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Chromosomal locations of genes related to flavonoid and anthocyanin production in leaf sheath of shallot (*Allium cepa* L. Aggregatum group)

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High-performance liquid chromatography (HPLC) analyses using a series of alien monosomic addition lines (AMALs) of Japanese bunching onion (*Allium fistulosum* L.) having extra chromosomes from shallot (*A. cepa* L. Aggregatum group) were performed to determine the chromosomal locations of the genes for flavonoid and anthocyanin production in leaf sheaths of *A. cepa* Aggregatum group. In HPLC profiles both at 360 and 520 nm, several peaks were observed in *A. cepa* Aggregatum group and AMAL with chromosome 5A from *A. cepa* Aggregatum group but no peak was observed in *A. fistulosum* and other AMALs. Four of the compounds observed at 360 nm were identified as known flavonoids, i.e., apigenin, kaempferol, quercetin, and rutin. Five out of the total 18 compounds at 520 nm were identified as known anthocyanins, i.e., cyanidin-3-glucoside, cyanidin-3-laminariobioside, peonidin-3-glucoside, cyanidin-3-malonylglucoside, and cyanidin-3-malonyllaminariobioside. These results reveal that a group of the genes related to the flavonoid and anthocyanin production in the leaf sheath of *A. cepa* Aggregatum group are located on the chromosome 5A.

INTRODUCTION

A series of alien monosomic addition lines (AMALs) of Japanese bunching onion (*Allium fistulosum* L., genomes FF, $2n = 2X = 16$) having extra chromosomes (1A – 8A) from shallot (*A. cepa* L. Aggregatum group, AA, $2n = 2X = 16$) was established in our previous study (Shigyo et al., 1996). The chromosomal locations of 10 isozyme and one 5S rRNA genes were determined by isozyme and rDNA analyses using the series (Shigyo et al., 1994; Shigyo et al., 1995a; Shigyo et al., 1995b; Shigyo et al., 1996). These studies demonstrated that the series was useful to determine the chromosomal locations of genes and chromosome mapping of *A. cepa* Aggregatum group.

The results of a study on morphological characteristics in the series revealed that all the three plants of the AMAL with chromosome 5A from *A. cepa* Aggregatum group (FF + 5A) showed reddish-yellow leaf sheaths (Shigyo et al., unpublished data). It seems that the genes related to the pigment production are obviously located on the chromosome 5A. The pigments were presumed to be flavonoids and anthocyanins, similarly produced in the skin of the bulbs of common onion (*A. cepa* L. Common onion group) (Fenwick and Hanley, 1990).

In the present study, the pigments produced in the leaf sheath of the AMAL FF + 5A were analyzed in detail using high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

A series of the AMALs of *A. fistulosum* possessing the extra chromosomes from *A. cepa* Aggregatum group (FF + 1A – FF + 8A) was used in the present study. Two plants were selected in each type of AMALs. *A. fistulosum* and *A. cepa* Aggregatum group were also used as controls.

The skin of the basal part of leaf sheath was collected from each plant material. The pieces of skin were lyophilized and cut, and 15–20 mg of them were extracted at room temperature with 0.5 ml of methnol for 24 h for flavonoid and with 1 ml of TMW (1% trifluoroacetic acid in 80% methnol) for 48 h for anthocyanin. After filtrating with a millipore filter (0.45 μm), the extract was injected into HPLC (2–5 μl for flavonoids, 20 μl for anthocyanins).

HPLC apparatus were SHIMADZU LC-6A system with UV spectro-photo meter (SPD-6AV) for flavonoid analysis and HITACHI L-6200 pumping system with UV spectro-

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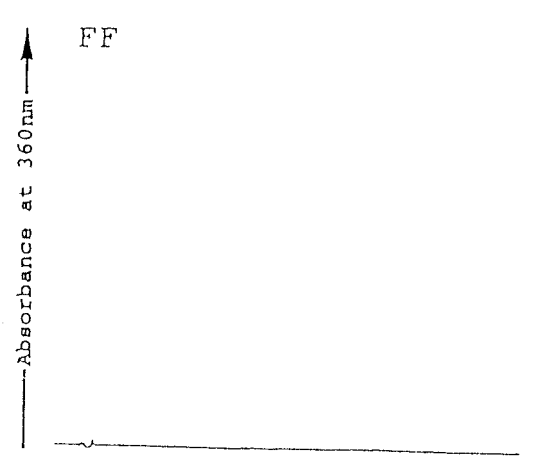
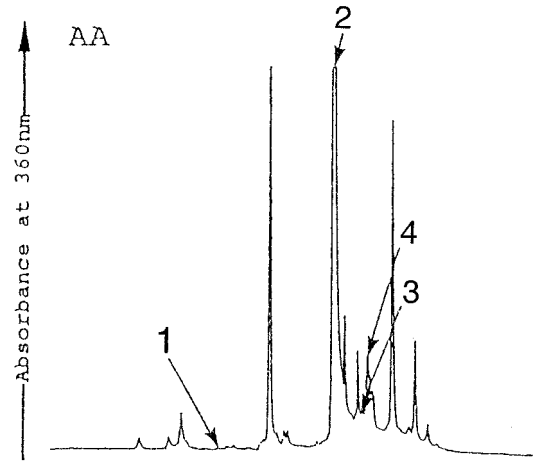




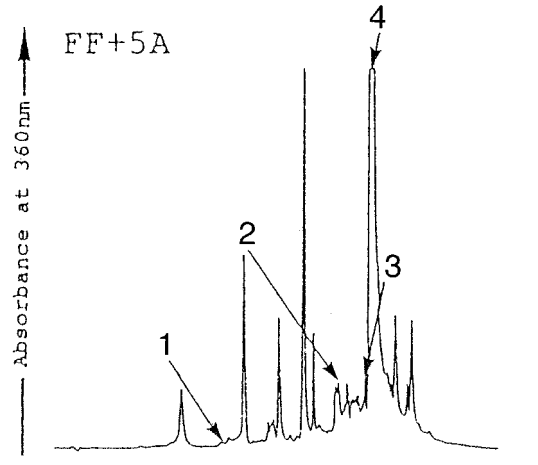
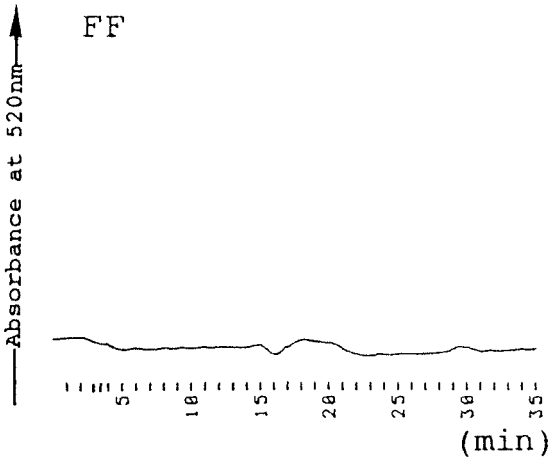
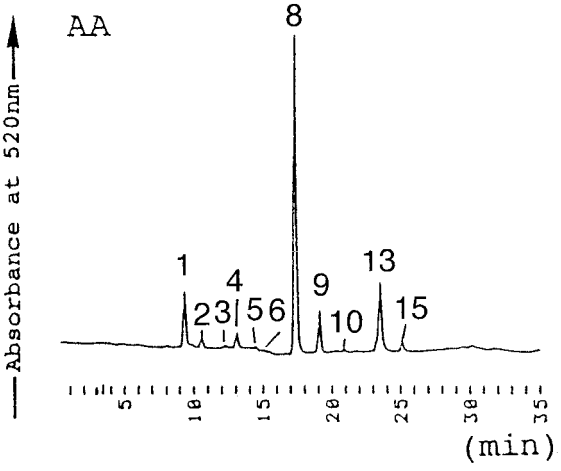


Fig. 1. HPLC profiles of methanol extracts of *A. fistulosum*, *A. cepa* Aggregatum group, and alien monosomic addition line FF + 5A (detection : 360 nm). 1, rutin (Rt = 14.9); 2, quercetin (Rt = 23.0); 3, apigenin (Rt = 25.0); 4, kaempferol (Rt = 25.4).





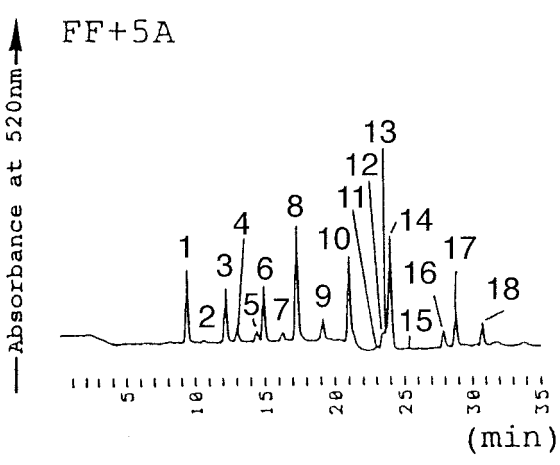


Fig. 2. HPLC profiles of TMW extracts of *A. fistulosum*, *A. cepa* Aggregatum group, and alien monosomic addition line FF + 5A (detection: 520 nm). 1, cyanidin-3-glucoside (Cy3-Glc); 2, cyanidin-3-laminariobioside (Cy3-Lam); 5, peonidin-3-glucoside (Pn3-Glc); 8, cyanidin-3-malonylglucoside(Cy3-MaGlc); 9, cyanidin-3-malonyllaminariobioside (Cy3-MaLam). Other peaks, 3, 4, 6, 7, 10, 11, 12, 13, 14, 15, 16, 17, and 18 were not identified.

photometer (SHIMADZU S-2000) for anthocyanin analysis. Analyses of flavonoids were carried out using a CAPCELL PAK C18 column (4.6 mm × 250 mm) with 1 mM tetrabutylammonium (adjusted to pH 2.9 with acetic acid) – acetonitrile (9 : 1 → 1 : 4, in 32 min) as the mobile phase, and monitored by the absorbance at 360 nm. The flow rate was 0.6 ml/min throughout the analysis. The column temperature was 40°C. The HPLC conditions for anthocyanin analyses were as follows: column, Inertsil ODS-2 column (4.6 mm × 250 mm); mobile phase, 1.5% phosphate –1.5% phosphate, 20% acetic acid and 25% acetonitrile (3 : 1 → 2 : 3, in 35 min); flow rate, 1 ml/min; column temp., 35°C; detect, 520 nm.

The peaks obtained in the detection at 360 nm and 520 nm were respectively identified by comparisons of the retention time with that of standard compounds.

RESULTS AND DISCUSSION

In HPLC profile detected at 360 nm, a large number of peaks attributable to flavonoids were observed in *A. cepa* Aggregatum group but no peak was observed in *A. fistulosum* (Fig. 1). Although a large number of peaks were also observed in two plants of AMAL FF + 5A, no peak was observed in any of other AMALs (FF + 1A, FF + 2A, FF + 3A, FF + 4A, FF + 6A, FF + 7A, and FF + 8A). Four of the compounds observed at 360 nm were identified as known flavonoids, i.e., apigenin, kaempferol, quercetin, and rutin. These flavonoids were detected in all plants of *A. cepa* Aggregatum group and the AMAL FF + 5A (Table 1). A fairly high content of kaempferol appeared in the AMAL FF + 5A but a low one in *A. cepa* Aggregatum group. Conversely, FF + 5A had a low content of quercetin but *A. cepa* Aggregatum group had a high one. The results revealed that the genes for the production of kaempferol were located on the chromosome 5A. Further, they also indicate that the kaempferol-3'-hydroxylase gene acting in the

Table 1. Flavonoid contents in *A. fistulosum*, *A. cepa* Aggregatum group, and alien monosomic addition lines (AMALs)

Flavoniod	HPLC peak area / 1 mg-sample (μv. sec)					
	FF 1, 2	AA 1	AA 2	FF + 5A 1	FF + 5A 2	Other AMALs
Apigenin	–	3.5	20.7	13.3	16.8	–
Kaempferol	–	9.3	47.0	644.3	312.9	–
Quercetin	–	432.9	458.9	18.4	26.7	–
Rutin	–	0.4	–	0.9	0.4	–

–, Not detectable.

Table 2. Anthocyanin contents in *A. fistulosum*, *A. cepa* Aggregatum group, and alien monosomic addition lines (AMALs)

Peak number	HPLC peak area / 1 mg-sample (μv. sec)						Retention time(min)	Anthocyanin[a]
	FF 1, 2	AA 1	AA 2	FF + 5A 1	FF + 5A 2	other AMALs		
1	–	321	482	112	30	–	9.3	Cy 3-Glc
2	–	59	68	tr	tr	–	10.5	Cy 3-Lam
3	–	14	19	80	20	–	12.2	
4	–	90	121	25	16	–	13.1	
5	–	18	17	17	14	–	14.4	Pn 3-Glc
6	–	tr	23	83	35	–	14.9	
7	–	–	–	17	tr	–	16.3	
8	–	1625	1965	175	69	–	17.3	Cy 3-MaGlc
9	–	227	192	75	12	–	19.1	Cy 3-MaLam
10	–	12	21	177	52	–	20.9	
11	–	–	–	tr	tr	–	22.7	
12	–	–	–	sh	39	–	22.9	
13	–	449	625	sh	–	–	23.0	
14	–	–	–	232	105	–	23.7	
15	–	55	50	tr	–	–	25.0	
16	–	–	–	29	tr	–	27.6	
17	–	–	–	66	24	–	28.5	
18	–	–	–	46	62	–	30.0	

–, Not detectable.
tr, Trace.
sh, Shoulder.
[a], See legend in Fig. 2.

synthesis of quercetin is not on the chromosome 5A. A double monosomic alien chromosome addition lines of *A. fistulosum* with the chromosome 5A and with a chromosome other than 5A (FF + 5A + nA) will be useful to determine the chromosomal locations of the gene. There are several unidentified peaks in *A. cepa* Aggregatum group and in the AMAL FF + 5A. Identification of the compounds corresponding to the peaks will provide information necessary for determining the chromosomal locations of genes for the enzymes acting in the flavonoid biosynthetic pathway. It is also important to examine the effects of genes from *A. fistulosum* on the pigment production in the AMALs used in this study.

In the detections at 520 nm, there was a tendency similar to those at 360nm; a large number of peaks were observed in *A. cepa* Aggregatum group and the AMAL FF + 5A (Fig. 2) but no peak was observed in *A. fistulosum* and any of other AMALs (FF + 1A, FF + 2A, FF + 3A, FF + 4A, FF + 6A, FF + 7A, and FF + 8A). The AMAL FF + 5A had larger number of peaks than *A. cepa* Aggregatum group. The compounds peak nos. 7, 11, 12, 14, 16, 17, and 18 were detected only in the AMAL FF + 5A (Table 2). The peak nos. 1, 2, 5, 8, and 9 were identified as five different anthocyanins (Fig. 2). These results revealed that the genes for the anthocyanin production were also located on the chromosome 5A. Seven peaks were observed in the AMAL FF + 5A but not in *A. cepa* Aggregatum group, and several of which have not yet been characterized. If the structural determinations of the unknown compounds are completed, the chromosomal locations of genes for the enzymes in anthocyanin biosynthetic pathway will be determined.

From the results mentioned above, it is concluded that a group of the genes related to both the flavonoid and anthocyanin production in the leaf sheath of *A. cepa* Aggregatum group are located on the chromosome 5A. A concentration of the genes related to both pigments production on one chromosome is a matter of great interest to trace the chromosomal evolution in *Allium* and is convenient to manipulate the genes. A large number of structural genes coding enzymes and regulatory genes seems to be involved in the pigment biosynthetic pathways. It will be possible to determine the chromosomal localization of these genes by means of the methods of molecular genetics in future.

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