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Morphological and Cytological Characteristics of Haploid Shallot

(*Allium cepa* L. Aggregatum group)

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Summary

Morphological and cytological characteristics were investigated on the haploid plant of shallot ($2n = 8$, genome A) derived from the crossing between diploid ($2n = 16$, AA) and triploid ($2n = 24$, AAA) plants. The haploid plant was smaller than the diploid plant in most morphological characters such as plant height, leaf blade diameter, flower stalk length, inflorescence diameter and sizes of flower organs. The beginnings of sprouting, bulb formation, dormancy and bolting were all later for the haploid plant than for the diploid plant. However, the haploid plant had larger numbers of tillers and bulbs. Epidermal cells and stomata of the haploid plant were smaller than those of the diploid plant. The haploid plant had 8 univalent chromosomes in meiosis and indicated that no homologous chromosome pair was in genome A. The haploid plant showed sterilities both in pollen formation and seed set.

Introduction

Shallot, *Allium cepa* L. Aggregatum group, is one of the important vegetables for the people who live in southeast Asia[8)12)16)]. It is valued for their distinctive pungency. Commonly shallot is used as an essential ingredient of dishes and soup and a remedy for various ailments.

Shallot has a high adaptability to tropical zone. However, this plant is a low land crop in the tropical regions such as Indonesia since the elevation of the growing site (above 450m) affects the growth and development of the plants and stimulates the bolting which causes a low yield and poor quality of the bulbs[13)]. Storage of the seed bulbs up to 5 months was only possible in low land[15)]. The bulb size of shallot is extremely small as compared with that of common onion. Therefore, shallot has many economic characters to be improved.

F_1 hybrids with superior characters may expand the utilization of shallot. True seed shallot will be a new breeding material. Shallot is also an important genetic resource for improvement of other allium crops such as common onion (*Allium cepa* L. Common onion group) and wakegi (*Allium* × *wakegi* Araki)[1)2)17)]. However, shallot has so far been a vegetatively propagated plant though it can set seeds. Self progenies of shallot and hybrid

plants between shallot and common onion showed variations in many characters[16]. Therefore, shallot is in a heterozygous state. This will hinder the progress of the new breedings in this crop mentioned above.

Haploid plants followed by chromosome doubling will offer the pure lines and solve the limitation of the breedings in shallot. Haploid plant induction of common onion via in vitro gynogenesis have been successfully reported by many researchers[3)4)5)6)9)10)11)]. We also succeeded to obtain a haploid plant of shallot ($2n=8$, genome A) in 1993 through the crossing between diploid ($2n=16$, AA) and triploid ($2n=24$, AAA) plants.

The objective of this study is to analyze the morphological and cytological characteristics in the haploid plant of shallot.

Materials and methods

Bulbs of the haploid and diploid plants of shallot were planted in a plastic house using polyethylene mulch. Plant density was 25 × 20 cm. The experimental plot consisted of 2 treatments of planting time (July 20 and August 20, 1996) and was replicated 8 times. Plant height, number of leaves, number of tillers were recorded every month until April 20, 1997. For recording dates of beginnings of sprouting, bulb formation, dormancy and bolting, observations on them were done every 3 days. Before harvesting the bulbs, number of flower stalks, length of flower stalk, diameter of inflorescence were observed. Flower organ characters were also observed including length of perianth, length of anther and diameter of ovary. At harvest, weight and number of bulbs were recorded. Leaves for cytological measurement were taken when the plants entered the bolting stage and the fourth leaves from the tips of plants were sampled and fixed in the mixture of acetic acid and ethyl alcohol (1:3 v/v). Types of epidermal cells and sizes of epidermal cells and stomata were recorded. The evaluation of variability was qualified by calculating the standard error on all data of all replications.

Root tip cells for cytological studies were taken from pot-grown plants. At approximately 1 cm from the tips, roots were cut and pretreated with 0.05% colchicine for 2.5 hours at 20 °C and fixed in the mixture of acetic acid and ethyl alcohol (1:3 v/v) before hydrolyzing at 60°C for 7 minutes in 1N HCl. Then they were stained with leucobasic fuchsine and squashed in 45% acetic acid. Karyotypic analysis was based on the characters of metaphase chromosomes such as chromosome lengths and arm ratios. Meiosis was observed with the smear preparation of pollen mother cells (PMCs) from fresh anthers in iron-acetic carmine. Iron-acetic carmine smears of pollen grains were also made for determining pollen fertility. To estimate the seed setting capacity, the haploid plants were crossed with the diploid plants of shallot and *Allium fistulosum* L., and also open pollinations were done in a green house. The seeds obtained were cultured in the hormone free MS agar medium, then germination and survival rate were evaluated.

Table 1 Beginnings of sprouting, bulb formation and bolting in haploid and diploid shallot

Material	Days after planting			
	Sprouting	Bulb formation	Dormancy	Bolting
Haploid	7.1±1.4	42.4±2.7	226.0±0.0	111.5±2.1
Diploid	5.0±0.3	32.0±0.0	48.6±2.9	174.5±0.2

Results and discussion

Morphological characters

Bulbs of the haploid plant sprouted later than those of the diploid plant (Table 1). The beginning of bulb formation of the haploid plant was also later than that of the diploid plant. For one year growth, the haploid plant had evergreen leaves even if the temperature was high enough for the diploid plant to begin dormancy. The haploid plant entered the dormancylike stage 226 days after planting while the diploid plant entered clear dormancy stage about 50 days after planting. Inversely, the beginning of bolting of the haploid plant was earlier than that of the diploid plant. Different times of planting seemed not to alter the morphological features of the haploid plant except number and weight of bulbs. Plant height of the haploid plant was smaller than that of the diploid plant by nature (Figs. 1,2). There was an

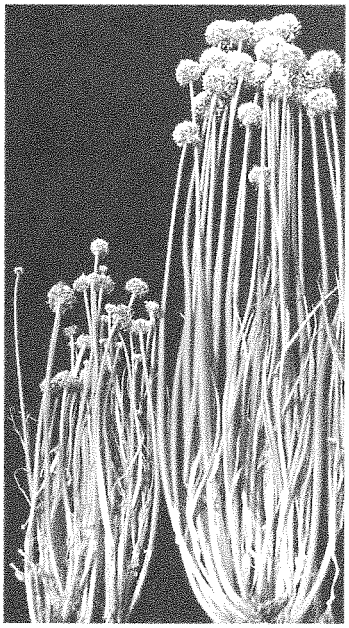

Fig. 1. Haploid (left) and diploid (right) plants of shallot at flowering stage.

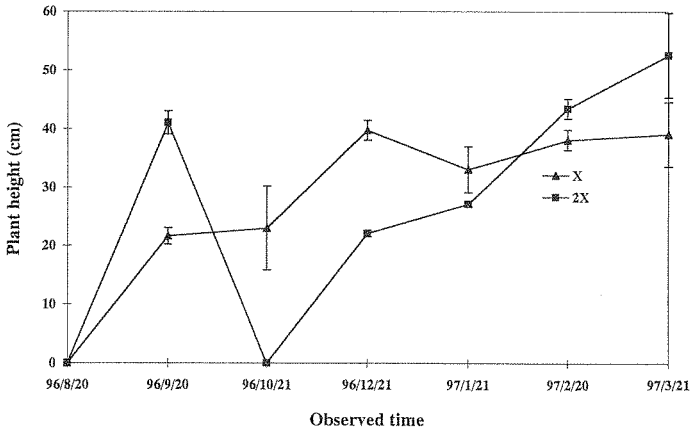

Fig. 2. Plant height of haploid (x) and diploid (2x) shallot.

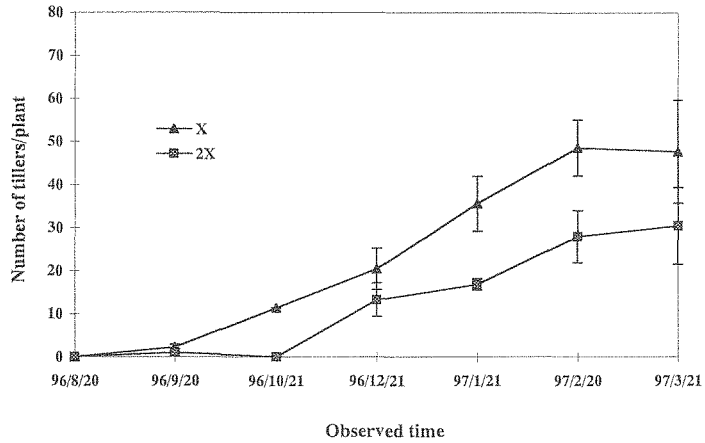

Fig. 3. Number of tillers of haploid (x) and diploid (2x) shallot.

exception in October-December 1996 : the haploid plant was larger than the diploid plant because the diploid plant entered dormancy stage while the haploid plant continuously developed vegetative organs. Number of tillers of the haploid plant was clearly larger than that of the diploid plant (Fig.3). The feature of inflorescence and flower organs of the haploid plant was clearly smaller than those of the diploid plant except number of flower stalks (Figs. 1,6, Table 2). Sizes of flower stalk, inflorescence and flower organs of the haploid plant were nearly half of those of the diploid plant. However, the haploid plant produced flower stalks more than two times of those produced by the diploid plant. The quantity of bulbs of the haploid plant reflected on number and weight of bulbs per plant and varied considerably depend on planting time (Figs. 4,5). The haploid plant planted in July 20, 1996 produced larger number and weight of bulbs per plant than that planted in August 20, 1996. The diploid plant planted in the same times showed different tendencies of bulb production from the haploid plant. Most of the epidermal cells of the haploid plant were surrounded by 3 stomata while those of the diploid plant were surrounded by 4 stomata (Fig. 7). The sizes of epidermal cells and stomata of the haploid plant were clearly smaller than those of the diploid plant (Table 3).

Cytological characters

The haploid plant had 8 somatic chromosomes (Fig. 8). Relative lengths and arm

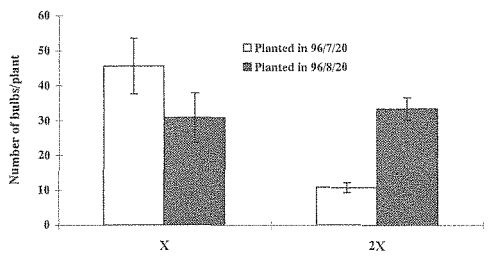

Fig. 4. Number of bulbs of haploid (x) and diploid (2x) shallot.

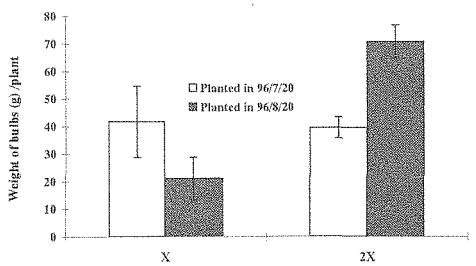

Fig. 5. Weight of bulbs of haploid (x) and diploid (2x) shallot.

Table 2 Flower stalks, inflorescences and flower organs of haploid and diploid shallot

Material	Number of flower stalks per plant	Length of flower stalk (cm)	Diameter of infloresence (cm)	Length of perianth (mm)	Length of anther (mm)	Diameter of ovary (mm)
Haploid	54.5±1.3	47.0±1.5	3.2±0.6	2.4±0.1	1.1±0.1	1.5±0.0
Diploid	20.5±1.3	84.1±1.9	6.4±0.3	4.6±0.1	1.8±0.0	3.0±0.0

Table 3 Length and width of epidermal cells and stomata of haploid and diploid shallot

Material	Length of epidermal cell (μm)	Width of epidermal cell (μm)	Length of stomata (μm)	Width of stomata (μm)
Haploid	263.3± 9.0	27.0±1.5	36.1±0.4	30.7±0.4
Diploid	355.5±10.9	30.0±1.1	46.8±0.6	35.3±0.4

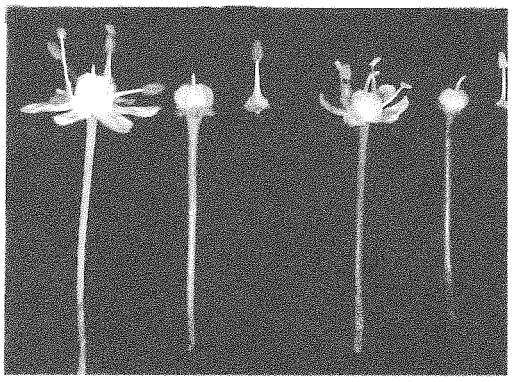

Fig. 6. Flower organs of haploid (right) and diploid (left) shallot.

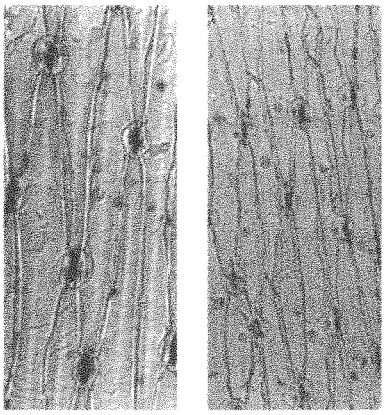

Fig. 7. Epidermal cells and stomata of haploid (right) and diploid (left) shallot. x 50.

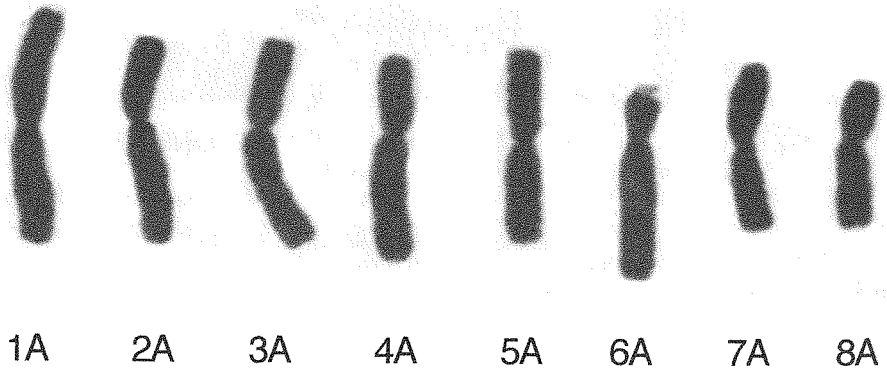

Fig. 8. Karyotype of haploid shallot.

ratios of the chromosomes are shown in Table 4. All the chromosomes could be identified with these two characters. Eight univalent chromosomes were observed at meiotic metaphase in all the PMCs examined in the haploid plant (Fig.9, Table 5). These results indicate that there is not any homologous chromosome pair in the genome A. The haploid plant could not form any fertile pollen grains. The seed fertility of the haploid plant was extremely low (Table 6). Two seeds were obtained in crossing with *A. fistulosum* and in open pollination. These seeds could germinate but could not survive. Spontaneous chromosome doubling was rarely observed, and the plants of doubled haploid occurred could be selected by morphological observations.

Table 4 Length and arm ratio of chromosomes of haploid shallot.

Chromosome	Relative length	Arm ratio
1A	14.99±0.19	0.91±0.01
2A	14.75±0.13	0.59±0.03
3A	13.08±0.30	0.76±0.07
4A	12.95±0.20	0.68±0.02
5A	12.65±0.20	0.88±0.05
6A	11.44±0.27	0.29±0.01
7A	10.27±0.18	0.91±0.03
8A	9.52±0.28	0.67±0.07

The results obtained in this study revealed that the haploid plant of shallot had many morphological and cytological characteristics. The haploid plant was vigorous and could withstand even the unfavorable climates such as high and low temperatures. Therefore, it will be easy to maintain the haploid plant for long time. The feature of the haploid plant was clearly smaller than that of the diploid plant while the tillering of the haploid plant was more active. The haploid plant was evergreen. The haploid plant was almost complete

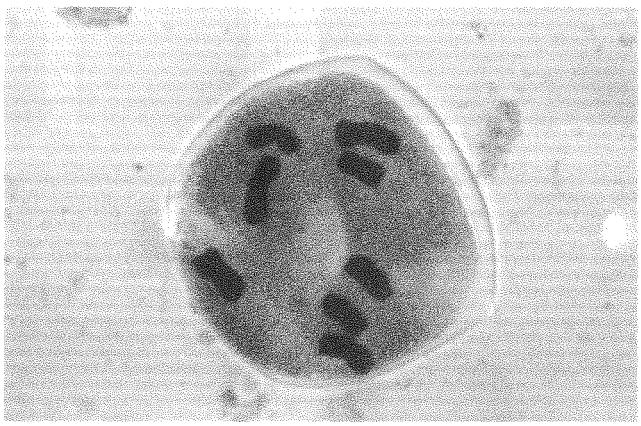

Fig. 9. Chromosomes at meiotic metaphase-I in a PMC of haploid shallot. Eight univalent chromosomes are observed.

Table 5 chromosome pairing and pollen fertility in haploid shallot

Number of PMCs observed	Number of PMCs showing 8I*	Percentage of fertile pollen
510	510	0

*Eight univalents

Table 6 Seed fertility in haploid shallot.

Pollen parent	Number of florets pollinated	Number of seeds obtained	Seed set (%)	Number of seeds that germinated	Number of seedlings that survived
Shallot (Diploid)	1101	0	0	—	—
A. fistulosum	1096	1	0.02	1	0
Open pollination	15148	1	0.001	1	0

sterile both in pollen formation and seed set. However, it was easy to propagate vegetatively this plant by division. Since the haploid plant of shallot has these characteristics, it is interesting to make use of this plant as a new green onion cultivar.

A haploid plant described in this report seem to be an exellent material for genome analysis and construction of genetic map in shallot because it has single genome A. Shigyo et al. succeeded to establish a series of alien monosomic addition lines of Japanese bunching onion (*Allium fistulosum*) with extra chromosomes from shallot[14]. Using these alien monosomic addition lines, they determined the chromosomal locations of many genes and genetic markers. The haploid plant will be helpful to this type of genetic works.

The doubled haploid line derived from the haploid plant of shallot will greatly decrease the time required to develop the pure line. The line can be selfed to produce true seed shallot and crossed with other strains or species to produce F1 hybrids if its homozygosity is proved. Homogeneity in the selfed progenies from the doubled haploid of common onion was examined by isozyme and RAPD markers[7]. The results indicated a high level of homogeneity in the progenies. A doubled haploid line of shallot have already been obtained from the haploid plant reported in this study by in vitro colchicine treatment of the shoot tips. Studies on the characteristics of the doubled haploid line are undergoing. Selfing and crossings of the doubled haploid line are also trying now. The results will be described in the future report.

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シャロットの半数体の形態的及び細胞遺伝学的特性

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摘　　要

シャロット（*Allium cepa* L. Aggregatum group）の二倍体（$2n=16$，ゲノム AA）と三倍体（$2n=24$，AAA）の交配実生の中から見いだした半数体（$2n=8$，A）について形態的及び細胞遺伝学的特性を調査した．

半数体は二倍体と比較して草丈が低く，葉，花茎，花序及び花器がいずれも小さかった．しかし，半数体は生育が旺盛で，良く分けつし，分球により容易に増殖することができた．半数体の8本の染色体の長さ及びくびれの位置は連続的に異なり，同形・同大の染色体は一組も観察されなかった．また，観察した花粉母細胞の全てが減数分裂第一中期において8個の一価染色体を形成した．したがって，シャロットのゲノム A の中には相同性が高い染色体の組は存在しないと考えられる．花粉稔性は全く無かった．また，半数体の多数の小花にシャロットの二倍体の花粉を受粉したが，種子は全く得られなかった．さらに，ネギの花粉を受粉した場合及び放任受粉をさせた場合にそれぞれ1粒づつの種子が得られ，これらの種子は倍地上で発芽したが，植物体へ生長することはできなかった．これらの結果から，シャロットの半数体は雄性，雌性ともに完全不稔であると考えられる．