園学雑. (J. Japan. Soc. Hort. Sci.) 71 (4) : 504-508. 2002.

Genetic Characteristics of the Indonesian White Shallot

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Summary

The bulb color of tropical shallot is commonly red, but there is a shallot with white bulb color in Indonesia. Genetic information to classify the white shallot and utilize it as breeding material is non-existent, so that we decided to genetically characterize the Indonesian white bulb shallot. Except for the colors of the bulbs, the leaves at sprouting and the anthers, the white shallot shared all morphological characters with the red bulb shallot (*Allium cepa* L. Aggregatum group, red shallot). The white shallot, which has eight homologous somatic chromosome pairs, possessed the same karyotype and meiotic behaviour as the red shallot. There was also a high cross compatibility between the white shallot and red shallot. Furthermore, PCR-RFLP analyses of chloroplast DNA and mitochondrial DNA showed that these cytoplasmic components were similar for both shallot types. We conclude that the white shallot belongs to the *A. cepa* L. Aggregatum group; we suppose that the white shallot originated from the red shallot through natural mutation processes.

Key Words: *Allium cepa*, bulb color, Indonesia, shallot, white shallot.

Introduction

Shallot (*Allium cepa* L. Aggregatum group) and common onion (*A. cepa* L. Common onion group) represent an important vegetable group enjoyed in many parts of the world (Currah and Proctor, 1990). Shallot similar to common onion in appearance and usage, has adapted to grow well under a greater variety of climatic conditions, ranging from tropical to temperate. Because of this adaptability, shallot is more predominant than the common onion as an essential vegetable in tropical lowlands such as Indonesia.

The characteristic pungency of the shallot has made it an important spice and seasoning; it is an essential ingredient in the preparation of many dishes. The bulbs are also pickled, and the young leaves and flowers are used for soup and various dishes (Heyne, 1927; Ochse, 1931; Jones and Mann, 1963; Hartuti and Sinaga, 1995). Shallot is not only used in foods but it is also an important ingredient for many medicinal remedies used to treat common ailments.

Typically, the color of shallot bulbs, cultivated in the tropical and subtropical climatic zones, varies from light to dark red (Fig. 1). However, there is a shallot with white bulbs (white shallot) endemic to the mountainous areas e.g., Java, Sumatra (Minangkabau, Lampung), Roti, Timor, Tanimbar, Kai, and Ternate islands of Indonesia (Heyne, 1927; Ochse, 1931). It is presently cultivated in the mountainous areas in Timor island, Papua island, and Brebes in central Java.

In Indonesia, the white shallot is mainly used for pickles similar to the rakkyo (*A. chinense* G. Don.) in Japan. Farmers in Java believe that the white shallot plant is a charm for their farms. Therefore, a small number of plants of white shallot have been grown among plants of red shallot. The white shallot has been preserved by continuous vegetative propagation for long time although it was first described in the literature by Heyne in 1927.

Indonesian people identify the red shallot and wakegi onion (*A.* x *wakegi* Araki, an interspecific hybrid between *A. fistulosum* L. and *A. cepa* L. Aggregatum group) in the same category and call both of them 'Bawang merah'. However, they strictly distinguish red shallot and white shallot; the latter is called 'Bawang acar'. Since it is difficult for the white shallot to flower in Indonesia, no researcher has observed its flower morphology. Moreover, genetic information that is available to classify the white shallot and utilize it as breeding material has never been documented. This paper provides such genetic evidence to the Indonesian white shallot.

Materials and Methods

Morphological analysis

The shallot strains used in this study were the white shallot 'Dili-white' collected from Dili in Timor island,

Received; August 28, 2001. Accepted; November 26, 2001.

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A part of this study was presented at the 2000 Autumn Meeting of the Japanese Society for Horticulture Science.

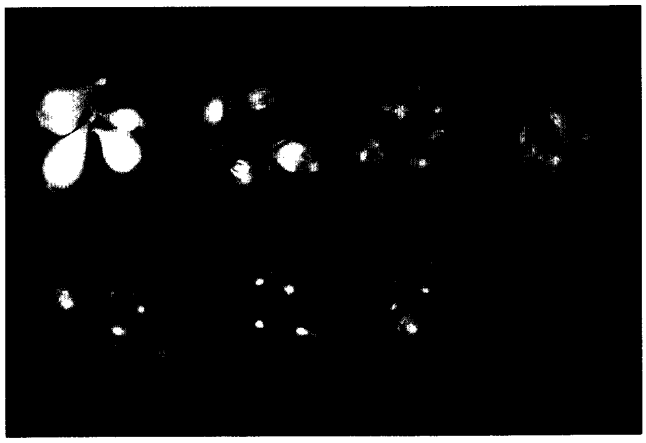

Fig. 1. Bulbs of shallot strains from Indonesia.
Top panel (from left to right): 'Dili-white', 'Dili-red', 'Bantul' and 'Ambon'. *Bottom panel* (from left to right): 'Wonosobo', 'Temanggung-1', and 'Temanggung-2'.

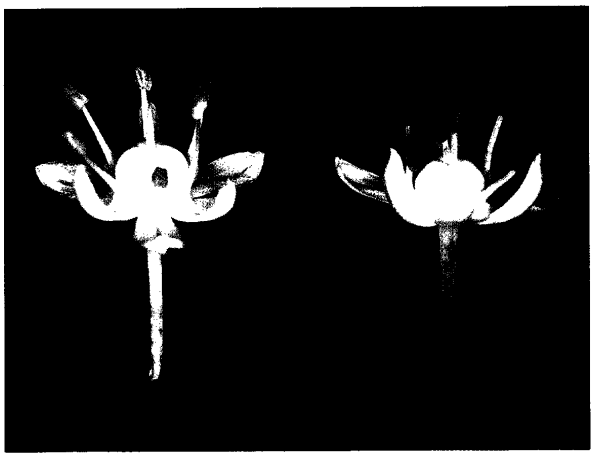

Fig. 2. Florets of 'Dili-white'(left) and 'Bantul'(right).

and the red shallot 'Bantul' from Bantul in Java island. Both strains were grown and maintained in pots under optimal greenhouse conditions. Bolting times and the following morphological characters were recorded: (1) leaf color; (2) number of tillers; (3) bulb color, shape, and size; (4) colors, shapes and sizes of inflorescences, florets and anthers.

Cytological analysis

Root tip cells, taken from the pot-grown plants, were pretreated with 0.05% colchicine for 2.5 hr at 20 ℃ and fixed in a mixture of acetic acid and ethyl alcohol (1:3, v/v). After hydrolysis in 1 N HCl at 60 ℃ for 7 min, the cells were stained with leucobasic fuchsine and squashed in 45 % acetic acid. Karyotypic analysis was done using karyotyping soft Chantal (Leica Co., Ltd.).

Meiotic behavior was monitored by using smear preparations of pollen mother cells (PMCs) obtained from fresh anthers in iron-acetic carmine. Similar smears were also used to determine pollen fertility. Selfings and reciprocal crossings were made; seed fertility was determined as percentage of seed set and seed germination rate.

PCR-RFLP analyses of chloroplast and mitochondrial DNAs

Total DNA from 1-2 g of fresh leaves was extracted, using the CTAB method described by Murray and Thompson (1980) as modified by Yamashita et al. (2000). For chloroplast DNA (cpDNA) analysis, the region between ribulose-1,5-bisphosphate carboxylase gene (*rbcL*) and open reading frame 106 (ORF 106) was amplified by PCR using 5'-ATGTCACCACAAACAGAAACTAAAGCAAGT-3'(*rbcL*) and 5'-ACTACAGATCTCATACTACCCC-3'(ORF106) as primers as described by Arnold et al. (1991) with minor modifications (Yamashita and Tashiro, 1999). The amplified products were digested with 6 restriction enzymes, *Alu* I, *Hinf* I, *Rsa* I, *Xba* I, *Ase* I and *Xho* I at 37 ℃ for 4 hr.

For mitochondrial DNA (mtDNA) analysis, the region between *nad4* exon1 (NADH dehydrogenase subunit 4 exon 1) and *nad4* exon2 (NADH dehydrogenase subunit 4 exon 2) was amplified by PCR using 5'-CAGTGGGTTGGTCTGGTATG-3' (*nad4* exon1) and 5'-TCATATGGGCTACTGAGGAG-3' (*nad4* exon2) as primers (Demesure et al., 1995) with minor modifications. Briefly, the reaction mixture (50 μl) contained: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.1 μM of each primer, and 0.4 units of *Taq* DNA polymerase (Takara Shuzo Co., Ltd.). The amplification was carried out for 4 min at 94 ℃, followed by 30 cycles of 45 sec at 92 ℃, 45 sec at 57.5 ℃, 2.5 min at 72 ℃, with a final 10 min incubation at 72 ℃ using the program temp control system PC800 (Astec Co., Ltd.). The amplified product was digested with the same restriction enzymes as above.

The digested cpDNA and mtDNA products were electrophoresed on 1.5 % agarose gels containing ethidium bromide in TAE buffer. The restriction patterns of cpDNA and mtDNA were observed on a UV transilluminator.

Results and Discussion

Morphological analysis

Flower stalks of 'Dili-white' and 'Bantul' began to elongate in mid-December in the greenhouse condition. Anthesis was occured at the beginning of April. Morphologically, 'Dili-white' was similar to 'Bantul' except for the colors of the bulbs, the leaves at sprouting, and the anthers. Bulb color of 'Dili-white' was white from harvesting time up to the end of storage, while those 'Bantul' were red from the beginning of bulb formation (Fig. 1). Young sprouts of 'Dili-white' displayed yellowish green leaves which turned green two weeks latter, whereas leaves of 'Bantul' were always green. Anthers of 'Dili-white' were yellow or yellowish- green, whereas those of 'Bantul' were green

Fig. 3. Somatic metaphase chromosomes of 'Dili-white'.

(Fig. 2). Both strains, bolting started in the beginning of December, and formed many tillers and inflorescences.

Cytological analysis

Karyotypically, both 'Dili-white' and 'Bantul' possess 16 somatic chromosomes grouped into 8 chromosome pairs (Fig. 3). Based on positions of the centromere, 4 pairs of chromosomes (No. 1, 3, 5 and 7) were metacentric, 3 pairs (No. 2, 4, and 8) were submetacentric, and 1 pair (No. 6) was subtelocentric and had small satellites. Of 84 PMCs observed for the 'Dili-white', 89.3 % had 8 bivalent chromosomes (Fig. 4). A small percentage of the PMCs (10.7 %) were found to have 7 bivalent and 2 univalent chromosomes. 'Bantul' showed 8 bivalents in all 34 PMCs observed. These results indicate that 'Dili-white' and 'Bantul' have 8 homologous chromosome pairs and have the same karyotype as several other red shallot strains previously examined (Tashiro et al., 1982).

'Dili-white' was found to have lower pollen fertility (48 %) as compared to 'Bantul' (92 %). The low pollen fertility in 'Dili-white' is attributed to pollen grains with undifferentiated nuclei and empty ones.

In selfing, the seed set of 'Dili-white' was lower than that of 'Bantul', but the seeds of both strains had high germination rates (Table 1). Twenty-one percent of seedlings from self-pollinated 'Dili-white' were albino. These albino seedlings grew only under aseptic condition.

In crossing between 'Dili-white' and 'Bantul', the seed set was 16.8% which was higher than those in selfings of'Dili-white' and 'Bantul'. The seeds also had high germination rates similar to those of the parent plants. However, the reciprocal crossing exhibited low seed sets. Thus, low pollen fertility of 'Dili-white' caused the low seed set in the reciprocal crossing. These

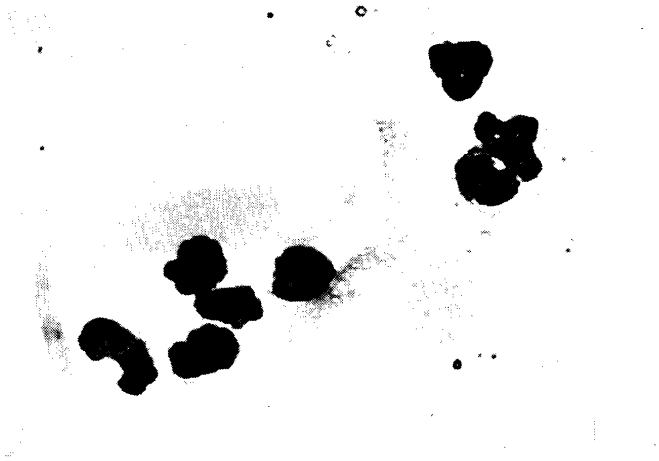

Fig. 4. Meiotic metaphase-I chromosomes of 'Dili-white'.

results also revealed the high fertility of the female organs of 'Dili-white' plants. Therefore, there is a high cross compatibility between 'Dili-white' and 'Bantul'.

PCR-RFLP analysis of cpDNA and mtDNA

The regions between *rbcL* and ORF 106 in cpDNA, and *nad4* exon1 - *nad4* exon2 in mtDNA previously showed polymorphism among the species in section *Cepa* of *Allium* (Yamashita et al., 1998 and unpublished data by Yamashita et al.).

PCR-RFLP analysis of cpDNA from 'Dili-white' and 'Bantul' revealed that both shallot plants had the same polymorphic bands (1200 bp) and the same restriction enzyme patterns (Fig. 5). Moreover, the restriction patterns of cpDNA of 'Dili-white' and 'Bantul' were the same as that of another red shallot strain called the 'Chiang Mai' previously examined (Yamashita et al., 1998). PCR-RFLP analysis of mtDNA also showed that 'Dili-white' and 'Bantul' had the same polymorphic bands (200 bp) and identical restriction enzyme patterns (Fig. 6). The results of cpDNA and mtDNA analyses showed that the cytoplasm of white shallot was quite similar to that of red shallot.

Heyne (1927) described that the white shallot in Indonesia might be a form of shallot, but to date no morphological or genetic evidence has ever been provided to confirm this description. This paper provides such morphological and genetic evidences linking white shallot to the *A. cepa* L. Aggregatum group. Based on our results, we suppose that the white shallot originated

Table 1. Seed fertility in selfing and reciprocal crossings of white shallot 'Dili-white' and red shallot 'Bantul'.

Cross combination	Number of plants examined	Total number of florets pollinated	Total number of seeds obtained	Seed set (%)	Germination rate (%)	Percentage of albino seedlings
'Dili-white' self	2	435	84	3.2	92.9	20.5
'Bantul' self	2	268	92	5.7	80.4	0
'Dili-white' × 'Bantul'	3	477	482	16.8	86.9	0.5
'Bantul' × 'Dili-white'	3	543	139	4.3	83.5	0

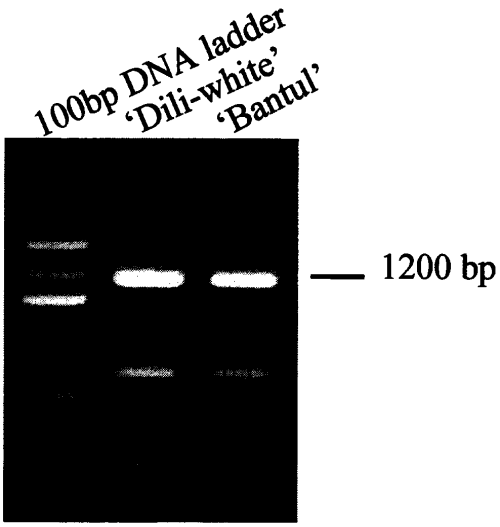

Fig. 5. Restriction patterns of *Alu* I digested *rbcL* - ORF 106 region of cpDNA in 'Dili-white' and 'Bantul'.

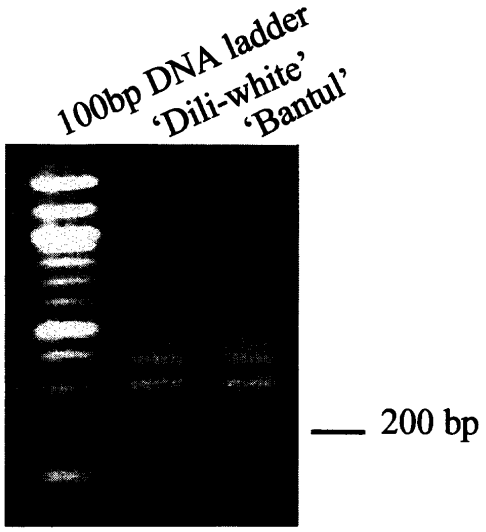

Fig. 6. Restriction patterns of *Alu* I digested *nad4* exon1-exon2 region of mtDNA in 'Dili-white' and 'Bantul'.

from the red shallot by natural mutation because the two have similar characters. Because the white shallot grows in tropical areas, this form can be a potentially important genetic resource to improve other shallot and common onion strains. Current breeding programs on the tropical shallot and common onion are advancing by using white shallot. Studies on genetic basis of the mutation of bulb color including pigment synthesis in shallot are in progress by using selfed, reciprocal F_1 and F_2 progenies between red and white shallot plants.

The name 'shallot' has been loosely used, e.q., the true shallot is little known in Japan, and the 'Echallote' on the market in Japan is actually a form of the rakkyo (Tashiro et al., 1982). In the United States, small onion bulbs are sometimes sold as shallot but they may actually be a hybrid between the shallot and common onion. The 'Lousiana Evergreen' shallot and the 'Delta Giant' shallot are interspecific hybrids between shallot and Japanese bunching onion, *A. fistulosum* L. (John and Mann, 1963; Currah and Proctor, 1990). In France, there are 2 types of shallot, i.e. the grey shallot and the Jersey shallot (Cohat et al., 2001). The grey shallot was proved to belong to *A. oschaninii* (Maass, 1996; Friesen and Klaas, 1998). Arifin and Okubo (1996) reported that Indonesian people did not distinguish wakegi onion from shallot. Thus, there are different 'shallots' in the world. This revealed the necessity to accumulate additional scientific information on the shallot. Our report on the Indonesian white shallot is one step towards clearing the confusion in the shallot.

Acknowledgement

The Indonesian white shallot bulbs used in this study was a gift from Ir. Gagat Taryono. We thank to Dr. A. H. Permadi (Lembang Vegetable Research Institute) and Dr. N. S. Arifin (Brawijaya University) for their information of white shallot in Indonesia. We are also indebted to Prof. Dr. Dennis J. Grab (Johns Hopkins University) and Dr. Shiro Isshiki (Saga University) for reading the manuscript.

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インドネシア産白玉シャロットの遺伝的特性

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摘　要

東南アジアで栽培されるシャロット(*Allium cepa* L. Aggregatum group)は一般に赤い球根を形成するが，インドネシアの一部の地域では白色の球根を形成するシャロットが栽培されている．しかし，この白玉シャロットを厳密に分類したり，育種素材として利用するための遺伝的情報はない．そこで，本研究ではこの白玉シャロットの遺伝的特性を調査した．

白玉シャロットと対照として供試した赤玉シャロットは，球根の色，やくの色および萌芽時の葉の色が互いに異なる点を除けば，形態的形質は同じであった．両者の核型および減数分裂時の染色体行動は同じであった．また，両者は高い交雑和合性を持っていた．さらに，葉緑体DNAおよびミトコンドリアDNAのPCR-RFLP分析から，両者は同質の細胞質を持つことが明らかになった．

以上の結果から，インドネシア産白玉シャロットは，*Allium cepa* L. Aggregatum groupに属すると結論づけられ，赤玉シャロットから自然突然変異によって生じたと推定された．