

Genetic Variation of Shallot (*Allium cepa* L. *Aggregatum* Group) in Vietnam

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To evaluate Vietnamese shallot strains as genetic resources, genetic variation among those collected from three main (northern, central and southern) regions in Vietnam was studied, based on morphology, physiology, and polymorphisms of nuclear, chloroplast, and mitochondrial DNAs. Strains from the northern region had spreading and dark green leaves, bolted late, and formed bulbs early. The bulb skin was white during bulb formation but turned brown after harvest. By contrast, strains from the central and southern regions, that had semi-spreading leaves, bolted early and formed bulbs late. The leaves were yellow green when young, but turned dark green as they matured. The bulb skin was pink when the bulbs were young but became red after harvest. In the dendrogram based on RAPD analysis of total DNA, two groups of strains were formed. One included the strains from the northern region and the other the strains from the central and southern regions. PCR-RFLP analyses of chloroplast and mitochondrial DNAs showed no variation among the strains from the three regions. From the results, two different types of shallot were identified, namely the North type (distributed in the northern region) and the South type (in the central and southern regions). Different characteristics of these two types will be useful for future breeding of shallot, common onion, and wakegi onion in tropical and sub-tropical countries.

**Key Words:** *Allium cepa* L. *Aggregatum* group, genetic variation, shallot, Vietnam.

**Introduction**

Shallot (*Allium cepa* L. *Aggregatum* group) is a crop that is closely related to common onion (*A. cepa* L. Common onion group). It differs from the common onion by their very small stature, active tillering and formation of a cluster of small bulbs (Jones and Mann, 1963; Tashiro et al., 1982).

In the Southeast Asian countries, shallot is an economically important crop and much more in demand than common onion because of its pungent flavour and high adaptability to tropical and subtropical conditions (Buijsen, 1990; Permadi and van der Meer, 1994; Tashiro et al., 1982).

In Vietnam, shallot that has been cultivated for centuries is grown much more extensively than any other *Allium* crops. Since growth cycle is short (about 3 months), it is usually rotated with rice and other crops in the field or mixed or inter-cropped with herbs, lettuce or flowers in the home garden. It is sold in every market located in low to high lands all over the country, as fresh green or dry bulbs. For dietary consumption, it is usually used as a vegetable, spice, pickle or medicinally to reduce fever and cure wounds. The main cultivated areas are the Red River Delta (in the northern region), Mekong

Delta (in the southern region) and some provinces in the central region. These regions are geographically isolated and have different climatic conditions. This fact suggests that there is a wide genetic variation among shallot strains that adapt to these areas.

To evaluate Vietnamese shallot strains as a genetic resource for future breeding of onions, genetic variation among the strains collected from different regions in Vietnam was studied based on morphological and physiological characters and polymorphisms of nuclear, chloroplast and mitochondrial DNAs.

**Materials and Methods***Plant materials*

Twenty strains of shallot were collected from the northern, central and southern regions of Vietnam during trips in December 2001 and May 2002 (Fig. 1, Table 1). When a sample of each strain was collected, the information on cultivated place and condition of the sample was collected by an interview. All strains collected had been cultivated in the collection sites or nearby except strain A-70 that was shipped from Hanoi to a market in Hochiminh a day before collection. These strains were cultivated in a plastic house of Saga University from September 15, 2002 to May 1, 2003; 10 plants of each strain were used for morphological and physiological observations. The roof of the plastic house was covered with plastic film to protect from rainfall and frost, but both sides and doors were opened. The DNA from 13 of these strains and one additional one collected from Camau province in the southern

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Fig. 1. Collection sites (●) of shallot strains in Vietnam.

region, were analyzed.

#### Cytological analysis

Actively cell-dividing root tips were pre-treated with 0.05% colchicine aqueous solution for 2.5 h at 20°C and fixed in a mixture of acetic acid and ethyl alcohol (1:3 v/v) before hydrolyzing with 1N HCl for 7 min at 60°C. Then the root tips were stained with leucobasic fuchsin and squashed in 45% acetic acid. Karyotype analysis was made on the characters of metaphase chromosomes, such as chromosome number, chromosome length, arm ratio, and satellite chromosome according to Tashiro et al. (1982).

#### Morphological and physiological observations

For each strain, plant height, leaf diameter, and number of tillers per plant were recorded two and half months after planting, while leaf color was observed in all the growing cycle, and bulb skin color was noted at the beginning of bulb formation and 50 days after harvest. The number of days from planting to bolting was counted, and flower morphology was observed at full bloom.

#### RAPD analysis

Total DNA was extracted from 1–2 g of fresh leaves, by using the CTAB method described by Murray and Thompson (1980) with minor modification described by Yamashita et al. (2000). Amplification was carried out under the condition reported by Shigyo et al. (1997) by using 40 arbitrary 10 bp primers (OPA01, OPA02, OPA03, OPA04, OPA05, OPA06, OPA09, OPA10, OPA11, OPA12, OPA13, OPA14, OPA15, OPA16, OPE02, OPE05, OPE08, OPE09, OPE10, OPE11, OPE13, OPE15, OPE17, OPG01, OPG02, OPG03, OPG04, OPG05, OPG06, OPG07, OPG09, OPG10, OPG11, OPG12, OPG13, OPG14, OPG15, OPG16, OPG17 and OPG18), obtained from Operon Technologies, Alameda, California.

RAPD bands derived from three independent amplification reactions were scored as being present (1) or absent (0) for all strains. To estimate the genetic distance among the strains, the genetic similarities (F) of RAPD fragments were calculated by employing the formula of Nei and Li (1979),  $F = 2M_{xy}/(M_x + M_y)$ , where,  $M_{xy}$ , number of shared fragments between the strains “x” and “y”;  $M_x$ , number of scored fragment of strain “x”;  $M_y$ , number of scored fragment of strain “y”. Distant values were calculated as 1-F. These values were then used as input data for cluster analysis. To generate a dendrogram, the UPGMA clustering method in MEGA version 2.1 (developed by Kumar et al., Arizona State University, USA) was employed.

#### Chloroplast DNA analysis

For the chloroplast DNA (cpDNA) analysis, total DNA from the leaves was extracted as above. The identification of cpDNA involved the use of oligonucleotide primers homologous to the region containing the *ribulose-1,5 biphosphate carboxylase* gene (*rbcL*) and open reading frame 106 (ORF-106) of cpDNA. Sequences of two primers for the amplification were 5'-ATGTCACCACAAACAGAACTAAAGCAAGT-3' (*rbcL*) and 5'-ACTACAGATCTCATACTACCCC-3' (ORF106) (Arnold et al., 1991). PCR was carried out under the condition reported by Arnold et al. (1991) with minor modification by Yamashita et al. (1998). The amplified products were digested with 10 restriction enzymes *Alu* I, *Ase* I, *Bgl* II, *Bam*H I, *Eco*R V, *Hinf* I, *Pst* I, *Rsa* I, *Xho* I (at 37°C) and *Taq* I (at 65°C) for 3 hours and electrophoresed on 1.5% agarose gel containing ethidium bromide in TAE buffer. The digestion pattern was observed on UV transilluminator.

#### Mitochondrial DNA analysis

Total DNA extraction was done as above, and the V7 region of mitochondrial (mt) small ribosomal subunit RNA (SrRNA) gene was analyzed. The sequences of the 2 primers were 5'-TATGAACAACAAAACCTGTCTTTAACGGGATGG-3' (mtV7<sub>P1</sub>) and 5'-GCG-GACTTGACGTCATCCCCACCTTCCTCCAG-3' (mtV7<sub>P2</sub>). PCR was carried out according to Yamashita et al. (2000). Methods for digesting the amplification product and observing digestion patterns were the same as those for cpDNA except for absence of the restriction enzyme *Xho* I.

## Results

#### Cytological analysis

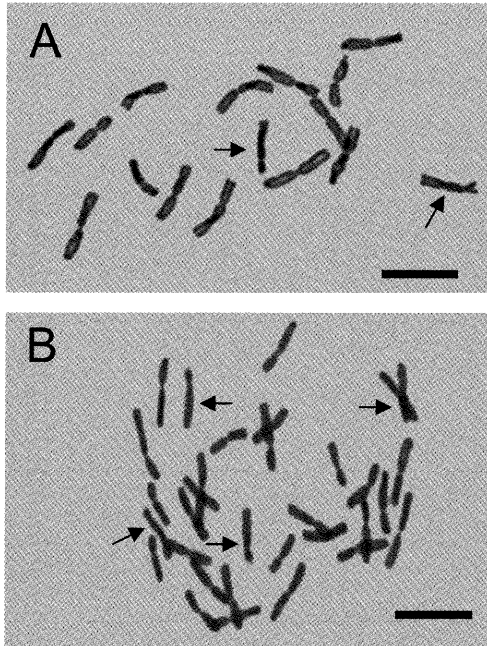
All strains collected were confirmed to be shallot based on the somatic chromosomes in the root tip cells: they were all diploid ( $2n = 16$ ) except the strain A-55 (Table 1). Of the strain A-55 collected in Hochiminh, some bulbs had 16 chromosomes while the others 32 chromosomes (tetraploid). Therefore, the strain A-55 was separated into two different strains as A-55-1

Table 1. Morphological and physiological characters of shallot strains from Vietnam.

Strain	Collection site	Market	Chromosome number (2n)	Plant height (cm)	Leaf diameter (mm)	No. of tillers per plant	Time for bolting (day)	Young leaf color	Bulb skin color <sup>z</sup>
A-04	Laocai	Baoyen	16	45.8±2.9 <sup>z</sup>	8.3±0.6	10.3±0.7	109.3±4.3	Dark green	Brown
A-19	Laocai	Baoyen	16	57.6±2.4	8.6±0.6	14.7±2.7	116.5±3.2	Dark green	Brown
A-20	Laocai	Baoyen	16	71.6±3.8	8.5±0.1	7.5±0.5	120.4±4.9	Dark green	Brown
A-13	Laocai	Cooleu	16	55.1±0.6	7.4±0.3	14.6±1.0	104.7±12.0	Dark green	Brown
A-23	Yenbai	Yenbinh	16	54.8±0.6	8.3±0.6	11.8±0.9	126.3±5.6	Dark green	Brown
A-10	Vinhphuc	Huogeanh	16	52.1±1.7	8.3±0.4	11.5±2.1	124.4±2.5	Dark green	Brown
A-01	Hanoi	Gialam	16	60.3±1.1	7.6±0.2	20.9±1.3	126.0±2.0	Dark green	Brown
A-47	Hanoi	Dongxuan	16	54.9±2.2	8.2±0.3	11.6±1.1	95.3±12.1	Dark green	Brown
A-49	Hanoi	Giangu	16	52.0±1.7	7.2±0.6	14.0±2.0	127.5±7.5	Dark green	Brown
A-52	Hanoi	Tuliem	16	49.4±0.1	8.3±0.3	12.1±1.1	119.2±1.5	Dark green	Brown
A-56	Hanoi	Tulicm	16	51.2±1.6	6.8±0.2	13.3±0.7	111.6±3.2	Dark green	Brown
Average of strains from North region									
				54.2±3.1	8.1±0.3	13.0±1.9	116.0±6.0		
A-36	Hue	Dongba	16	57.3±4.7	8.4±0.2	15.7±1.5	50.5±4.1	Yellow green	Red
A-42	Hue	Dongba	16	56.5±3.1	8.6±0.4	12.2±3.9	48.2±3.8	Yellow green	Red
A-02	Hue	Dongba	16	79.3±0.9	7.3±0.6	13.5±0.8	45.8±0.5	Yellow green	Red
Average of strains from Central region									
				64.4±7.5	8.1±0.4	13.8±1.0	48.2±1.4		
A-58	Lamdong	Dalat	16	51.7±0.3	9.9±0.4	6.3±1.2	67.1±4.0	Yellow green	Red
A-55-1 <sup>x</sup>	Hochiminh	Benthanh	16	54.8±0.8	7.4±0.3	11.3±0.6	56.1±1.2	Yellow green	Red
A-55-2 <sup>w</sup>	Hochiminh	Benthanh	32	76.4±1.1	10.1±0.4	9.3±1.1	51.5±3.6	Yellow green	Red
A-74	Hochiminh	Benthanh	16	61.2±3.5	8.9±0.6	11.2±2.5	59.4±3.4	Yellow green	Red
A-68	Hochiminh	Maixuan-thuong	16	55.7±0.3	8.9±0.4	5.0±1.0	57.5±3.7	Yellow green	Red
A-64	Hochiminh	Benthanh	16	49.3±5.1	8.0±0.4	13.7±1.3	60.4±5.9	Yellow green	Red
A-70 <sup>v</sup>	Hochiminh	Maedinhchi	16	52.0±1.0	8.3±0.1	13.8±2.4	125.0±3.0	Dark green	Brown
Average of strains from South region									
				54.5±2.8	8.6±0.4	9.5±1.7	60.1±1.9		

<sup>z</sup> Mean ± SE (n = 10)<sup>y</sup> Bulb skin color was observed 50 days after harvest.<sup>x, w</sup> Strains A-55-1 and A-55-2 were mixed and sold in a market in Hochiminh. The data of strain A-55-2 (tetraploid) were not included in the average of strains from the Southern region.<sup>v</sup> Cultivation place of these strain was Hanoi and the data of this strain were not included in the average of strains from Southern region.

(diploid) and A-55-2 (tetraploid). All the diploid strains observed had the karyotype formulas as  $K_{(2n)} = 16 = 14V + 2J^t$  or  $K_{(2n)} = 16 = 14V + J^t + J$ , in agreement with the result of Tashiro et al. (1982). The tetraploid strain had 4 satellite chromosomes, of which sizes, arm ratios and positions of satellites were similar to those of diploid strains (Fig. 2).

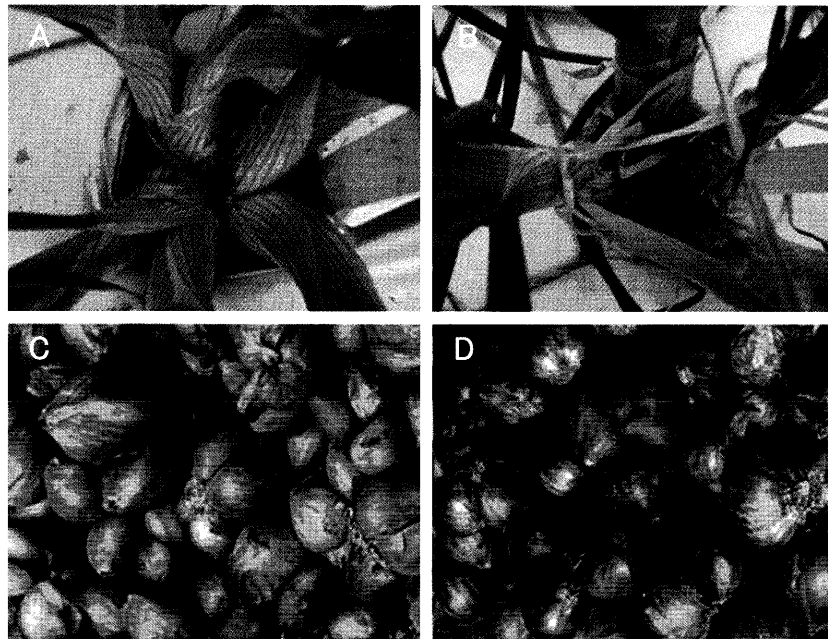


**Fig. 2.** Somatic chromosomes in root tip cells of diploid (A) and tetraploid (B) shallot strains from Hochiminh, Vietnam. Arrows indicate J-shaped chromosomes. One of the J-shaped chromosome of diploid strain does not have satellite. Scale bars indicate 10  $\mu$ m.

#### *Morphological and physiological observations*

Morphological and physiological characters of all strains observed are in Table 1. Except for strain A-70, which was cultivated in the northern region, but collected in the southern region, there was no variation in the young leaf and bulb colors among the strains collected in each region; there were some variation in plant height, leaf diameter, number of tillers and time for bolting. Morphological and physiological differences were detected between the strains of the northern region and those of the central and southern regions. The former had spreading dark green leaves, and bolted late; their bulb skin color was white during development, but it turned brown after harvest (Fig. 3). Whereas, the latter had semi-spreading young yellow green leaves, which turned dark green at maturity; they bolted early. The young bulb skin was pink but became red after harvest. Strains from northern region had fewer flower stalks per plant than had those from the central and southern regions (data not shown). The former began bulb formation two weeks earlier, entered into dormancy two months earlier and produced bigger bulbs than did the latter. All strains from three regions had the same flower morphology, typical of the shallot.

The strain A-70 had the same morphological and physiological characteristics as the strains from the North region where the strain A-70 was cultivated. The tetraploid strain A-55-2 had larger plant height and leaf diameter but had fewer tillers than the diploid strain A-55-1.



**Fig. 3.** Basal portions of leaf sheaths at the beginning of bulb formation (A and B) and dry bulbs (C and D) of the strains from Hanoi (A and C) and Hochiminh (B and D).

### RAPD analysis

Sixteen out of 40 primers used in RAPD analysis produced a total of 130 distinct bands in the diploid strains (Table 2). Seventy polymorphic bands were scored with fourteen primers. The two primers, OPA16 and OPG06, produced no polymorphic band. Significant variations in the presence or absence of the bands could be observed between the strains from the northern region and those from the central and southern regions. In the case of primer OPA01, a polymorphic band about 1.5 kbp was detected only in all strains from the central and southern regions (Fig. 4), while a polymorphic band about 0.65 kbp was found only in strains from the northern region. Polymorphism of banding patterns was also detected among the strains from the same region.

In the dendrogram based on the RAPD data, two

**Table 2.** Number of total and polymorphic bands detected in RAPD analysis of shallot strains from Vietnam.

Primer	Sequence	No. of total bands	No. of polymorphic bands
OPA-01	5'-CAGGCCCTTC-3'	13	7
OPA-02	TGCCGAGCTG	11	6
OPA-04	AATCGGGCTG	12	6
OPA-10	GTGATCGCAG	11	8
OPA-11	CAATCGCCGT	5	3
OPA-16	AGCCAGCGAA	2	0
OPG-03	GAGCCCTCCA	10	5
OPG-04	AGCGTGTCTG	5	3
OPG-05	CTGAGACGGA	5	1
OPG-06	GTGCCTAACC	4	0
OPG-10	AGGGCCGTCT	11	6
OPG-11	TGCCCGTCGT	8	6
OPG-12	CAGCTCACGA	12	8
OPG-17	AGCGTCCTCC	9	6
OPG-18	GGCTCATGTG	7	3
OPE-02	GGTGCGGGAA	5	2
Total		130	70

different groups were formed. One group (North type) included all strains from the northern region, meanwhile, the other (South type) all the strains from the central and southern region (Fig. 5). Strain A-70 was categorized with the North type.

### Chloroplast DNA analysis

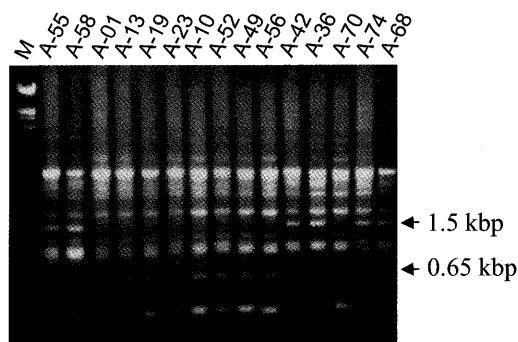
The size of the amplified region between *rbcL* and ORF-106 was about 3.2 kbp. Except for *Pst* I, all restriction enzymes used digested the amplified DNA and generated a total of 27 bands. None exhibited polymorphism.

### Mitochondrial DNA analysis

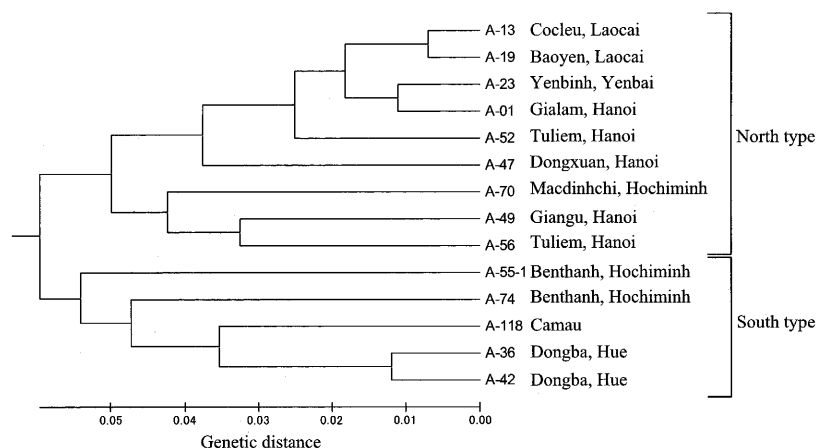
The size of the amplified region was about 550 bp. A total of 11 bands were produced by digesting the amplified DNA with 6 restriction enzymes (*Alu* I, *Ase* I, *Bgl* II, *EcoR* V, *Pst* I and *Rsa* I). Other three restriction enzymes (*Bam*H I, *Hinf* I and *Taq* I) did not digest the amplified DNA. No polymorphic band appeared among the strains.

### Discussion

In the present study, the results from morphological and physiological observations and RAPD analysis



**Fig. 4.** RAPD profiles generated by primer OPA-01 of shallot strains from Vietnam. M:  $\lambda$ Hind III-EcoR I.



**Fig. 5.** Dendrogram from RAPD patterns in shallot strains from Vietnam.

reveal that there are two distinguishable types of shallot in Vietnam, namely the North and South types. The former is distributed in the northern region and the latter in the central and southern regions. These two types of shallot can be easily distinguished in the market of Vietnam by their dry bulb skin color; the North and South types have brown and red bulb skin colors, respectively.

Human and natural selections have led to the development of different plant types present in several cultivated species (Fritsch and Friesen, 2002). Geographically, Vietnam is a country with two distinct climates; the northern region has four distinct seasons, while the central and southern regions have two seasons, dry and rainy. In the northern region, shallot is usually cultivated from October to February, when the weather is cool and dry, whereas in the central and southern regions, it is usually cultivated from November to March, when the weather is hot and dry. It seems that the two different types of shallot have occurred as a result of the selection in Vietnam. The two types of shallot—each type must have high adaptability to the cultivated region—will be useful as genetic resources for the breeding of shallot, common onion and wakegi onion in Vietnam and other tropical and sub-tropical countries.

In Vietnam, shallot does not show bolting and the local name “hanh cu” (bulb onion) has been applied to shallot in order to separate from “hanh hoa” (flower onion) for Japanese bunching onion. Under our observation in Saga University, however, all strains of shallot from Vietnam bore flower stalks. This result proves that the shallot in Vietnam has a potential to flower and that the growing condition has a remarkable effect to the flowering. In Saga, the short day length and/or low temperature during autumn and winter supposed to stimulate the bolting of both types of shallot, and the South type of shallot might have higher sensitivity than the North type (About 60 days and 120 days were necessary for bolting, respectively). Besides the change in bolting, the bulb size also had altered in Saga. Both types form almost the same size of bulbs in Vietnam, but in Saga the North type entered bulbing earlier and produced bigger bulbs than the South type. It seems that early bolting and continuous flower stalk formation resulted in the small size of bulbs in the South type. Being done in the field of Saga University, our research had pointed out variations in bolting and bulb formation among the shallot strains from Vietnam. In the future, advanced morphological and physiological studies of the two types of shallot will be done in Vietnam.

In the results of PCR-RFLP analyses, there was no obvious variation among restriction sites in the analyzed regions of chloroplast and mitochondrial DNAs of the shallot strains from Vietnam. It will be necessary to consider other method to detect polymorphisms in chloroplast and mitochondrial DNAs.

Shallot has been widely cultivated and consumed not only in Vietnam but also in other Southeast Asian countries (Arifin et al., 2000; Buijssen, 1990; Endang et al., 2002; Ochse, 1931; Permadi and van der Meer, 1994; Tashiro et al., 1982). Concerning to the bulb color, the red shallot was popular in these countries. However, other types of shallot have been cultivated with a small amount for local consume. In Thailand, there are white shallot “hom khao” cultivated in the North and North East regions and the orange color shallot “hom bua” in the North region (Buijssen, 1990). White shallot, which is cultivated in Indonesia, has been used as a charm for the fields. This shallot has the same morphological characteristics as red shallot except for the colors of bulb, leaf and anther. After genetic analyses, Endang et al. (2002) concluded that this shallot belongs to *Allium cepa* L. Aggregatum group. They supposed that the white shallot originated from red shallot through natural mutation processes. During our survey, brown shallot was found in the North region of Vietnam and this type was very popular in this region. Furthermore, some bulbs of tetraploid shallot were mixed with diploid ones and sold in a market in Hochiminh. Except having larger size in bulb, plant height and leaf diameter, most characters of the tetraploid were as the same as the diploid. Therefore, this strain seems to be an autotetraploid. The karyotype analysis supported this presumption. In Vietnam, shallot (*A. cepa* L. Aggregatum group) is not distinguished from wakegi onion (*A. wakegi* Araki) and the name “hanh ta” (native onion) or “hanh cu” are used for both of them. To avoid confusion between shallot and wakegi onion and to confirm the ploidy, karyotype analysis of the material plants will be essential in the comparative studies on shallot. With the presence of many types of shallot in Southeast Asian countries, it is interesting to study on the relationship between shallot strains cultivated in Vietnam and those in nearby countries.

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### ヴィエトナムのシャロット (*Allium cepa* L. *Aggregatum* group) の遺伝的変異

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ヴィエトナムのシャロットを遺伝資源として評価するために、北部、中部および南部から集めた系統の遺伝的変異を調べた。これらの系統を佐賀大学のプラスチックハウスで栽培し、形態および生理的形質を調査した。また、RAPD法で全DNAの多型を、PCR-RFLP法で葉緑体およびミトコンドリアDNAの多型を分析した。北部の系統はすべて、葉が開張性で、暗緑色であり、抽苔が遅く、球根形成が早かった。球根の皮の色は、球根形成時には白かったが、収穫後には褐色になった。中部と南部の系統はすべて、葉が半開張性で、若い葉は黄緑色であったが、成葉は暗緑色になり、抽苔が早く、球根形成が遅かった。球根の皮の色は、球根形成の始めにはピンクであったが、成熟すると赤くなった。RAPD分析の結

果にもとづいて系統間の遺伝的距離を計算し、デンドログラムを作成した結果、供試した系統は二つのグループに分かれた。一つは北部の系統からなり、他のグループは南部と中部の系統からなっていた。PCR-RFLP分析の結果、供試したすべての系統の葉緑体およびミトコンドリアDNAは、用いた制限酵素すべてで同じバンドパターンを示し、これらの系統は同様な細胞質を持つと考えられた。以上の結果から、ヴィエトナムには遺伝的に異なる二種類（北部型と南部型）のシャロットが存在することが明らかになった。これらが持つ異なる特性は熱帯および亜熱帯のシャロット、タマネギおよびワケギの育種に利用できると思われる。