 공시하고, 캡사이시노이드 함량을 조사했다.

그 결과, 캡사이시노이드 함량은 과피보다 태좌에 많이 함유되어 있었다. 태좌의 캡사이시노이드 함량에 있어서 'Yatsufusa', 'Taka no Tsume', '청양', '신흥' 그리고 '거성'은 건물중 100g당 1000mg이상 아주 매운 품종이었다. 그러나, 'California Wonder' 그리고 'Fushimi Amanaga'는 거의 함유되어 있지 않았다. F₁ 잡종에 있어서 캡사이시노이드 함량은 정역교잡간에 큰 차이가 있었다. 또한, 과실의 발육에 따라 캡사이시노이드 함량도 다양해, 개화 4~5주후까지는 급격히 증가하고, 그 후는 감소하는 경향이 있었다.