

## Inheritance of Bulb Formation in *Allium schoenoprasum* L.

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Chives (*Allium schoenoprasum*) continuously produce leaves during spring and summer, and continue to develop leaves in winter, although growth is slow (evergreen). They do not produce well-defined bulbs, whereas asatsuki (*A. schoenoprasum* var. *foliosum*) forms bulbs with leaf desiccation in summer. Bulb formation of F<sub>1</sub> progenies of chives × asatsuki and BC<sub>1</sub> plants obtained by crosses with asatsuki was investigated in this study. No F<sub>1</sub> progenies formed bulbs. The bulb formation of BC<sub>1</sub> was classified based on the bulbing ratio into two phenotypes, e.g. non-bulb-forming and bulb-forming types; however, the variation persisted in obvious bulb-forming and non-bulb-forming individuals. The index of the maximum thickness of scale leaves was an alternative to clearly segregate the BC<sub>1</sub> progenies into three types, e.g. no bulb formation, intermediate, and bulb-forming phenotypes. These results indicate that more than two recessive genes are involved in the bulb formation of chive.

**Key Words:** *Allium schoenoprasum*, BC<sub>1</sub>, bulbing ratio, maximum thickness scale leaves.

### Introduction

Genetic control of bulb formation in geophytes is poorly understood because of the difficulties of obtaining appropriate plant materials that have bulb-forming and non-bulb-forming phenotypes within species, crossability with fertile generations for inheritance analysis or the controllability of bulb formation by environmental factors. It has been shown that storage organ formation of Dutch iris (*Iris* × *hollandica*) and lotus (*Nelumbo nucifera*) plants is controllable by the environment (Masuda et al., 2007; Okubo and Uemoto, 1981). Recently obtained populations of garlic seedlings demonstrate a large variation in vegetative and reproductive characteristics, including bulbing ability and response to environmental conditions (Kamenetsky et al., 2003; Shemesh et al., 2008).

Many species form bulbs in the genus *Allium*. Morphological, ecological, and physiological studies on bulb formation and development in such *Allium* species, particularly in common onion (*Allium cepa*) and garlic (*Allium sativum*), have been performed because of their worldwide commercial importance. Long daylength is

generally the major environmental factor in bulb formation and development in *A. cepa* (Magruder and Allard, 1937). Although there is physiological understanding of bulb formation in the common onion, the inheritance mode of bulb formation not only in bulbous *Allium* species but also in other bulbous plants has been poorly investigated and is not well understood, maybe due to the sterility of F<sub>1</sub> hybrids in interspecific crosses, if obtainable, to obtain the next generation of F<sub>2</sub> or BC<sub>1</sub> for inheritance analysis.

Chives (*Allium schoenoprasum*) are used in many dishes (Poulsen, 1990) and their tufted appearance, attractive green foliage, and purple flowers make them useful as ornamental edging plants or for growth in clumps among other herbs (Jones and Mann, 1963). The species is highly polymorphous, being widely distributed throughout the world (Levan, 1936) and many ecotypes and varieties exist (Poulsen, 1990; Stearn, 1978).

The term “chives” is commonly used in Japan as the English name for *A. schoenoprasum* var. *foliosum*, the Japanese name of which is “asatsuki”. “Chives” is therefore restricted to *Allium schoenoprasum* var. *schoenoprasum* (type species) and the term “asatsuki” is used for *A. schoenoprasum* var. *foliosum* to discriminate the two varieties in this article.

Chives produce leaves abundantly during spring and summer, and continue to develop leaves even in winter when growth vigor weakens under our conditions

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(evergreen). They do not produce well-defined bulbs (as already described by Jones and Mann, 1963). Chives were therefore propagated by seeds. Asatsuki, also an edible minor crop in Japan, forms bulbs and ceases its growth with leaf desiccation under natural long-day conditions in summer (Takagi, 1987). It sprouts from bulbs in autumn and continues to develop leaves, with slow growth in winter, until the next year of bulb formation. Asatsuki was vegetatively propagated by bulbs.

$F_1$  progenies of reciprocal crosses between chives and asatsuki show the characteristics of continuous growth without bulb formation in summer, similar to chives (Inada, 1997); however, the inheritance mode of bulb formation has not been studied since no  $F_1$  plants formed bulbs. The inheritance of bulb formation in  $F_1$  seedlings of chives  $\times$  asatsuki and in  $BC_1$  seedlings backcrossed with asatsuki was investigated in this study.

## Materials and Methods

### Plant materials and culture conditions

The chives and asatsuki plants used in this study were conserved by vegetative propagation in an unheated plastic house at Iwate University (39.42°N latitude, 141.8°E longitude) for long years. Their origins were seeds obtained from seed companies in the Netherlands and Japan, respectively.

Each individual plant of chives and asatsuki was used as cross parents. Twenty-six individuals of the  $F_1$  hybrids obtained by a cross between chives (seed parent) and asatsuki (pollen parent) were used for morphological measurement. One  $F_1$  hybrid, which resembled chives, was selected and backcrossed with asatsuki as a pollen donor in 2005. Fifty-two  $BC_1$  individuals were obtained by the backcross. They were all grown in the plastic house and used for the following examinations.

### Observation and measurement of bulb formation traits

#### 1. Bulbing ratio (BR)

Each of five clonal plants of chives, asatsuki, and their  $F_1$  was taken from July to August 2005, and their bulbing ratios were measured. BR was obtained by the formula in which the maximum bulb diameter was divided by the maximum pseudostem (neck) diameter. Moreover, the BRs of each individual of chives, asatsuki, and the  $F_1$  for backcrossing were measured from 2006 to 2008

to evaluate the annual change; BRs of  $BC_1$  plants obtained by crossing the  $F_1$  with asatsuki were measured for three years, 2006–2008.

#### 2. Maximum thickness of scale leaves (MT)

The formed bulbs were horizontally cut at the maximum diameter and the cut surface was stained with toluidine blue for anatomical observation under a stereoscopic microscope. The greatest thickness scale was chosen and measured the maximum thickness of the scale leaf. The average of two years (2007–2008) of data was used for MT.

## Results

### Segregation of bulbing ratios in $F_1$ and $BC_1$ progenies

In summer (from July to August), chives did not form clear bulbs and continued to develop green leaves (Fig. 1). The average number of tillers per plant was 75 in chives. On the other hand, in asatsuki, the aerial part died back and clear bulb formation was observed. The average number of bulblets per plant was 22. The morphology of basal part of leaf sheaths in all  $F_1$  progeny obtained from a cross between chive (♀) and asatsuki (♂) resembled hat of chives, which did not form bulbs and the aerial part continued to grow. The average number of tillers was 60, similar to chives. The maximum bulb diameter ranges of chives, asatsuki, and  $F_1$  were 0.40–0.87 cm, 1.20–2.15 cm, and 0.56–0.92 cm, respectively. Over three years BRs were larger in asatsuki than in chives and  $F_1$  with significant differences at the 1% level (Table 1). BRs in the  $F_1$  population were from 1.02 to 1.19, close to the BR of chives (1.07).

Both bulb-forming and non-bulb-forming plants appeared in  $BC_1$  progenies (Fig. 1). All non-bulb-forming  $BC_1$  progenies and some bulb-forming progenies retained its aerial parts until summer and maintained growth. The average numbers of tillers (bulblets) ranged widely from 15 to 125. The maximum bulb diameter  $BC_1$  ranged from 0.21 to 1.99 cm. The BR of the  $BC_1$  was between 1.48 and 3.43 (Fig. 2). Since the average BR of asatsuki from 2006 to 2008 was  $4.52 \pm 1.50$  and that of chives was  $1.30 \pm 0.15$ , it was defined that  $BC_1$  progenies having BRs of 3.02–6.02 were bulb-forming types and 1.15–1.45 were non-bulb-forming types. Among all  $BC_1$  progenies (52 individuals), 6 individuals were classified as bulb-forming types and



Fig. 1. Morphology of the basal part of leaf sheaths (August, 6. 2007).

11 as non-bulb-forming types. The variation was, however, continuous from obvious bulb-forming to non-bulb-forming individuals (Fig. 2). No clear segregation of bulb-forming and non-bulb-forming plants was observed in BC<sub>1</sub> progenies based on BR values.

#### Segregation of maximum thickness of scale leaves in BC<sub>1</sub> progenies

Observation of the cross sections of bulbs at the maximum diameter in BC<sub>1</sub> progenies proved that there were two types of construction (Fig. 3D, E); one developed by tillering inside the bulbs in chives (Fig. 3A) and F<sub>1</sub> (Fig. 3C), the other by the swelling of scale leaves

in asatsuki (Fig. 3B). Three peaks of segregation patterns by MT values were recognized; a peak with an MT value range of 1.0–1.5 mm, very close to those of chives (0.7 mm) and the F<sub>1</sub> (1.1 mm), that of >5 mm including the value in asatsuki (6.4 mm), and that with intermediate values of 3.0–3.5 mm between the two peaks (Fig. 4). The intermediate peak was the highest.

#### Discussion

The results in this study that F<sub>1</sub> progenies of chives crossed with asatsuki resembled chives in the morphology of the basal part and did not form bulbs coincide with the previous report by Inada (1997). The morphology of the F<sub>1</sub> hybrid between asatsuki (bulbous) and Welsh onion (*Allium fistulosum*) (syn. Japanese bunching onion) (non-bulbous) resembled that of Welsh onion and did not form bulbs (Gonzalez et al., 1987; Umehara et al., 2006). The results together with those of this study suggest that the genetic trait of bulb formation is recessive in *A. schoenoprasum*. The hybrid of rakkyo (*A. chinense*) (bulb-forming) and Welsh onion forms bulbs (Nomura, 1996). *Allium* × *wakegi* forms bulbs and originated from natural interspecific hybridization between Welsh onion (*A. fistulosum*) as the maternal plant and shallot (*A. cepa* Aggregatum group) as the paternal plant (Hizume, 1994; Tashiro, 1984; Tashiro et al., 1995). Reciprocal crossings have also been performed by Arifin et al. (2000), and both formed bulbs. Furthermore, Masuzaki et al. (2007) revealed that the genetic trait of bulb formation is dominant in *A. cepa*. Bulb formation seems to be dominant in other *Allium* species but recessive in *A. schoenoprasum*.

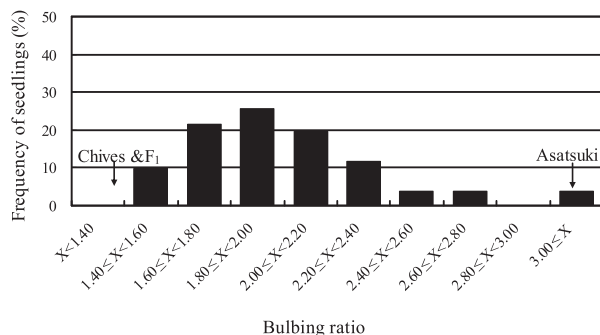
It was proved in this study that MT is more desirable than BR as an indicator of bulb formation in bulbs inside which tillering occurs since the swelling derived from tillering does not reflect the true swelling of scale leaves. BC<sub>1</sub> plants were segregated into three phenotypes, bulb-forming, non-bulb-forming and intermediate pheno-

**Table 1.** Difference in bulbing ratios of asatsuki, chives and the F<sub>1</sub> progeny over three years.

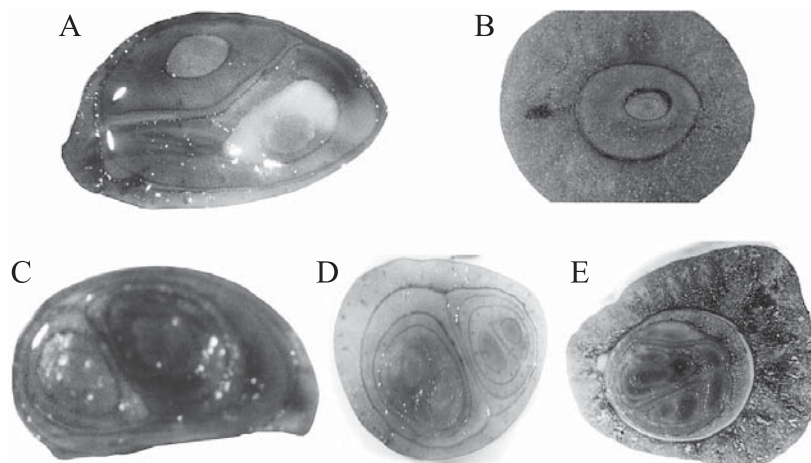
Accession	2006	2007	2008
Asatsuki	6.01 a <sup>z</sup>	4.54 a	3.02 a
Chives	1.27 b	1.19 b	1.47 b
F <sub>1</sub>	1.25 b	1.27 b	1.38 b

Bulbing ratio: Maximum bulb diameter/Maximum pseudostem (neck) diameter.

<sup>z</sup> Different letters represent significant difference by Tukey's test ( $P < 0.01$ ,  $n = 5$ ).



**Fig. 2.** Frequency of distribution of bulbing ratio in BC<sub>1</sub> progenies.



**Fig. 3.** Cross section of bulbs. A: Chives, Developed by tillering inside the bulb. (BR: 1.19, MT: 0.5 mm) B: Asatsuki, Developed by swelling of scale leaves. (BR: 4.54, MT: 6.2 mm) C: F<sub>1</sub>, Developed by tillering inside the bulb. (BR: 1.27, MT: 1.1 mm) D: BC<sub>1</sub>, Developed by tillering inside the bulb. (BR: 2.12, MT: 1.4 mm) E: BC<sub>1</sub>, Developed by swelling of scale leaves. (BR: 2.25, MT: 4.3 mm)

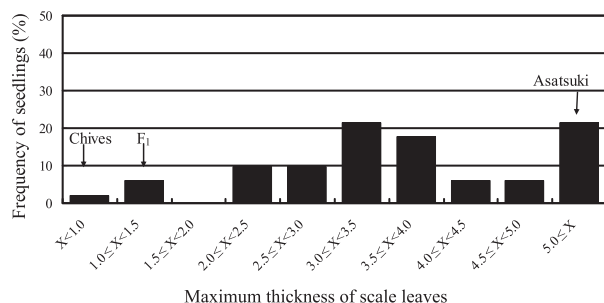


Fig. 4. Frequency of distribution of the value of maximum thickness of scale leaves in BC<sub>1</sub> progenies.

types, suggesting the existence of more than two genes controlling bulb formation. If two genes [A and B; A and B are dominant (non-bulb-forming) over a and b, respectively] are equally additive and control bulb formation, the genotype of asatsuki is aabb and that of chives is AABB. Their F<sub>1</sub> has the genotype of AaBb and the phenotype is non-bulb-forming. The BC<sub>1</sub> population is segregated into bulb-forming (aabb), intermediate (Aabb and aaBb) and non-bulb-forming (AaBb) with a segregation ratio of 1 : 2 : 1. Intermediate phenotypes appeared at the highest rate in this study, indicating that at least two genes control the bulb formation of *A. schoenoprasum*. If three genes controlled bulb formation, the segregation ratio would be 1 : 6 : 1. Considering the distribution of BC<sub>1</sub> plants (peak height) based on MT ratios, it seems more reasonable to consider that more than two genes control bulb formation in this species.

According to the classification proposed by Hanelt (1990), *A. cepa*, *A. fistulosum*, and *A. chinense* are in section *Cepa*, while *A. schoenoprasum* is in section *Schoenoprasum*. It is unknown whether there is any relationship between dominancy in bulb formation and the difference in section-level classification. There are opposite opinions on the evolution of bulb formation in the genus *Allium*. Kamenetsky and Rabinowitch (2006) cited Cheremushkina's opinion that rhizomes were ancestral and primitive traits of *Allium* species, whereas bulb formation represents an advanced evolutionary stage. Fritsch (2001) considered that bulbs are not an advanced product of evolution, but an ancestral characteristic in *Allium* development. Neither the inheritance of bulb formation nor its dominancy was considered from the viewpoint of the evolution of bulb formation because of the difficulties of obtaining fertile progenies. Two types of bulb formation (bulb-forming and non-bulb-forming phenotypes) within one species and the inheritance of bulb formation clarified in this study may contribute to the further understanding of bulb evolution not only in *Allium* species but also in other bulbous species. They may also contribute to molecular research on bulb formation and bulb dormancy, since fertile progenies of both types are available for comparison.

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