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ORIGINAL PAPER

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Direct comparison between genomic constitution and flavonoid contents in *Allium* multiple alien addition lines reveals chromosomal locations of genes related to biosynthesis from dihydrokaempferol to quercetin glucosides in scaly leaf of shallot (*Allium cepa* L.)

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Abstract The extrachromosome 5A of shallot (*Allium cepa* L., genomes AA) has an important role in flavonoid biosynthesis in the scaly leaf of *Allium fistulosum*–shallot monosomic addition lines (FF + nA). This study deals with the production and biochemical characterisation of *A. fistulosum*–shallot multiple alien addition lines carrying at least 5A to determine the chromosomal locations of genes for quercetin formation. The multiple alien additions were selected from the crossing between allotriploid FFA (♀) and *A. fistulosum* (♂). The 113 plants obtained from this cross were analysed by a chromosome 5A-specific PGI isozyme marker of shallot. Thirty plants were preliminarily selected for an alien addition carrying 5A. The chromosome numbers of the 30 plants varied from 18 to 23. The other extrachromosomes in 19 plants were completely identified by using seven other chromosome markers of shallot. High-performance liquid chromatography analyses of the 19 multiple additions were conducted to identify the flavonoid compounds produced in the scaly leaves. Direct comparisons between the chromosomal constitution and the flavonoid contents of the multiple alien additions revealed that a flavonoid 3′-hydroxylase (F3′H) gene for the synthesis of quercetin from kaempferol was located on 7A and that an anonymous gene involved in the glucosidation of quercetin was on 3A or 4A. As a result of supplemental SCAR analyses by using genomic DNAs from two complete sets of *A. fistulosum*–shallot monosomic additions, we have assigned F3′H to 7A and flavonol synthase to 4A.

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Introduction

Onion (*Allium cepa* L. Common onion group) contains a large number of flavonoids in coloured leaf sheaths (scales) of the edible part (Chu et al. 2000; Bahorun et al. 2004). Major flavonoids in onion are quercetin-4′-glucoside and 3,4′-diglucoside (Brandwein 1965), and these two quercetin glucosides account for more than 85% of all the flavonoids in onion grown in Japan (Tsushida and Suzuki 1995). Traces of flavonoids in onion are known as kaempferol and isorhamnetin derivatives (Bilyk et al. 1984; Tsushida and Suzuki 1995). Among the dietary flavonoids, quercetin has been extensively studied (Constantinou et al. 1995; Lee et al. 1998; Aligiannis et al. 2001). Similarly, there has been increased attention on the health benefits of kaempferol, which inhibits the cell proliferation of estrogen receptor-positive breast cancer cells (Hung 2004). Francis et al. (2004) reported that, among 13 flavonoids such as quercetin glucosides and chalcone, kaempferol from Easter lily (*Lilium longiflorum*) showed the highest inhibition of cyclooxygenase-1 and -2 enzymes, which convert arachidonic acid into the prostaglandins that are responsible for inflammation. Given the importance of flavonoids for human health, and the fact that onions are rich in these compounds, it is of importance to find ways to develop an efficient way for breeding onion cultivars that accumulate flavonoids to a larger extent than was hitherto possible. The present study tries to contribute to this challenge.

A complete set of Japanese bunching onion (*Allium fistulosum* L.)–shallot (*A. cepa* L. Aggregatum group) monosomic addition lines has been established (Shigyo et al. 1996). Pigmentation in leaf sheaths was observed only in a monosomic addition carrying chromosome 5A of shallot (FF + 5A) (Shigyo et al. 1997b). Analysis by high-performance liquid chromatography (HPLC) using the skins of leaf sheaths in a monosomic addition set revealed that anonymous genes for flavonoid and anthocyanin production are located on 5A (Shigyo et al. 1997a). We suggested in this context that an essential transcription factor for the flavonoid biosynthesis in onions is located on chromosome 5A. The FF + 5A is not sufficient to analyse the process of quercetin formation, as a reversal of the component ratio of the contents of two different flavonoids (quercetin and kaempferol) between FF + 5A and shallot has revealed that enzyme genes related to the synthesis of quercetin from kaempferol are located on other chromosomes. In other species, multiple alien addition lines have been used with monosomic additions as complementary materials for determining the chromosomal location of the S-specific glycoprotein locus in *Brassica nigra* (Chevre et al. 1997) and for facilitating the genetic linkage mapping of the *Gossypium* G genome (Brubaker and Brown 2003).

In the present study, we applied *A. fistulosum*–shallot multiple alien additions ($2n = 18$–23) to chromosomal assignments of the enzyme genes involved in the flavonoid biosynthesis of shallot.

Materials and methods

Production of multiple alien additions carrying chromosome 5A

Multiple alien addition lines of *A. fistulosum* ($2n = 2x = 16$, genomes FF) with extrachromosomes of shallot ($2n = 2x = 16$, AA) were obtained from the BC_2 of their amphidiploids with *A. fistulosum* as a pollinator. The parentage of the 30 BC_2 plants mainly used in this study were shown in Table 1. The percentage, suggested by Shigyo et al. (1996), of ovules that developed into seeds (PODS) was applied to evaluate the seed-setting characteristics of allotriploids between these two species ($2n = 3x = 24$, AFF). A chromosome 5A-specific isozyme marker, *Pgi-1*$^{A\text{-}1}$ (Shigyo et al. 1995b), was employed to select alien additions carrying this chromosome in the BC_2 population. Somatic chromosomes of root tips in the BC_2 plants were observed by Fulgen nuclear staining followed by the squash method.

Identification of extrachromosomes with the help of genetic markers

Chromosome-specific genetic markers of the three isozymes (2A, *Got-1*^{A}; 6A, *Got-2*^{A}; and 8A, *Gdh-1*^{A}) reported in Shigyo et al. (1996) and the one 5S ribosomal DNA (Ac5SL for 7A) described in Shibata and Hizume (2002), in addition to three newly developed microsatellites (1A, AMS16-261; 3A, AMS23-218; and 4A, AMS20-372), were available for the identification of seven other kinds of extrachromosomes in alien additions. For DNA analyses, total genomic DNA was isolated from fresh leaf tissue using a miniprep DNA-isolation method (van Heusden et al. 2000a). The PCR products of the 5S rDNA and microsatellites were electrophoresed through 5% denaturing polyacrylamide gels according to the methods of Martin et al. (2005). The isozyme analyses of the three enzyme systems were conducted according to the procedures of Shigyo et al. (1994, 1995a). Ribosomal DNA analysis was done according to the procedure of Shigyo et al. (1996) with the 5S rDNA primer set of Hizume (1993). The 30 pairs of microsatellite primers developed by Fischer and Bachmann (2000) were applied to determine their chromosomal locations by using two complete sets of *A. fistulosum*–shallot monosomic additions (Masuzaki et al. 2006). Consequently, the three microsatellite markers, AMS16-261, AMS23-218, and AMS20-372, designated according to the primer name and the molecular size of each band in base pairs, have been assigned, respectively, to chromosomes 1A, 3A, and 4A. The three primer pairs used were based on the GenBank™ database (Accession numbers, AJ391696 and AJ391697 for

Table 1 Parentage of BC_2 plants mainly used in this study

Seed parent *A. fistulosum* 'Kujyo-hoso'	Pollinator shallot 'Myanmar'	Amphdiploid	Pollinator *A. fistulosum* 'Kujyo-hoso'	BC_1 population	Pollinator *A. fistulosum* 'Kujyo-hoso'	BC_2 population selected by PGI isozyme analysis
S-3 ×	17-2 →	B40 ×	4-2 →	40-9 ×	4-2 →	$S_2$9, U140
	↑ colchicine			40-14 ×	4-2 →	U71, U72, U73, U75, U77, U81, U82, U87, U88
				40-18 ×	4-2 →	U53, U63
				40-19 ×	4-2 →	U13
				40-20 ×	4-2 →	$S_1$20, U138
				40-22 ×	4-2 →	U118, U120, U121
				40-25 ×	4-2 →	U7
				40-27 ×	4-2 →	$S_2$27, $S_5$27, $S_6$27
				40-29 ×	4-2 →	$S_2$29, $S_3$29, $S_4$29, U83, U107, U114, U159

AMS16; AJ391704 and AJ391705 for AMS20; AJ391710 and AJ391711 for AMS23). Moreover, karyotype analyses of somatic chromosomes in the alien additions were conducted to confirm their genomic constitution in an *A. fistulosum* diploid background.

Evaluation of phenotypic expressions in multiple alien additions

The skin and the outer scale of the basal part of the leaf sheath were collected from the multiple additions, along with the monosomic addition of *A. fistulosum* carrying shallot chromosome 5A (FF + 5A) and shallot, used as controls. The quercetin contents in the sheaths decrease from the dry skin to the inner rings (Bilyk et al. 1984; Patil and Pike 1995), so the skin and the outer scale were used. The pieces of skin and scale were lyophilised and cut, and 5 g was extracted with hot 74% ethanol (final concentration 70% ethanol) for 15 min. The extract was filtered through #2 filter paper (ADVANTEC, Tokyo, Japan) and then through a 0.5 μm filter (Katayama Chemical, Osaka, Japan) to remove pigments. Flavonoids in the filtrate were analysed by HPLC. An aliquot of filtrate (20 μl) was injected into the HPLC fitted with a LiChrospher C18 column (Merck), 4×250 mm. The column was eluted with a linear gradient of 40–70% methanol over 60 min and then maintained in an isocratic state for an additional 10 min; the flow rate was 0.6 ml per min. The absorbance, which was monitored at 370 nm, allowed kaempferol, quercetin, quercetin-4′-glucoside, and quercetin-3,4′-diglucoside to be distinguished by their retention times and the absorption spectra according to standards.

Sequence-characterised amplified region (SCAR) analyses for candidate genes related to flavonoid biosynthesis

Sequence-characterised amplified region analysis was performed using two complete sets of *A. fistulosum*–shallot monosomic additions (Shigyo et al. 1996) to assign a segment of the coding region for flavonoid biosynthesis to chromosomes of shallot. The isolation of total genomic DNA was conducted by the procedure described above. Outside- and inside-primer sets to amplify the objective were designed with the software GENETYX® 6.1.3 (Genetyx, Tokyo, Japan) based on the GenBank™ accession number of AY541035 (a full-length cDNA sequence of flavonoid-3′-hydroxylase, F3′H) and AY647262 (a full-length genomic sequence of flavonol synthase, FLS). The primer sets were as follows. F3′H: outside-forward 5′-CAA AGA AGC ACG AAG GCA-3′, outside-reverse 5′-AAA CAC CTC CCA AAG CAT AC-3′, inside-forward 5′-CGT CAA CGA AAC AAC CGA TAC-3′, and inside-reverse 5′-GGA ACG TCT CCT TCA CTA TGG-3′. FLS: outside-forward 5′-GGG GGC ATA CAT CCT AAA TA-3′, outside-reverse 5′-TCA TAC CCA AAC AGC CTT-3′, inside-forward 5′-TTA AGG ACG ACC ACT GGT T-3′, and inside-reverse 5′-CCA CGA CAT CCG TGA CT-3′. The first and second PCRs were both run at 94°C for 3 min, followed by 25 cycles of 94°C for 1 min, 65°C (FLS) or 67°C (F3′H) for 1 min, and 72°C for 1 min, with a final extension at 72°C for 7 min on a Program Thermal Cycler iCycler™ (Bio-Rad, Hercules, CA, USA). The ramp times were carried out in the default conditions that adjusted temperatures at the maximum ramp rate with the minimum ramp time. All PCR amplifications were done with template DNA (about 100 ng of genomic DNA for the first PCR and 5 μl of the first PCR products for the nested PCR), 1 μM for each of the primers, 0.25 mM dNTPs, 1× Ex *Taq* buffer, and 0.5U Ex *Taq* polymerase (Takara, Shiga, Japan) in a volume of 25 μl. The second PCR products were separated on 2.0% agarose gel. If the PCR products were monomorphic between *A. fistulosum* and shallot, amplicons were separated on 5% denaturing polyacrylamide gels according to the procedure of Martin et al. (2005). To demonstrate the reliability of PCR amplification, three different chromosome-specific DNA fragments of shallot were excised from the gel with a razor blade and the DNA extracted using the Quantum Prep™ Freeze 'N Squeeze DNA Gel Extraction Spin Columns kit according to the manufacturer's instructions (Bio-Rad). Ligation of the PCR products was carried out for 2 h at 16°C with the Ligation High (Toyobo, Osaka, Japan) and pGEM®-T Easy Vector System I kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. The ligation reactions were transformed with DH5α competent cells (Toyobo), and plasmid DNA was extracted using the GenElute™ Plasmid Miniprep kit according to the manufacturer's instructions (Sigma, Saint Louis, MO, USA). The DNA sequencing was performed using BigDye Terminator Cycle Sequencing ver. 3.1 (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions with an ABI 310 DNA sequencer (Applied Biosystems). Homology searches were performed in the GenBank™ database using the BLAST program.

Results

Selection and identification of multiple alien additions

After crossing with *A. fistulosum*, the allotriploid showed a very low seed set [PODS = (2,093/55,080)×100 = 3.8%], and the germination rate of the seeds obtained was also low [(272/2,093)×100 = 13.0%]. However, the survival rate of the germinated seeds was relatively high [(113/272)×100 = 41.5%], and 113 BC_2 plants were obtained. Thirty out of the 113 BC_2 plants showed a positive genotype $Pgi\text{-}1^{A\text{-}1}/Pgi\text{-}1^{F\text{-}1}/Pgi\text{-}1^{F\text{-}1}$ (af^1f^1) or another positive genotype $Pgi\text{-}1^{A\text{-}1}/Pgi\text{-}1^{F\text{-}1}/Pgi\text{-}1^{F\text{-}2}$ (af^1f^2) (Fig. 1). These 30 plants were, therefore, presumed to possess chromosome 5A of shallot. The chromosome

numbers of these 30 plants varied from 18 to 23 ($2n = 18$, 3 plants; 19, 2; 20, 6; 21, 12; 22, 2; 23, 5).

From the results of more isozyme analyses on three shallot alleles, *Got-1*^{A}, *Got-2*^{A}, and *Gdh-1*^{A}, out of the 30 BC_2 aneuploid plants, 20 had *Got-1*^{A}, 21 had *Got-2* ^{A}, and 14 had *Gdh-1*^{A} (Table 2), so these plants were presumed to possess the chromosome of the shallot on which each of these three enzymes was located. In the amplification profile of 5S rDNAs, all the samples showed a certain band, Ac5SS, common to *A. fistulosum* and shallot. Ac5SL of a chromosome-7A-specific rDNA band was observed in 14 BC_2 plants (Fig. 2a). These two 5S rDNA bands were coined by Shibata and Hizume (2002). The amplification profiles for AMS16 indicated that 28 BC_2 plants had a microsatellite marker, AMS16-261 (Fig. 2b). The other two microsatellite markers (AMS23-218, AMS20-372) were also observed in 16 and 8 BC_2 plants, respectively (Fig. 2c, d).

To determine the chromosomal arrangements in the primarily selected 30 BC_2 plants, segregation data on eight chromosome markers of shallot were summarised (Table 2). In 19 of the 30 plants investigated, the number of positive genetic markers corresponds well to the chromosome number. However, the remaining 11 plants showed several patterns of conflict between the positive marker and the chromosome numbers. Through karyotype analyses based on the results of the molecular markers, it was determined that all extrachromosomes were derived from shallot in the 19 integral BC_2 plants (Fig. 3, Table 2).

Chromosomal assignments of genes involved in flavonoid biosynthesis by HPLC analysis

In the HPLC profile, several peaks attributable to flavonoids were observed in both shallot and the monosomic addition FF + 5A (Fig. 4a–d). Four of the compounds observed at 370 nm were identified as known flavonoids, i.e., kaempferol (K), quercetin aglycone (Q), quercetin-4′-*O*-glucoside (4′-Qmg), and quercetin-3,4′-*O*-diglucoside (3,4′-Qdg). In shallot, a fairly high content of Q and a low content of 4′-Qmg appeared in the skin, but high contents of 4′-Qmg and 3,4′-Qdg appeared in the outer scale. This result indicates that genes related to the glucosidation of flavonoids in shallot are predominantly expressed in the outer scale. In FF + 5A, a fairly high content of K and a low content of Q appeared in the skin as well as in the outer scale. The 19 multiple additions that the genomic constitution was identified were used for HPLC analysis (Fig. 4e–h). A simple comparison of the two main flavonoid contents in both the skin and outer scale allowed us to divide the 19 multiple additions into two initial groups (Fig. 5). Group I has a higher content of K than Q, and Group II has a higher content of Q than K. In comparing the genomic constitution between the two groups, chromosome 7A was found only in Group II. There has been no report that Q is directly converted from K in *A. cepa*. After F3′H converts dihydrokaempferol into dihydroquercetin, FLS changes dihydroquercetin into Q (Fig. 6). Furthermore, an F3′H gene acting in the synthesis might be located on chromosome 7A. The chromosomal location of FLS could not be determined by these HPLC results. Group II was further divided into two subgroups by different component ratios between Q and quercetin glycosides in the outer scale. The subgroup II-b showed excess amounts of quercetin glycosides. Two kinds of chromosomes, 3A and 4A, were found only in this subgroup. Consequently, some of the genes related to the glucosidation of Q might be located on chromosome 3A or 4A.

Chromosomal assignment of candidate genes for flavonoid biosynthesis

The primer sets for F3′H amplified a single PCR product with the template DNA of shallot but not that with *A. fistulosum* (Fig. 7a). The amplicon of F3′H was larger than expected. An amplicon the same size as shallot was

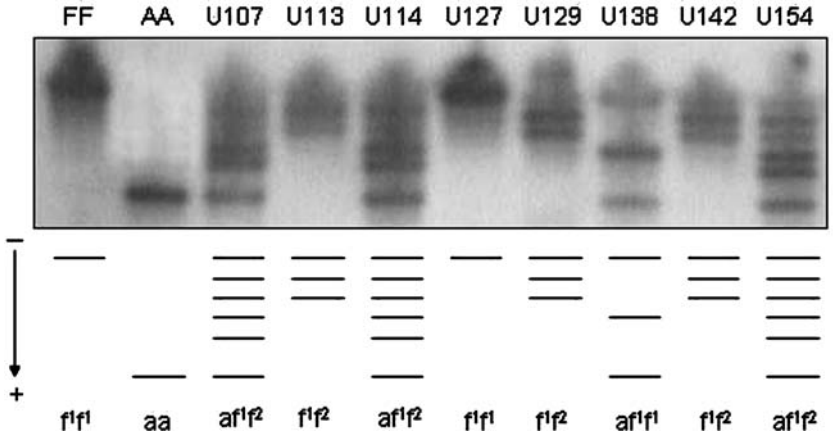

Fig. 1 Phosphoglucoisomerase zymograms of *A. fistulosum* (FF), shallot (AA) and BC_2 generations. Schematic illustration is shown in the bottom half of the figure. aa and f^1f^1 indicate homozygous genotypes. f^1f^2, af^1f^1 and af^1f^2 show heterozygous genotypes. f^2f^2 stands for the parent used for the original cross. f^1f^1 stands for recurrent parent (data not shown)

Table 2 Segregation of chromosome-specific molecular markers of shallot in BC_2 plants with chromosome 5A

Plant number	Chromosome number (2*n*)	Chromosome-specific molecular marker of shallot						
		AMS16-261	*Got-1*^{A}	AMS23-218	AMS20-372	*Got-2*^{A}	Ac5SL	*Gdh-1*^{A}
		1A	2A	3A	4A	6A	7A	8A
$S_2$27	23	■	■	■	■	□	■	■
U82	23	■	■	□	■	■	■	■
U120	23	■	■	■	■	■	□	■
U121	23	■	■	■	□	■	■	■
U138	23	■	□	■	■	■	■	■
$S_1$20	22	■	■	□	□	■	■	■
U88	22	■	■	■	□	■	■	□
$S_5$27	21	■	■	□	□	■	■	□
$S_6$27	21	■	■	□	□	■	■	□
U7[a]	21	■	□	■	□	■	■	■
U53	21	■	■	■	□	□	□	■
U63[a]	21	■	□	□	□	■	□	■
U71[a]	21	■	■	■	□	■	■	□
U72[a]	21	■	■	■	□	■	□	■
U77[a]	21	■	■	□	□	■	□	□
U81[a]	21	□	■	■	■	■	□	■
U87	21	■	□	■	□	□	■	■
U107	21	■	■	□	□	■	■	□
U114	21	■	■	□	■	■	□	□
$S_2$9	20	■	■	□	□	□	□	■
$S_2$29[a]	20	■	■	■	□	■	■	□
U13	20	■	■	□	□	■	□	□
U73[a]	20	■	■	■	□	■	□	□
U75[a]	20	■	□	■	■	■	□	□
U140[a]	20	■	□	■	■	□	■	□
U83	19	■	□	□	□	■	□	□
U159	19	■	■	□	□	□	□	□
$S_3$29	18	■	□	□	□	□	□	□
$S_4$29	18	■	□	□	□	□	□	□
U118[a]	18	□	□	■	□	□	□	■

[a] The footnotes indicate aneuploids showing a conflict between positive marker and chromosome numbers

■ : presence □ : absence

observed only in the monosomic addition FF + 7A. The amplicons of shallot and FF + 7A were cloned and sequenced. The BLASTn of the sequences showed 99% homology with the original sequence (AY541035). These results revealed that the F3′H gene was located on chromosome 7A. An alignment between AY541035 and

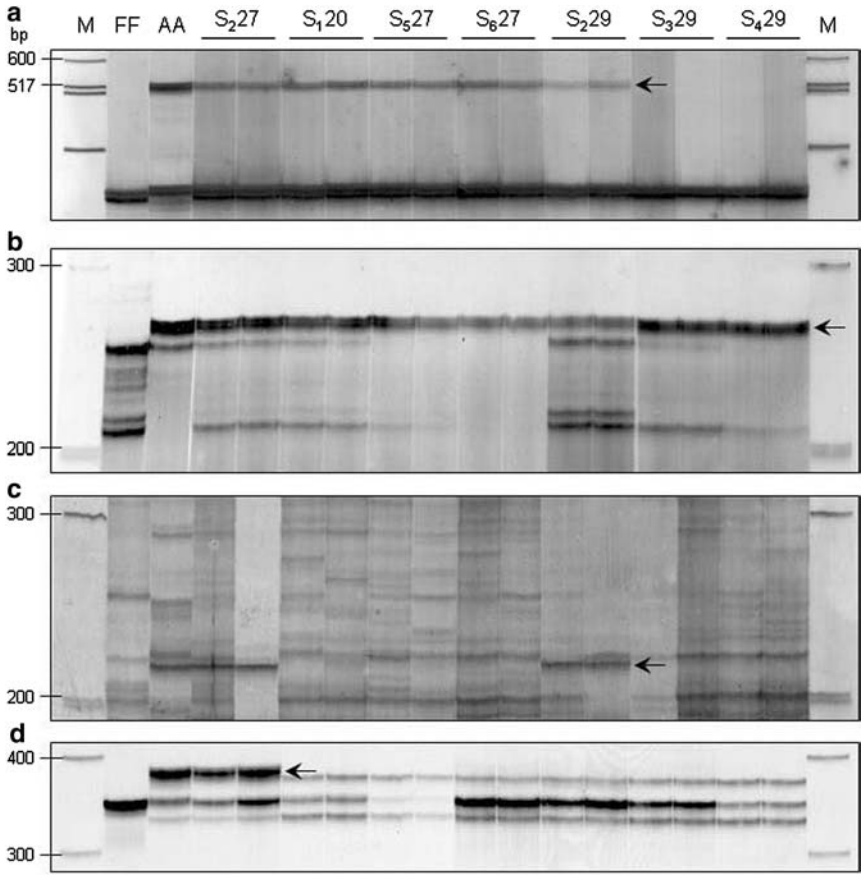

Fig. 2 Amplification profiles for 5S rDNA and microsatellites in *A. fistulosum* (FF), shallot (AA) and BC_2 plants selected by PGI isozyme analysis. *M* molecular size marker (100 bp DNA ladder). The two lanes per test plant represent two repetitions for verifying reproducibility. Arrows indicate chromosome specific markers. **a** Ac5SL for 7A. **b** AMS16-261 for 1A. **c** AMS23-218 for 3A. **d** AMS20-372 for 4A

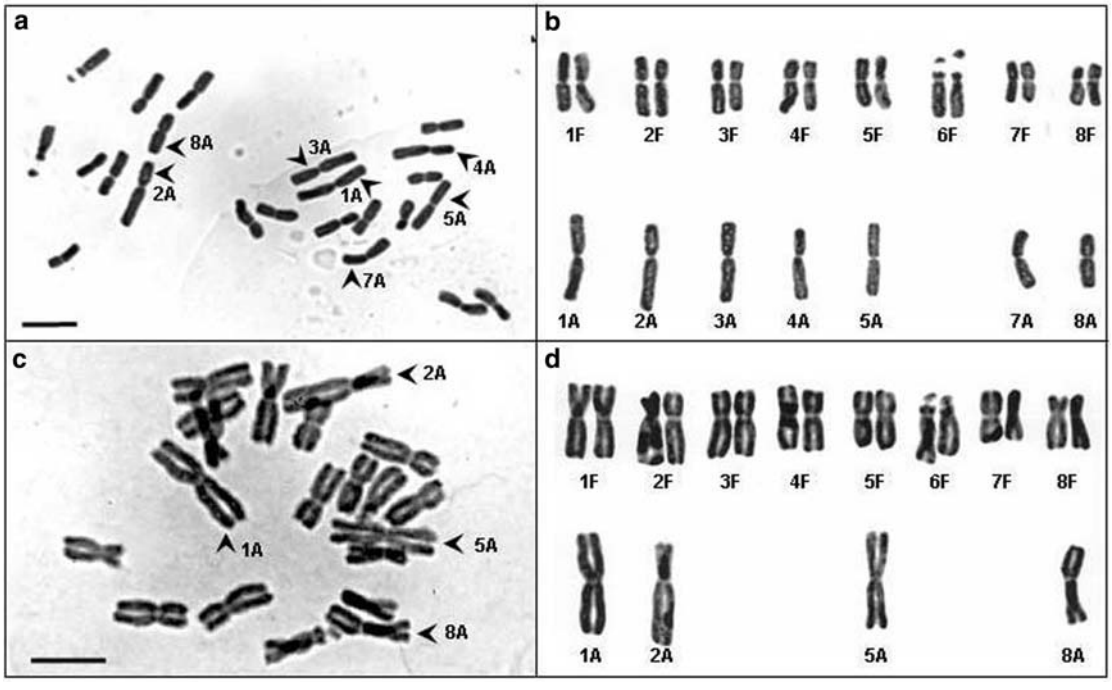

Fig. 3 Somatic metaphase chromosomes of multiple alien addition lines [**a-b** $S_2$27 ($2n = 23$), **c-d** $S_2$9 ($2n = 20$)]. **a**, **c** *Arrowheads* point to the extrachromosomes. *Scale bar* = 10 μm. **b**, **d** Eight pairs of *A. fistulosum* chromosomes (1F–8F), showing similar sizes and shapes, and solitary chromosomes of shallot (1A–8A) are distinguished

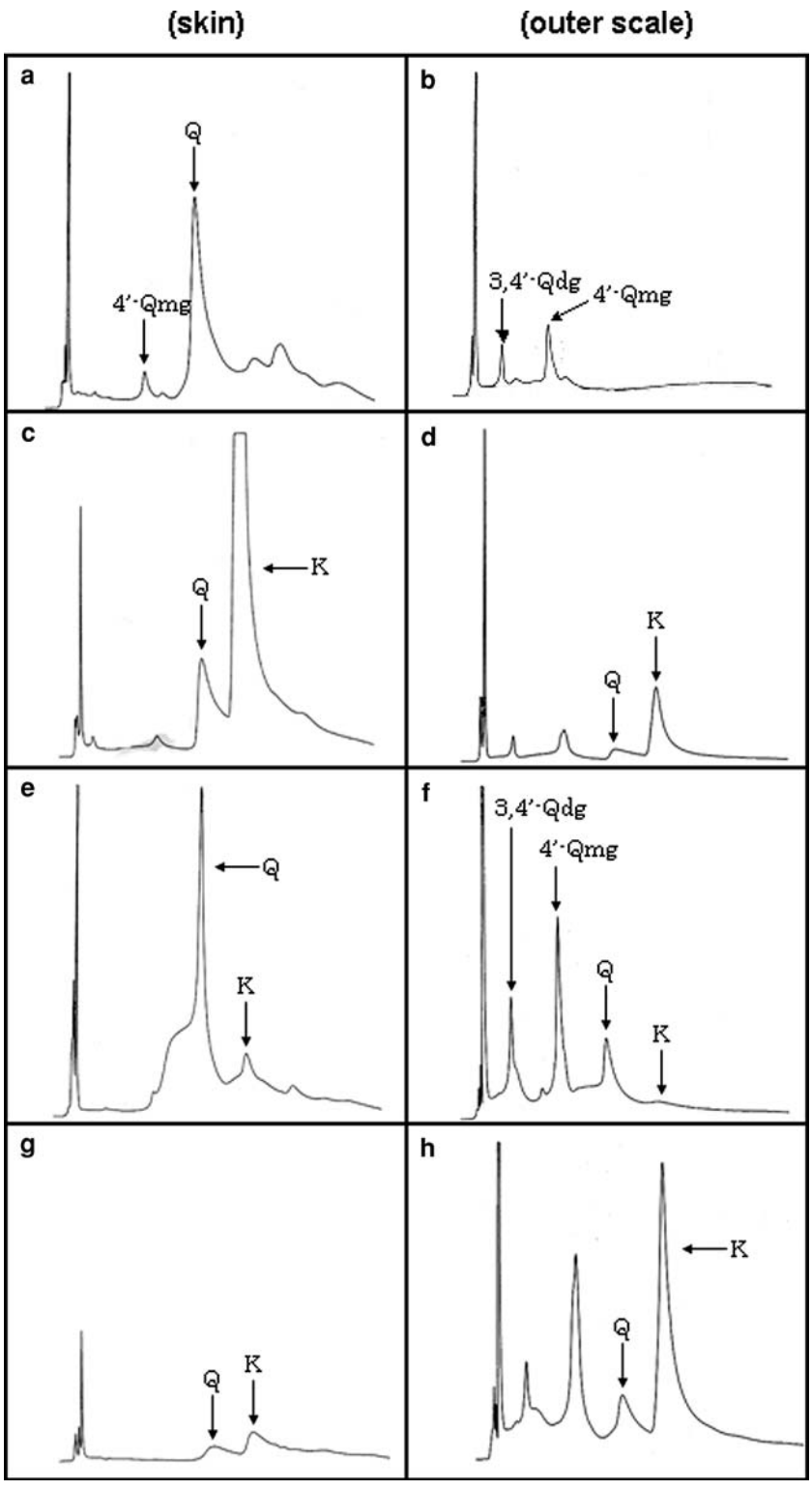

Fig. 4 High-performance liquid chromatography profiles of ethanol extracts (detection: 370 nm). **a–b** Shallot. **c–d** A monosomic addition FF + 5A. **e–f** U88 [multiple alien addition ($2n = 22$) carrying chromosome 7A]. **g–h** U159 [multiple alien addition ($2n = 19$) deleting chromosome 7A]. *Q* quercetin, *4′*-Qmg quercetin-*4′-O*-glucoside, *3,4′-Qdg* quercetin-3,*4′-O*-diglucoside, *K* kaempferol

our sequence data indicated that 63-bp indel existed as an intron (Fig. 8). The amplicons that were larger than the expected size resulted from this indel. In addition, at least three SNPs were found between onion and shallot. This sequence has been deposited with GenBank (Accession number, DQ160213).

The primer sets of FLS amplified the single fragment of the expected size with the DNA of shallot. The same primer sets produced another single fragment in *A. fistulosum*. After the separation of their amplicons via 5% denaturing PAGE, an interspecific polymorphism was detected as a slight

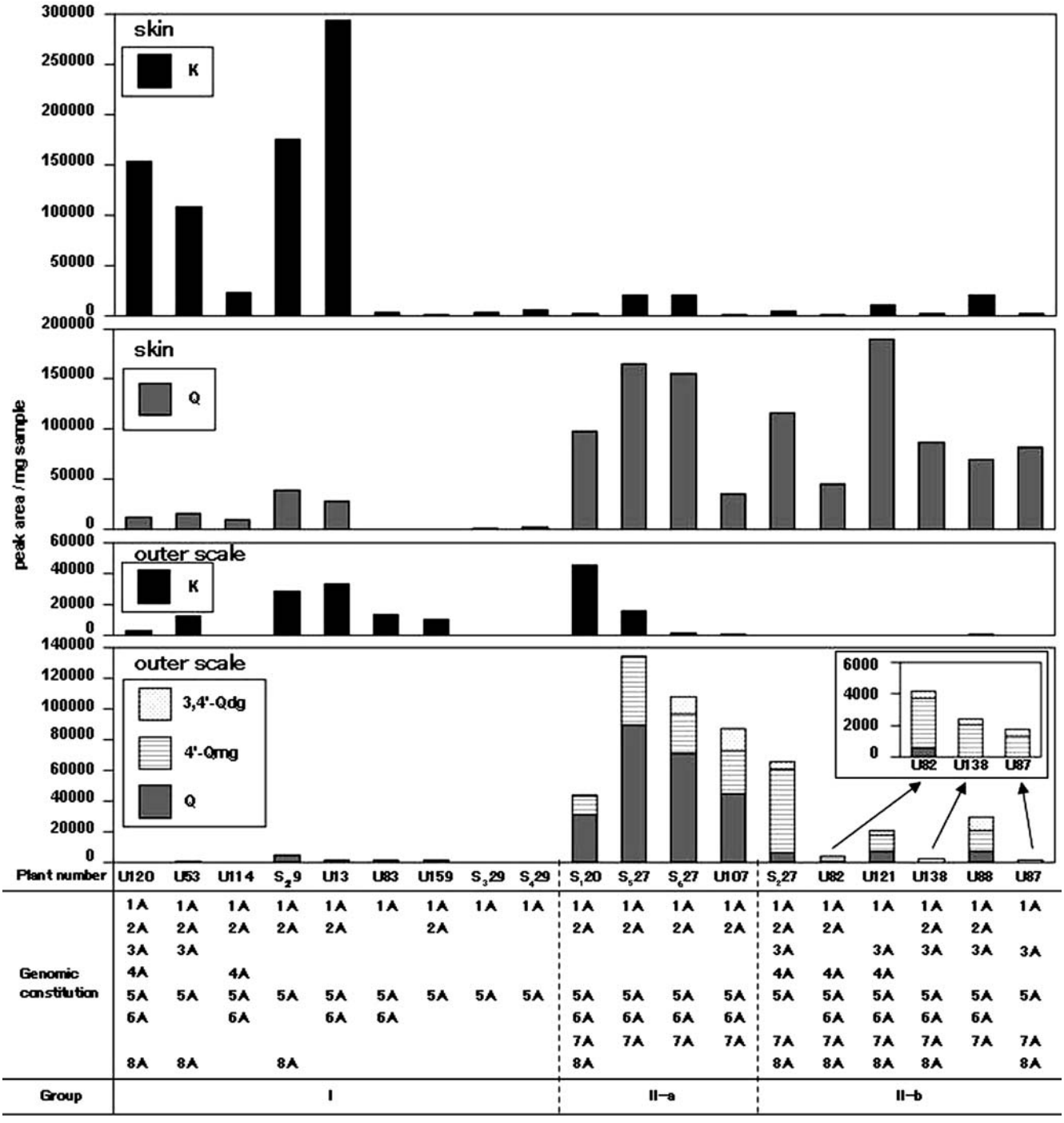

Fig. 5 Flavonoid contents in skin and outer scale of multiple alien addition lines

size difference between the single fragments. In the two complete sets of monosomic additions, the amplicon derived from shallot was present only in FF + 4A (Fig. 7b). BLASTn of the sequence data from the amplicon of shallot had a homology of 99% to the original sequence (AY647262). These results revealed that the FLS gene of shallot is located on chromosome 4A.

Discussion

Inheritance of bulb colour is very complex, and previous genetic studies have revealed that five major genes (*I*, *C*, *G*, *L*, and *R*) are involved in the pigmentation (Reiman 1931; Clarke et al. 1944; Davis and El-Shafie 1967). *I* and *C* are the genes concerned

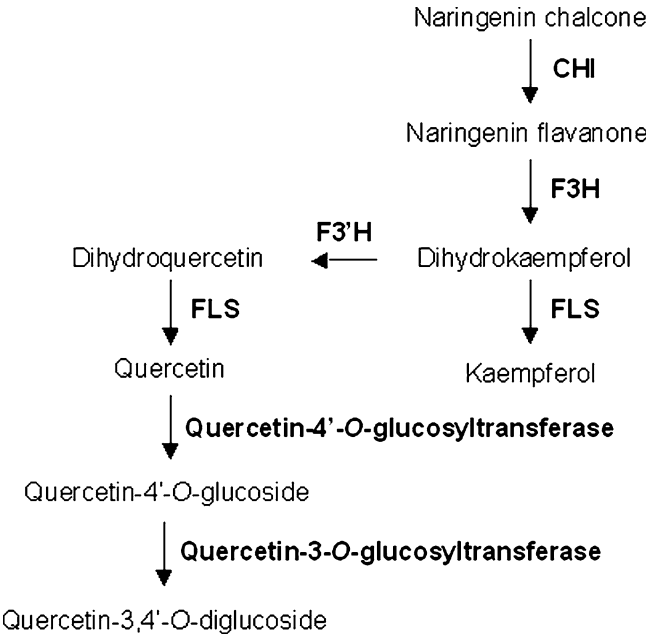

Fig. 6 Flavonoid biosynthesis pathway in *A. cepa*. *CHI* chalcone isomerase, *F3H* flavanone 3-hydroxylase, *F3′H* flavonoid 3′-hydroxylase, *FLS* flavonol synthase

with the expression of the pigmentation. The reduced transcription of chalcone synthase (CHS) was observed only in white onions among onions with various colours (Kim et al. 2004b, 2005) and *C* of the basic colour factor seems to be a regulatory gene controlling CHS gene transcription (Kim et al. 2005). *I* is presumed to be a regulatory gene because *I* inhibits pigment formation regardless of the other dominant genes (Kim et al. 2004a). FLS and F3′H seemed not to be on chromosome 5A. There is a possibility that the regulatory gene or CHS is located on 5A.

In the present study, we found that FLS and F3′H are located on chromosome 4A and 7A, respectively. Furthermore, we showed that genes for glucosidation of quercetin are located on chromosome 3A or 4A. Previously, we suggested that it is likely that an essential transcription factor or the CHS gene is located on chromosome 5A (Shigyo et al. 1997a). Although the FLS gene related to the conversion of flavanonol (dihydrokaempferol and dihydroquercetin) to flavonol (kaempferol and quercetin) was located on 4A, a fairly high content of kaempferol was produced in the skin and outer scale of monosomic addition FF + 5A. In such a case, the regulatory gene derived from shallot seems to stimulate some structural genes from CHS to FLS, excluding F3′H of *A. fistulosum*. Molecular analyses to determine the chromosomal locations of the epistatic genes derived from shallot are needed. The enzymes involved in the glucosidation of Q were reported by Tsushida and Suzuki (1996). Quercetin-4′-*O*-glucosyltransferase catalyses the synthesis of 4′-Qmg from Q, while quercetin-3-*O*-glucosyltranferase is related to the synthesis of 3,4′-Qdg from 4′-Qmg as well as that of quercetin-3-*O*-glucoside from Q. Kramer et al. (2003) also reported that one (*UGT73G1*; Accession No., AY62062) out of the four flavonoid glucosyltransferase genes was isolated from yellow onion glucosylated aglycones or monoglucosides of various flavonoids, including quercetin. Several isozymes for the glucosidation of flavonoids seem to catalyse the same substrates. In the future, we will determine the chromosomal locations of the remaining genes involved in the flavonoid biosynthesis pathway and allocate the flavonoid genes to the two different onion genetic maps thus far developed (King et al. 1998; van Heusden et al. 2000b).

As mentioned in the review by Kik (2002), many researchers have reported that the genomes of *A. cepa* and *A. fistulosum* can exchange genetic material. Peffley and Mangum (1990) reported that chromosome 8F of *A. fistulosum* substituted for 1C (a homologous chromosome of 1A) of *A. cepa* in the backcross progeny of allotriploid to an *A. cepa* diploid and that recombination occurred between chromosome 6 of *A. cepa* and *A. fistulosum* in the same progeny. The 11 BC_2 plants showing disagreements between cytogenetics and molecular data perhaps resulted from the chromosomal substitution and recombination. However, this is yet to be confirmed by GISH analysis.

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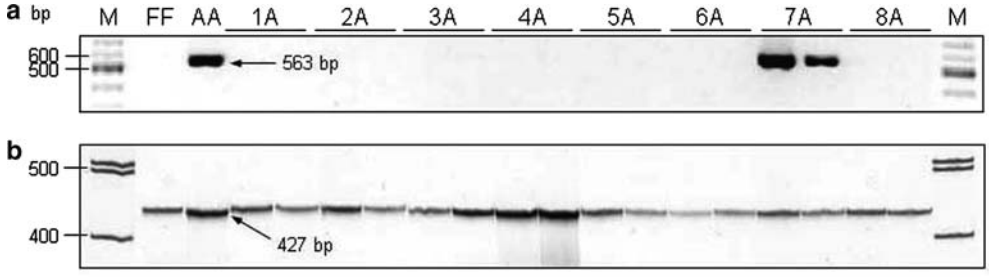

Fig. 7 Amplification profiles for flavonoid 3′-hydroxylase (**a**) and flavonol synthase (**b**) in *A. fistulosum* (FF), shallot (AA) and two complete sets of *A. fistulosum*–shallot monosomic addition lines (1A–8A). *M* molecular size marker (100 bp DNA ladder)

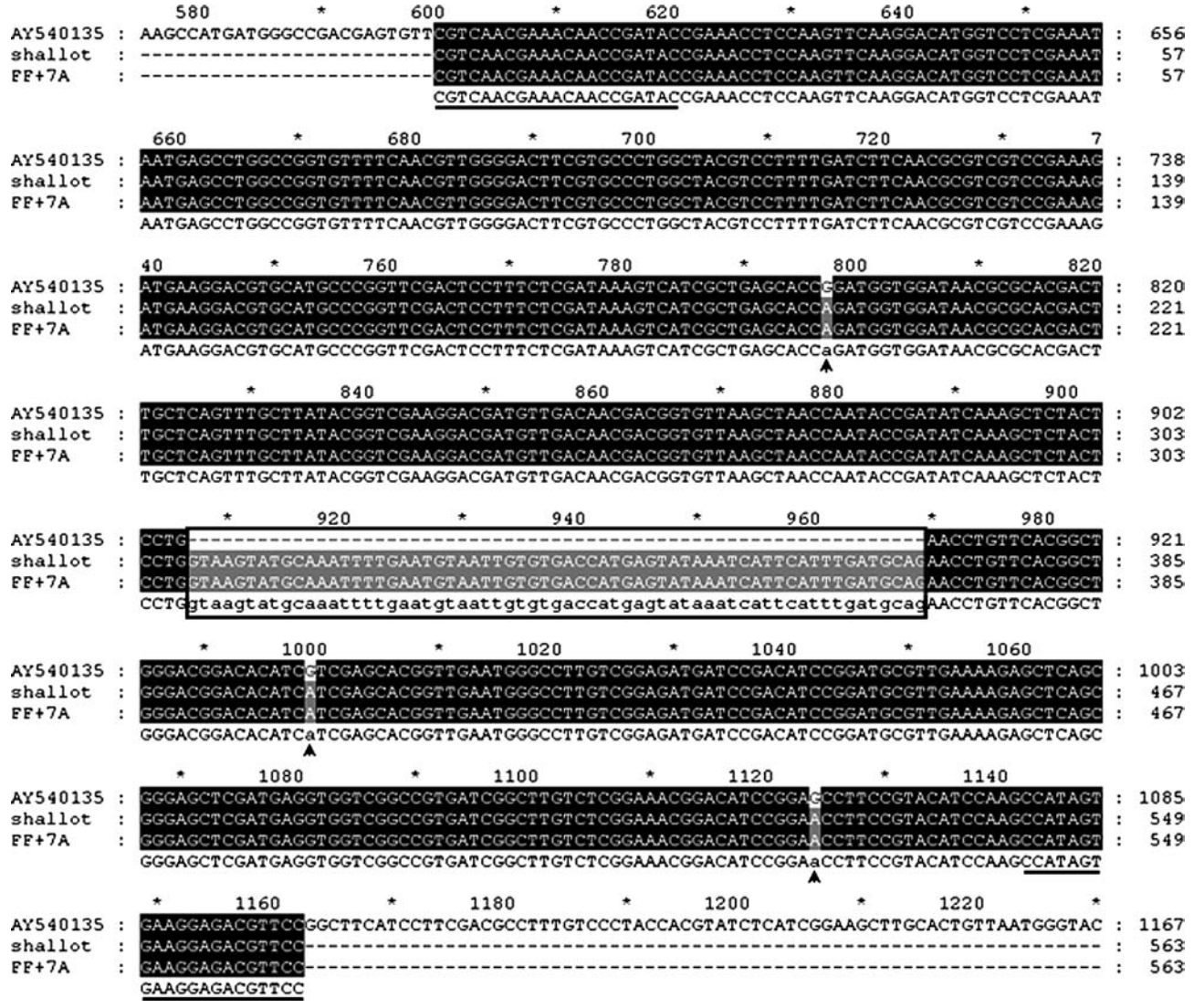

Fig. 8 Alignment of nucleic acid sequences of flavonoid 3′-hydroxylase. The sequences used for the alignment are F3′H mRNA of onion (AY540135) and sequence dates in this study (shallot and FF + 7A). The *single bars* indicate the sequence of the primer sets used for the second PCR. The *box* shows the 63-bp indel. *Arrowheads* point to SNPs between onion and shallot

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