Breeding Science 62: 160–169 (2012)
doi:10.1270/jsbbs.62.160

# Modes of inheritance of two apomixis components, diplospory and parthenogenesis, in Chinese chive (*Allium ramosum*) revealed by analysis of the segregating population generated by back-crossing between amphimictic and apomictic diploids

**Ken-ichiro Yamashita*[1)], Yoshiko Nakazawa[2)], Kiyoshi Namai[2)], Masayuki Amagai[2)], Hikaru Tsukazaki[1)], Tadayuki Wako[1)] and Akio Kojima[1)]**

[1)] *Institute of Vegetable and Tea Science, National Agriculture and Food Research Organization*, 360 Kusawa, Tsu, Mie 514-2392, Japan
[2)] *Tochigi Prefectural Agricultural Experiment Station, Utsunomiya*, 1080 Utsunomiya, Tochigi 320-0002, Japan

To investigate the mode of inheritance of apomixis in Chinese chive, the degrees of diplospory and parthenogenesis were evaluated in $F_1$ and $BC_1$ progenies derived from crosses between amphimictic and apomictic diploids ($2n = 16$, 2x). The $F_1$ population was generated by crossing three amphimictic diploids 94Mo13, 94Mo49 and 94Mo50 with an apomictic diploid KaD2 and comprised 110 diploids and 773 triploids. All the diploid $F_1$ plants examined were completely or highly eusporous and completely syngamic. All the triploid $F_1$ plants examined were highly diplosporous and highly parthenogenetic. KaD2 could not transmit its high level of apomixis via monoploid pollen grains. The $BC_1$ population, generated by crossing 94Mo49 with apomictic triploids found in the $F_1$ offspring, exhibited heteroploidy; it comprised haploid, diploid, triploid, tetraploid and various aneuploid individuals. In this generation, clear segregation was observed between diplospory and parthenogenesis. Analysis of the $BC_1$ population suggests that diplospory and parthenogenesis are each controlled by single dominant genes, *D* and *P*, respectively. However, all the $BC_1$ plants characterized as parthenogenetic were diplosporous. The absence of phenotypically eusporous parthenogenetic plants can be explained by assuming that the presence of diplospory gene is a prerequisite for the parthenogenesis gene expression in Chinese chive.

**Key Words:** *Allium ramosum*, apomixis, Chinese chive, diploid, diplospory, parthenogenesis, triploid.

## Introduction

Apomixis (gametophytic apomixis) is a mode of reproduction in which genetic recombination is bypassed allowing only the maternal germplasm to be transmitted to offspring. Apomixis is therefore called “clonal propagation via seeds” (Asker and Jerling 1992, Koltunow and Grossniklaus 2003, Nogler 1984a). This trait occurs in more than 400 flowering plant taxa representing 40 families (Carman 1997, Nogler 1984a). Because almost all varieties of major crops are amphimictic, many researchers have focused on introducing apomixis into corn, rice and other crops. The major concern in these studies of apomixis is the fixation of heterosis, which remarkably reduces both the time required for hybrid variety breeding and the cost of hybrid seed production. The potential economic impact that could be achieved by fixing heterosis via apomixis has been estimated at over US $2.5 billion per year in hybrid rice alone (McMeniman and Lubulwa 1997).

Communicated by T. Sato
Received November 4, 2011. Accepted March 25, 2012.
*Corresponding author (e-mail: kenyamas@affrc.go.jp)

Apomixis comprises two genetic components, apomeiosis (unreduced embryo sac formation) and parthenogenesis (embryogenesis without fertilization of the egg cell) and is categorized into diplosporous and aposporous types according to the mechanism of apomeiosis. In diplosporous apomixis, unreduced embryo sacs arise from embryo sac mother cells (EMCs), whereas those in aposporous apomixis develop from somatic cells of the nucellus or integument and replace immature eusporous embryo sacs. The endosperm develops autonomously or pseudogamously and in the latter case, fertilization of polar nuclei is required for seed development (Asker and Jerling 1992, Koltunow and Grossniklaus 2003, Nogler 1984a).

Diplosporous apomixis is further categorized into three major types based on the cause of unreduced megaspore formation: 1) *Allium*-type, which is characterized by endoreduplicational meiosis; 2) *Taraxacum*-type, which is characterized by first-division restitution; and 3) *Antennaria*-type, which lacks both the first and the second meiotic divisions (Nogler 1984a). Recent genetic studies have revealed that diplospory and parthenogenesis are independently regulated by different genes in some diplosporous apomicts, such as

*Erigeron annuus* (*Antennaria*-type) (Noyes 2006, 2007, Noyes and Rieseberg 2000), *Tripsacum dactyloides* (*Antennaria*-type) (Blakey *et al.* 2001) and *Taraxacum officinale* (*Taraxacum*-type) (Vijverberg *et al.* 2004). In contrast, aposporous apomixis is inherited monogenically in *Pennisetum squamulatum* (Akiyama *et al.* 2004, Goel *et al.* 2006, Ozias-Akins *et al.* 1998), *Cenchrus ciliaris* (Roche *et al.* 1999) and *Panicum maximum* (Savidan 1981).

Chinese chive (*Allium ramosum* L., syn. *A. tuberosum* Rottl. ex Spr.) is an important vegetable crop in east and central Asian countries, especially Japan and China (Fritsch and Friesen 2002, Kumazawa and Katsumata 1965). Almost all Chinese chive varieties and landraces are autotetraploid (2n = 32, 4x) (Kurita 1962, Mathur and Tandon 1965) and have a high degree (over 90%) of diplosporous apomixis with pseudogamous seed development (Kojima *et al.* 1991, Kojima and Nagato 1997). From segregation analysis of parthenogenesis in progenies of crosses between tetraploid varieties, Nakazawa *et al.* (2006) speculated that parthenogenesis in Chinese chive is regulated by a single dominant gene. In our previous studies, both an apomictic diploid (2n = 16, 2x) and amphimictic diploids were discovered (Kojima and Nagato 1997, Kojima *et al.* 2001). These diploid materials are useful for producing populations segregating for apomixis at a lower ploidy level. Using such populations permits elucidation of the mode of inheritance for apomixis, including information on the number of loci controlling apomixis (monogenic or polygenic), the genotypes at those loci, the relationship between the phenotypic expression of diplospory and parthenogenesis, and the presence or absence of gametophytic selection.

In the present study, both the degrees of diplospory and parthenogenesis in numerous $F_1$ and $BC_1$ progenies derived from crosses between the amphimictic and apomictic diploids were evaluated to elucidate the mode of inheritance of apomixis in Chinese chive in detail.

## Materials and Methods

*Generation of progeny populations*

Two successive cross programs were conducted to generate progeny populations segregating for apomixis at ploidy levels lower than tetraploid.

*1) $F_1$ offspring*: Three amphimictic diploids (2n = 16, 2x) found among Mongolian landraces by Kojima *et al.* (2001), 94Mo13, 94Mo49 and 94Mo50, were crossed as the seed parent with KaD2, the apomictic diploid found by Kojima and Nagato (1997) as a dihaploid among seedlings of the tetraploid variety 'Kaoshung', as the pollen parent. The obtained $F_1$ seeds were sown on 200-cell plug trays and the seedlings were transplanted to pots. The ploidy level of seedlings was evaluated by flow cytometry with the ploidy analyzer (Partec GmbH, Münster, Germany) and Partec CyStain UV Precise P kit using a small amount of green leaf

**Table 1.** DNA markers used in this study

| Marker name | Marker size (bp) | | Primer sequence | Reference or origin |
|---|---|---|---|---|
| KaD2-specific RAPD markers used for evaluating hybrid status of $F_1$ and $BC_1$ | | | | |
| $A30_{1430}$ | 1430 | | 5′-GACCTGCGATCT-3′ | Nippon Gene Co., Ltd. |
| $A30_{590}$ | 590 | | 5′-GACCTGCGATCT-3′ | Nippon Gene Co., Ltd. |
| $A30_{500}$ | 500 | | 5′-GACCTGCGATCT-3′ | Nippon Gene Co., Ltd. |
| $OPC07_{900}$ | 900 | | 5′-GTCCCGACGA-3′ | Operon Co. Ltd. |
| $OPC07_{550}$ | 550 | | 5′-GTCCCGACGA-3′ | Operon Co. Ltd. |
| Tender Pole-specific SCAR markers used for diplospory test | | | | |
| SCAR-1 | 850 | Forward | 5′-TTTCGCTCCACCAAAGCACT-3′ | OPB01 (Nakazawa *et al.* 2006) |
| | | Reverse | 5′-CCTCTAGCGGACATCACTAT-3′ | |
| SCAR-2 | 500 | Forward | 5′-CTCAAGCTCGCTCTTTAACT-3′ | A48 (Nippon Gene Co., Ltd.) |
| | | Reverse | 5′-CCCTCAAGCTTAAACACTTC-3′ | |
| SCAR-3 | 220 | Forward | 5′-GCAACAACACACTAGTCCTC-3′ | OPB18 (Operon Co., Ltd.) |
| | | Reverse | 5′-GATGGAGATGGAAGCTGGAA-3′ | |
| SCAR-4 | 400 | Forward | 5′-GATGGGTCAAAATGCGTTGG-3′ | OPC14 (Operon Co., Ltd.) |
| | | Reverse | 5′-CTCGTGTGATTATTCGGCAC-3′ | |
| Tender Pole-specific RAPD markers used for diplospory test | | | | |
| $OPA08_{550}$ | 550 | | 5′-GTGACGTAGG-3′ | Operon Co. Ltd. |
| $OPA10_{700}$ | 700 | | 5′-GTGATCGCAG-3′ | Operon Co. Ltd. |
| $OPB10_{870}$ | 870 | | 5′-CTGCTGGGAC-3′ | Nakazawa *et al.* (2006) |
| $OPB15_{1160}$ | 1160 | | 5′-GGAGGGTGTT-3′ | Operon Co. Ltd. |
| RAPD markers used for confirmation of amphimixis in 94Mo13, 94Mo49 and 94Mo50 | | | | |
| $A07_{1100}$ | 1100 | | 5′-TGCCTCGCACCA-3′ | Nippon Gene Co., Ltd. |
| $A42_{1250}$ | 1250 | | 5′-TCCAAGCTACCA-3′ | Nippon Gene Co., Ltd. |
| $A43_{890}$ | 890 | | 5′-AAGTGGTGGTAT-3′ | Nippon Gene Co., Ltd. |
| $A61_{810}$ | 810 | | 5′-GACTGCTATACA-3′ | Nippon Gene Co., Ltd. |
| $OPA10_{700}$ | 700 | | 5′-GTGATCGCAG-3′ | Operon Co. Ltd. |

tissue collected from seedlings according to the manufacturer's protocol. The number of somatic chromosomes was determined by the Feulgen staining and squash method (Nishiyama 1961) after treating seedling root tips with 0.05% colchicine at 20°C for 4 h.

*2) $BC_1$ offspring*: Apomictic triploids (2n = 24, 3x) found in the $F_1$ offspring between 94Mo13 and KaD2 were backcrossed as the pollen parent to the amphimictic diploid 94Mo49 to generate $BC_1$ offspring. The number of somatic chromosomes in individuals of the $BC_1$ offspring was determined by the Feulgen staining and squash method.

To identify and eliminate selfing-derived seedlings from the $F_1$ and $BC_1$ populations, we developed KaD2-specific random amplified polymorphic DNA (RAPD) markers. Detection of polymorphisms among KaD2, 94Mo13, 94Mo49 and 94Mo50 was conducted using random primers A01-72 (Nippon Gene Co., Ltd.) and OPC01-20 (Operon Co., Ltd.) under the PCR condition reported by Nakazawa *et al.* (2006). Consequently, five KaD2-specific markers (Table 1 and Fig. 1) were developed and used for this purpose.

*Evaluation of the degrees of diplospory and parthenogenesis*

In our previous study, the degree of diplospory in Chinese chive was evaluated directly by observing chromosome configuration at meiotic metaphase I in EMCs (Kojima and Nagato 1992a). This microscopic observation to check the degree of diplospory is not suitable for large-scale screening. To evaluate the degree of diplospory in a large number of $F_1$ and $BC_1$ plants, we applied a progeny testing method using the tetraploid variety 'Tender Pole' as a tester (Fig. 2). Theoretically, the ploidy level of progeny plants varies according to a combination of the mode of embryo sac development (diplospory or euspory) and the mode of embryogenesis (parthenogenesis or syngamy; the latter occurs upon fertilization of the egg cell with a sperm cell from 'Tender Pole'). We examined the ploidy level of more than 10 progeny seedlings per subject plant by flow cytometry.

The degree of diplospory in diploid plants was calculated as the percentage of the sum of 2x and 4x plants in the progeny, because 2x and 4x plants were hypothesized to originate from diplosporous parthenogenesis (D-P) and diplosporous syngamy (D-S), respectively (Fig. 2A).

In triploid plants, some progeny plants derived from eusporous syngamy (E-S: hybrid progeny plants) may have nearly 24 chromosomes, therefore they are not distinguishable by flow cytometry from the progeny plants arising from diplosporous parthenogenesis (D-P: apomictic, maternal progeny plants) (Fig. 2B). RAPD markers had been efficiently used for identifying the origin of progeny plants between apomictic and sexual parents in combination with flow cytometry in *Poa pratensis* (Barcaccia *et al.* 1997, Huff and Bara 1993). In the present study, to characterize the two types of progeny plants, we developed 'Tender Pole'-specific RAPD and sequence-characterized amplified region (SCAR) markers. Detection of polymorphism among 'Tender Pole', KaD2, 94Mo13, 94Mo49 and 94Mo50 was

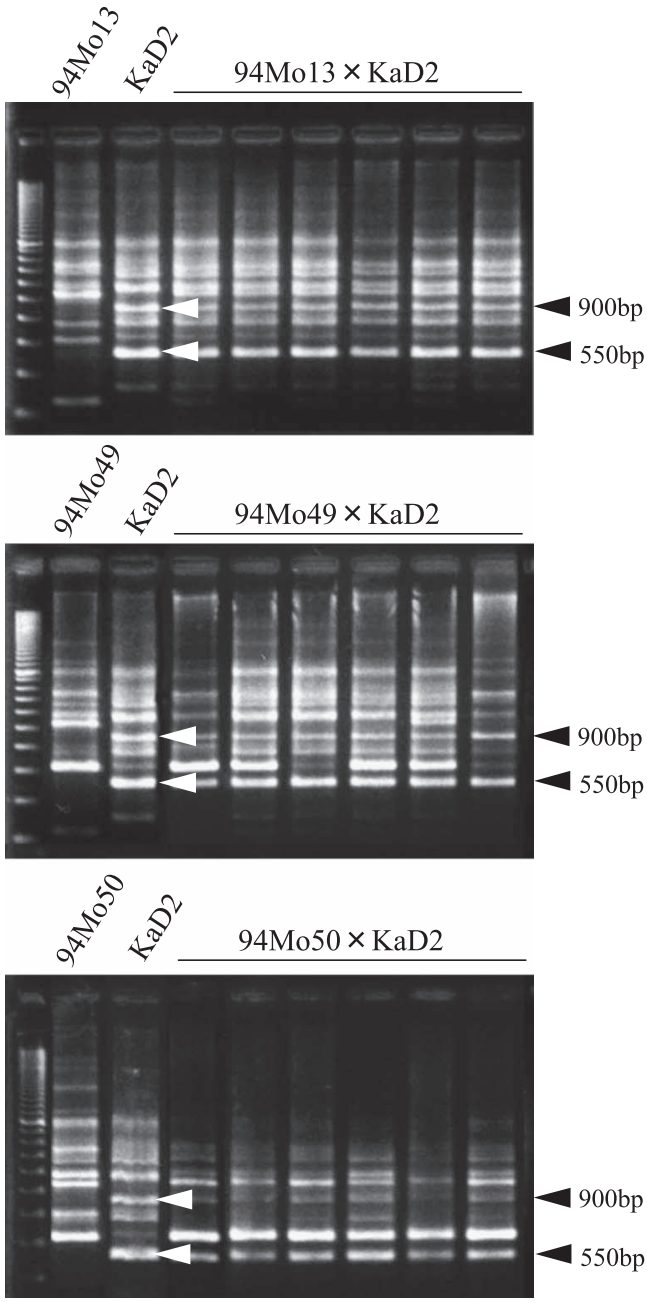


**Fig. 1.** PCR patterns of KaD2-specific RAPD markers ($OPC07_{900}$ and $OPC07_{550}$) in 94Mo13, 94Mo49, 94Mo50, KaD2 and $F_1$ plants between the three amphimictic diploids and KaD2. The hybrid status of the $F_1$ plants was confirmed by the markers.

conducted using primers A01-72 (Nippon Gene Co., Ltd.), OPA01-20, OPB01-20 and OPC01-20 (Operon Co., Ltd.) under the PCR condition reported by Nakazawa *et al.* (2006). Eight RAPDs were selected as 'Tender Pole'-specific markers (Table 1) and three of these RAPDs were newly converted to SCAR markers according to the method reported by Nakazawa *et al.* (2006) (Fig. 3). Reaction mixture (15 µl) for SCAR amplification contained 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 11 pmol of each primer, 40 ng of template DNA and

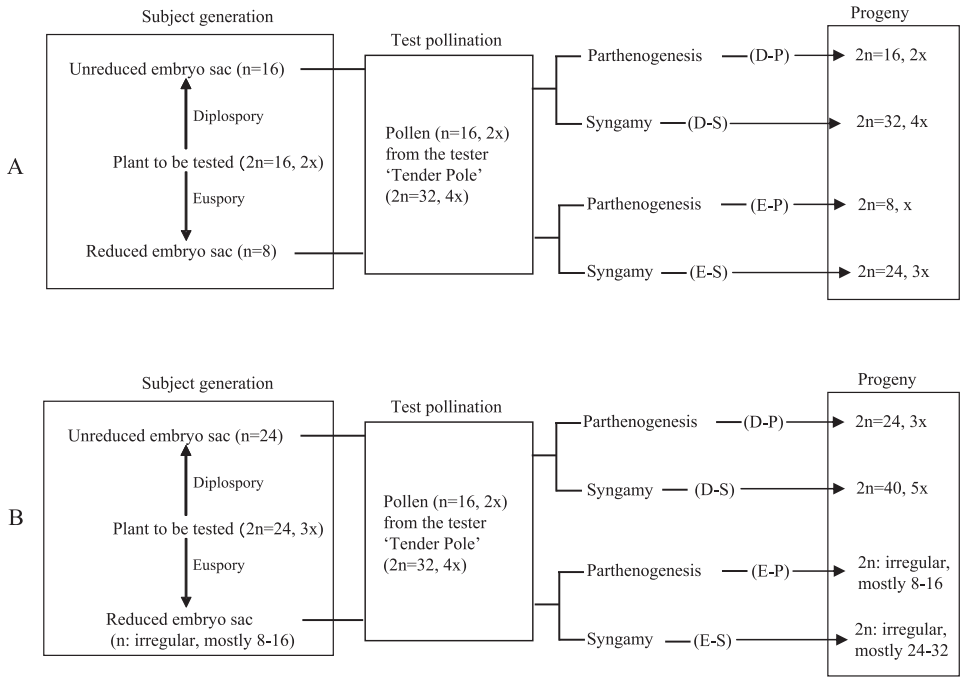


**Fig. 2.** Progeny testing method for evaluating the degree of diplospory in diploid (A) and triploid (B) plants.

0.35 units of Taq DNA polymerase (Takara Co., Ltd.). The PCR program for detection of SCAR-3 was the same as previously published for SCAR-1 (Nakazawa *et al.* 2006). For amplification of SCAR-2 and SCAR-4, the annealing temperature was modified to 64.5°C and 64.0°C, respectively. The 'Tender Pole'-specific DNA markers were applied to test samples from progeny plants in which the number of chromosomes was estimated to be close to 24 by flow cytometry to confirm whether these seedlings were of maternal (D-P) or hybrid (E-S) origin.

The degree of diplospory was estimated in hypotriploid plants (2n = 21–23) in the $BC_1$ population by progeny testing with both flow cytometry and amplification of 'Tender Pole'-specific DNA markers described above. In addition, four RAPDs commonly amplified in 'Tender Pole' and KaD2 were used for confirming amphimixis of 94Mo13, 94Mo49 and 94Mo50 along with the $OPA10_{700}$ (Table 1).

The degree of parthenogenesis can be evaluated indirectly by the progeny test as described above. However, observation of cleared ovules enables direct visualization of parthenogenesis, and is neither time- nor labor-intensive (Kojima and Nagato 1992b, Kojima *et al.* 1994). Embryo sacs were observed in ovules using Herr's clearing method (1982) to directly evaluate the degree of parthenogenesis in individuals of $F_1$ and $BC_1$ populations. Unpollinated florets at four days after flowering were fixed in $FPA_{50}$ solution (formalin : propionic acid : 50% ethanol, 5 : 5 : 90, vol : vol) devised by Herr (1982). Ovules were excised from ovaries and stored in 70% ethanol. After dehydration in 90% to 99.5% ethanol series, ovules were cleared in BB-4-1/2 fluid (lactic acid : chloral hydrate : phenol : clove oil : xylene : benzyl benzoate, 2 : 2 : 2 : 2 : 1 : 1, wt : wt) devised by Herr (1982), and observed with a differential interference-contrast microscope. Ten to 20 apparently fertile ovules per subject plant were observed, and the degree of parthenogenesis was calculated as the percentage of ovules with egg-derived embryos from among apparently fertile ovules.

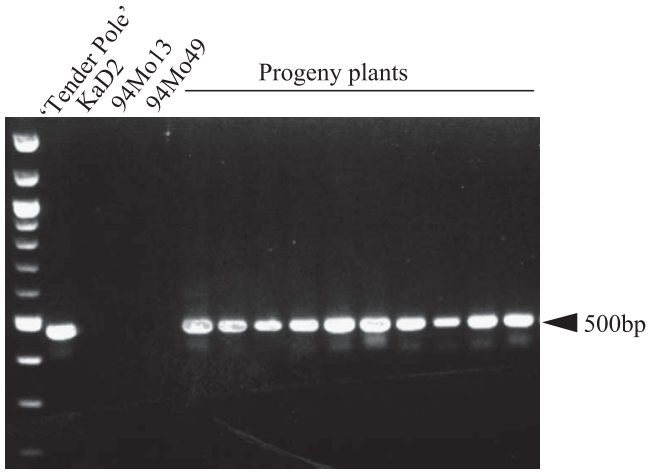


**Fig. 3.** PCR patterns of SCAR2 in 'Tender Pole', KaD2, 94Mo13, 94Mo49 and progeny plants from a triploid plant in the progeny testing method using 'Tender Pole' as a tester.

## Results

*94Mo13, 94Mo49 and 94Mo50*

The eusporous syngamic nature of these Mongolian diploid accessions was confirmed by progeny testing methods

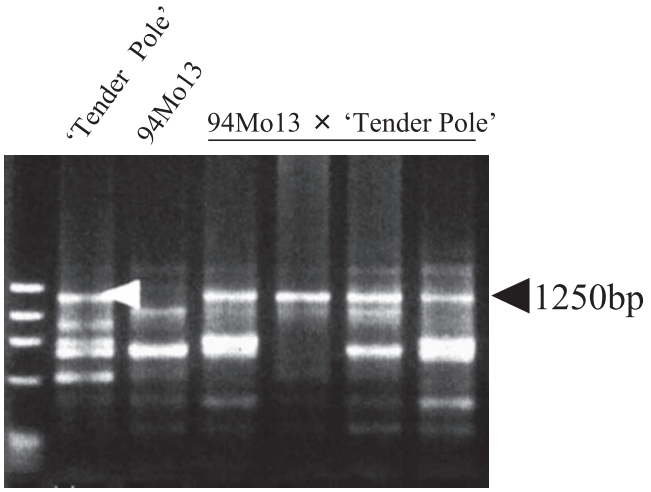


**Fig. 4.** PCR patterns of the marker $A42_{1250}$ in 'Tender Pole', 94Mo13 and $F_1$ hybrids between 94Mo13 and 'Tender Pole'.

described above using the tetraploid variety 'Tender Pole' as a tester. After test crossing, 28, 75 and 24 progeny plants were obtained from 94Mo13, 94Mo49 and 94Mo50, respectively. All of these $F_1$ progeny plants were triploid, and each carried at least one RAPD marker from 'Tender Pole' described in Table 1, which indicated that these $F_1$ plants were hybrids with 'Tender Pole' (Fig. 4).

*$F_1$ offspring*

By crossing the three amphimictic diploids (94Mo13, 94Mo49 and 94Mo50) with apomictic diploid (KaD2), 901 seedlings were obtained. Female parents were emasculated by hand before crossing; however, 18 seedlings were found to have been derived from selfing, although 883 individuals were confirmed as $F_1$ hybrids. Flow cytometry and observation of Feulgen-stained squash preparations revealed that the $F_1$ offspring comprised two distinct ploidy level groups: diploids (110 plants, 12.5%) and triploids (773 plants, 87.5%) (Table 2). We applied the KaD2-specific RAPD markers (Table 1) to 110 diploids and 122 (33, 24, 65 plants from each cross between 94Mo13, 94Mo49, 94Mo50 and KaD2) of 773 triploids to confirm their hybrid status. Because all the diploid and triploid plants examined had at least one KaD2-specific markers (Fig. 1), these plants were confirmed to be hybrids between the amphimictic diploids and KaD2. Kojima and Nagato (1997) reported that endoreduplication occurred in microsporogenesis in KaD2 at a frequency of 9%. Therefore it was speculated that the triploid $F_1$ plants was originated from fertilization between reduced egg cell (x) of the amphimictic diploids and unreduced sperm cells (2x) of KaD2. Although examination of most diploid plants for degrees of diplospory and parthenogenesis was attempted, 25 and 8 plants were eliminated from tests of diplospory and parthenogenesis, respectively, due to the low number of flower and/or seed setting frequency, or early degradation of unpollinated florets. Because no segregation of apomixis components is expected in triploid $F_1$ plants, if they are transmitted via unreduced pollen from KaD2, not all of 122 triploid $F_1$ plants were tested. Consequently, 84 diploid and 41 triploid plants were tested for both diplospory and parthenogenesis. Additionally, one diploid plant was examined for degree of diplospory, and 18 diploid and 42 triploid plants were tested for degree of parthenogenesis (Table 2).

Sporadic occurrence of diplospory was detected by the progeny test in two diploid $F_1$ plants (Table 2). In the progeny of each of these two plants, a few (3/90, 4/45) seedlings were tetraploids, which may have been derived from diplosporous syngamy and the remainder triploids originating from eusporous syngamy. The other 83 diploid $F_1$ plants had only triploid seedlings among their test cross progenies and were therefore all characterized as completely eusporous. The syngamic origin of these tetraploid and triploid seedlings was confirmed using 'Tender Pole'-specific DNA markers. Moreover, the syngamic nature of diploid $F_1$ plants was directly demonstrated by observation of cleared ovules (Fig. 5). Of the 102 diploid $F_1$ plants examined, 96 were completely syngamic; some, if not all, of the ovules observed had well-

**Table 2.** Degrees of diplospory and parthenogenesis in diploid and triploid $F_1$ plants produced by crosses between amphimictic and apomictic diploids

| Ploidy of $F_1$ hybrid | Parents | | No. of plants produced | Degree of diplospory | | | | | Degree of parthenogenesis | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ♀ | ♂ | | No. of plants examined | 0% | 1–10% | 11–90% | 91–100% | No. of plants examined[a] | Sterile[b] | 0% | 1–50% | 51–60% | 61–70% | 71–80% | 81–90% | 91–100% |
| 2x | 94Mo13 | KaD2 | 11 | 6 | 6 | 0 | 0 | 0 | 6 | 1 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 94Mo49 | KaD2 | 87 | 72 | 70 | 2 | 0 | 0 | 84 | 5 | 79 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 94Mo50 | KaD2 | 12 | 7 | 7 | 0 | 0 | 0 | 12 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Total | | 110 | 85 | 83 | 2 | 0 | 0 | 102 | 6 | 96 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3x | 94Mo13 | KaD2 | 98 | 14 | 0 | 0 | 0 | 14 | 33 | 1 | 0 | 0 | 1 | 0 | 1 | 6 | 24 |
| | 94Mo49 | KaD2 | 475 | 16 | 0 | 0 | 0 | 16 | 24 | 1 | 0 | 0 | 0 | 0 | 0 | 4 | 19 |
| | 94Mo50 | KaD2 | 200 | 11 | 0 | 0 | 0 | 11 | 26 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 21 |
| | Total | | 773 | 41 | 0 | 0 | 0 | 41 | 83 | 2 | 0 | 0 | 1 | 0 | 3 | 13 | 64 |

[a] Including all but one (a diploid derived from 94Mo13 × KaD2) of the $F_1$ plants examined for the degree of diplospory.

[b] All of the embryo sacs observed had degenerated.

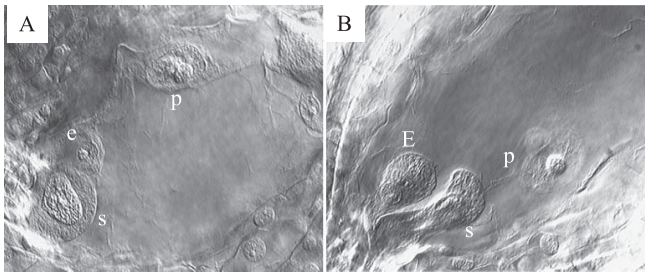


**Fig. 5.** Embryo sacs in syngamic (A) and parthenogenetic (B) plants four days after flowering. Pollination was prevented by means of pinching immature style on the day of flowering. E: embryo, e: egg cell, s: synergid, p: polar nuclei.

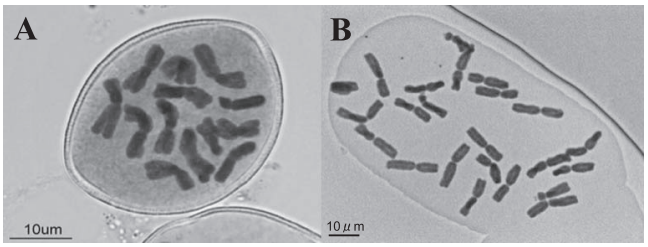


**Fig. 6.** Mitotic metaphase chromosomes in a microspore (A, n = 13) from a triploid $F_1$ plant and in a root-tip cell (B, 2n = 23) of a $BC_1$ plant.

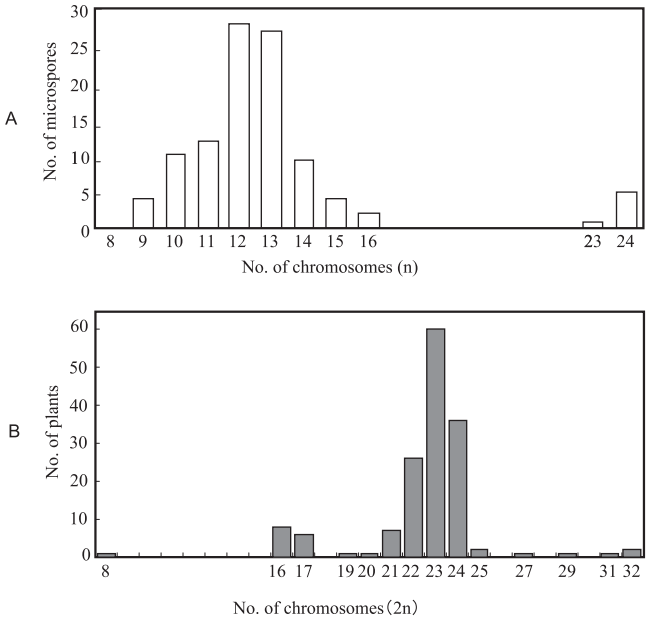


**Fig. 7.** Distribution of number of chromosomes in microspores (A) from an apomictic triploid $F_1$ plant and in root-tip cells of $BC_1$ offspring (B) generated by crosses between the amphimictic diploid 94Mo49 and apomictic triploid $F_1$ plants. A total of 106 microspores were observed in A, and 153 $BC_1$ plants were examined in B.

developed embryo sacs, but no parthenogenetic embryos were observed in any embryo sacs. The remaining 6 diploids had degenerative embryo sacs only. Thus, all of the diploid $F_1$ plants examined, except for sterile ones, were completely or highly eusporous and completely syngamic.

In contrast, all of the 41 triploid $F_1$ plants examined using the progeny test showed very high degrees of diplospory (>90%) (Table 2). Of the 83 triploids examined with the ovule-clearing method, 81 showed high degrees of parthenogenesis (>50%) and 2 were completely sterile. Thus, all of the triploid $F_1$ plants examined, except for sterile ones, were very highly diplosporous and highly parthenogenetic.

Microspores from a triploid $F_1$ plant were observed at mitotic metaphase (Fig. 6A) and the modal number of chromosomes was 12 (Fig. 7A). As much as 64% of the microspores observed had from 11 to 13 chromosomes.

*$BC_1$ offspring*

A total of 153 progeny plants were obtained by backcrossing between the amphimictic diploid 94Mo49 and five of the apomictic triploid $F_1$ plants derived from crossing of 94Mo13 with KaD2. The number of chromosomes in these $BC_1$ plants ranged from 8 (1x) to 32 (4x) (Figs. 6B, 7B). KaD2-specific RAPD markers verified the hybrid status of all of these, except for one haploid. Since the majority (64%) of microspores derived from a triploid $F_1$ plant carried from 11 to 13 chromosomes (Fig. 7A), the number of chromosomes in the majority of the $BC_1$ generation should be between 19 and 21 because these pollen grains fertilize with 94Mo49 female gametes with eight chromosomes. However, $BC_1$ individuals carrying 19–21 chromosomes comprised only 6% (Fig. 7B) of the total. In contrast, most $BC_1$ plants (80%) had 22–24 chromosomes. These results imply that pollen grains with 11–13 chromosomes had a distinct competitive disadvantage compared to those with 14–16 chromosomes.

The haploid $BC_1$ plant was excluded, because it must have inherited no genes from the apomictic parent. We also excluded hyper-triploid and tetraploid $BC_1$ plants (2n = 25–32) to avoid difficulties in genetic analyses. The remaining 145 plants were examined for degrees of diplospory and parthenogenesis. Eventually, 6 diploids, 43 aneuploids and 30 triploids were tested for the degree of both diplospory and parthenogenesis (Table 3 and Fig. 8). Additionally, three plants were examined for the degree of diplospory, and 40 plants were tested for degree of parthenogenesis. The remaining 23 $BC_1$ plants could not be examined for either component of apomixis because of very low flower set or early degeneration of unpollinated florets.

Table 4 shows an example of estimation of the degree of diplospory by progeny testing a triploid $BC_1$ plant. In this case, the number of chromosomes in 10 seedlings produced from the cross between this triploid and 'Tender Pole' was estimated to be between 23.9 and 30.8 by flow cytometry, suggesting two possibilities for the origin of each seedling: either diplosporous parthenogenesis or eusporous syngamy (see Fig. 2B). Further examination with 'Tender Pole'-specific DNA markers revealed that all the seedlings were of syngamic origin (Fig. 3). The most logical explanation for this data is that these seedlings were derived from eusporous

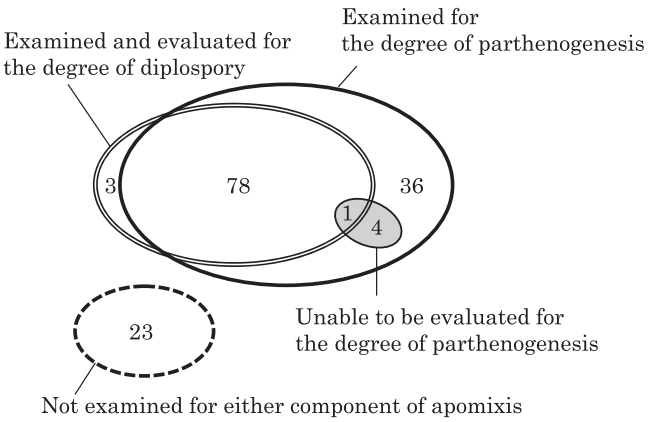


**Fig. 8.** Venn diagram showing the overlap between subpopulations of $BC_1$ plants (2n = 16–24) examined for the degree of diplospory and parthenogenesis.

syngamy; the degree of diplospory was estimated at 0% in this case.

The degree of diplospory in the $BC_1$ population showed a widely separated bi-modal distribution (Table 3). Of the 82 $BC_1$ plants examined, 26 were completely eusporous, while 54 showed a high degree of diplospory (>70%). Sporadic occurrence of diplospory was detected in the remaining 2 $BC_1$ plants. The degree of parthenogenesis also showed a bi-modal distribution. Of the 119 $BC_1$ plants examined, 67 were completely syngamic, and 44 showed high degrees of parthenogenesis (>50%). Another 3 plants showed intermediate degrees (18, 42 and 43%) of parthenogenesis, and the remaining 5 could not be evaluated due to early degeneration of the embryo sacs in all the ovules examined.

Of the 114 $BC_1$ plants evaluated for the degree of parthenogenesis, 36 plants could not be evaluated for the degree of diplospory due to low seed set. Half (18) of them exhibited the non-parthenogenetic (syngamic) phenotype. Another 15 were highly parthenogenetic (60-100%). The remaining 3 were the above-mentioned plants that showed intermediate degrees of parthenogenesis (18–43%).

Segregation of the apomixis components in $BC_1$ offspring is illustrated in Fig. 9, where plants with a degree of diplospory greater than 70% were categorized as diplosporous, and plants with a degree of parthenogenesis greater than 50% were categorized as parthenogenetic. Of the 78 $BC_1$ plants examined for both degree of diplospory and parthenogenesis, 28 were diplosporous and parthenogenetic, 24 were diplosporous and completely syngamic, and 25 were completely eusporous and completely syngamic. The remaining plant, a hyper-diploid (2n = 17, 2x + 1), showed a very low degree of diplospory (8.3%) and was completely syngamic. We did not categorize this plant as diplosporous but omitted it from Fig. 9. Interestingly, there were no plants showing eusporous parthenogenesis at any frequency.

## Discussion

In the present study, the $F_1$ population between amphimictic (94Mo13, 94Mo49 and 94Mo50) and apomictic (KaD2) diploids and the $BC_1$ population between 94Mo49 and apomictic triploid $F_1$ plants were used to evaluate the mode of inheritance of apomixis. Because the parental diploid plants belong to the same species, production of these populations was accomplished without concern for hybrid sterility attributable to interspecific or intergeneric crosses. There was no clear segregation of apomixis components in the $F_1$ population either at the diploid or triploid level in this study. All of the diploid $F_1$ plants characterized were either completely or highly eusporous and completely syngamic, while all the triploid ones were very highly diplosporous and highly parthenogenetic (Table 2). If the genes responsible for apomixis in Chinese chive are dominant, as reported in many other plant taxa (Nogler 1984b, Noyes and Rieseberg 2000, Ozias-Akins *et al.* 1998, van Dijk *et al.* 1999), no segregation of the components of apomixis would be expected to appear in the triploids, because the unreduced diploid pollen grains should have a genotype identical to that of the apomictic parent KaD2. In contrast, it is notable that neither diplospory nor parthenogenesis was observed in the diploid $F_1$ plants. In

**Table 3.** Degrees of diplospory and parthenogenesis in diploid (2n = 16), aneuploid (2n = 17–23) and triploid (2n = 24) $BC_1$ plants

| No. of chromosomes | No. of plants produced | Degree of diplospory | | | | | | | Degree of parthenogenesis | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of plants examined | 0% | 1–10% | 11–70% | 71–80% | 81–90% | 91–100% | No. of plants examined[a] | Sterile[b] | 0% | 1–10% | 11–20% | 21–30% | 31–40% | 41–50% | 51–60% | 61–70% | 71–80% | 81–90% | 91–100% |
| 16 | 8 | 6 | 6 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | 6 | 3 | 2 | 1 | 0 | 0 | 0 | 0 | 5 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 1 | 0 | – | – | – | – | – | – | 0 | – | – | – | – | – | – | – | – | – | – | – | – |
| 20 | 1 | 0 | – | – | – | – | – | – | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 | 7 | 0 | – | – | – | – | – | – | 3 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 22 | 26 | 6 | 1 | 0 | 0 | 0 | 0 | 5 | 21 | 1 | 10 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 7 |
| 23 | 60 | 35 | 10 | 0 | 0 | 0 | 1 | 24 | 50 | 2 | 30 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 3 | 3 | 10 |
| 24 | 36 | 32 | 7 | 1 | 0 | 2 | 3 | 19 | 33 | 0 | 15 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 1 | 2 | 12 |
| Total | 145 | 82 | 26 | 2 | 0 | 2 | 4 | 48 | 119 | 5 | 67 | 0 | 1 | 0 | 0 | 2 | 2 | 1 | 4 | 8 | 29 |

[a] Including all but one hypotriploid (2n = 23) and two triploids of the $BC_1$ plants examined for the degree of diplospory.

[b] All of the embryo sacs observed had degenerated.

**Table 4.** Example of estimation of the degree of diplospory in a triploid $BC_1$ plant (2n = 24) by progeny testing (see Fig. 2B); in this case, the degree of diplospory was estimated as 0% (0/10)

| Progeny seedling | Flow cytometry score (arbitrary unit) | | | Origin of seedling confirmed with DNA markers | Logical explanation for origin |
|---|---|---|---|---|---|
| | Mean | CV (%) | Estimated no. of chromosomes | | |
| 1 | 230.44 | 2.28 | 24.7 | S[a] | E[a]-S[a] |
| 2 | 238.41 | 3.04 | 25.5 | S | E-S |
| 3 | 237.77 | 3.68 | 25.5 | S | E-S |
| 4 | 233.97 | 3.10 | 25.1 | S | E-S |
| 5 | 264.99 | 3.11 | 28.4 | S | E-S |
| 6 | 239.51 | 3.24 | 25.7 | S | E-S |
| 7 | 287.32 | 2.18 | 30.8 | S | E-S |
| 8 | 222.80 | 2.58 | 23.9 | S | E-S |
| 9 | 260.48 | 2.40 | 27.9 | S | E-S |
| 10 | 230.11 | 2.06 | 24.6 | S | E-S |
| Tender Pole (Control) | 298.77 | 3.77 | 32.0 | | |

[a] E: euspory, S: syngamy.

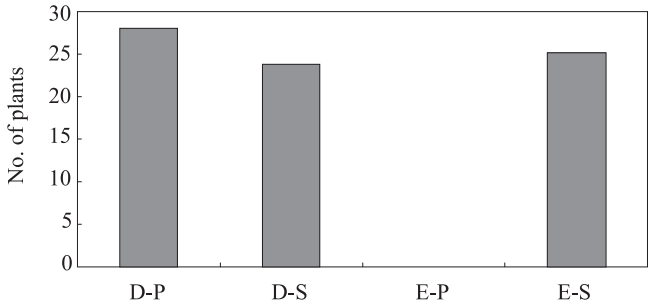


**Fig. 9.** Segregation of apomixis components in $BC_1$ population. D: diplosporous (degree of diplospory >70%), E: eusporous (degree of diplospory = 0%), P: parthenogenetic (degree of parthenogenesis >50%), S: syngamic (degree of parthenogenesis = 0%).

other words, KaD2 was unable to transmit its high level of either diplospory or parthenogenesis via monoploid pollen, while it could transmit both components of apomixis via diploid pollen. These results mean that KaD2 is heterozygous for the dominant genes responsible for a high level of apomixis (both diplospory and parthenogenesis). Furthermore, severe gametophytic competition exists among monoploid pollen grains with and without the apomixis genes in KaD2.

Regardless of plant taxa, apomictic diploid plants have not yet been obtained from crossing experiments because of the lack of transmission of apomixis via monoploid gametophytes. Ozias-Akins *et al.* (1998) postulated that a gametic lethal factor would be linked to apomixis genes in *Pennisetum squamulatum.* Nogler (1984b) interpreted the results of crossing experiments in *Ranunculus* as indicating that a dominant apomixis gene expresses pleiotropic, recessive lethal activity, when the gene is carried by monoploid pollen grains. The present results in Chinese chive may be explained either by close linkage of apomixis genes in KaD2 to recessive lethal factors (either as genes or segmental chromosome deficiencies) or by a pleiotropic, recessive lethal effect of the genes.

Gametophytic competition also seemed to exist between diploid and monoploid pollen grains from KaD2. Although the percentage of pollen mother cells showing endoreduplication was only 9% in KaD2 (Kojima and Nagato 1997), the ratio of 3x to 2x $F_1$ plants was as high as 7 : 1 (Table 2). To explain this segregation distortion by only a lethal effect associated with apomixis genes, more than six such loci should be assumed:

[frequency of endoreduplicational pollen mother cells] : ([frequency of meiotic pollen mother cells] × [expected rate of monoploid microspores carrying non-apomixis alleles at 6 loci]) = $0.09 : ([1 - 0.09] \times [0.5]^6) <$ observed rate of 3x to 2x $F_1$ plants (7 : 1).

This assumption is however not valid because the analysis of $BC_1$ offspring indicates that there are two major genes responsible for apomixis in Chinese chive as described below. Instead, it is likely that the poor viability of monoploid pollen grains is caused not only by some lethal factors associated with apomixis genes but also by other deleterious recessive mutations accumulated in a heterozygous state throughout the genome of apomicts, by the mechanism known as Muller's ratchet (1932). Such accumulation of deleterious mutations is also inferred from the fact that pollen grains with 13 or fewer chromosomes inherited from triploid apomictic $F_1$ plants had a distinct competitive disadvantage compared to those with 14 to 16 chromosomes.

In the $BC_1$ offspring, clear segregation was observed between the two components of apomixis, diplospory and parthenogenesis (Table 3). The widely separated bi-modal distributions of the degree of diplospory and parthenogenesis indicate that each apomixis component is regulated by one or a very few genes with a strong dominant effect. Fig. 9 presents a clearer view of this model. Of the 52 $BC_1$ plants characterized as highly diplosporous, 28 were highly parthenogenetic and the remaining 24 were completely syngamic. This result supports the view that a single dominant gene *P* (parthenogenesis) plays a key role in parthenogenesis in Chinese chive (Nakazawa *et al.* 2006), suggests that the *P*

gene is unlinked to the diplospory gene, and confirms that KaD2 is heterozygous for the *P* gene. Similarly, 24 of the 49 completely syngamic $BC_1$ plants were highly diplosporous and the other 25 were completely eusporous. This suggests that diplospory is probably controlled to a large extent by a single dominant gene *D* (diplospory) in Chinese chive and that KaD2 is also heterozygous for the *D* gene. It is therefore likely that the apomictic diploid KaD2, the Mongolian amphimictic diploid accessions and the apomictic triploid $F_1$ plants have the genotypes *DdPp*, *ddpp* and *DddPpp*, respectively.

However, the segregation of apomixis components was apparently still distorted in the $BC_1$ offspring. No $BC_1$ plants exhibited eusporous parthenogenesis; all of the parthenogenetic $BC_1$ plants subjected to the progeny test were diplosporous (Fig. 9). It is unlikely that *dPp* and *ddPp* male gametes from the triploid $F_1$ plants are all lethal, because: 1) *d* and *dd* male gametes are unlikely to be lethal, as half of the syngamic $BC_1$ plants were eusporous; 2) even if *P* has a pleiotropic recessive lethal effect, *Pp* male gametes may be highly competitive, as more than half of the diplosporous $BC_1$ plants were parthenogenetic; and 3) the *D* locus does not appear to be linked to the *P* locus. Hence, a significant number of plants with the either *ddPpp* or *dddPpp* genotype must have been included in the $BC_1$ population. The phenotype of such plants might be expressed as eusporous parthenogenetic with seed abortion or seedling lethality or as eusporous syngamic. The former explanation is, however, unlikely. Of the 36 $BC_1$ plants that produced very few seeds or seedlings that could be evaluated only for the degree of parthenogenesis, only 18 showed a comparatively parthenogenetic phenotype upon observation of cleared ovules. If the *Ppp* genotype had expressed the parthenogenetic phenotype, even in combination with *dd* or *ddd*, $BC_1$ plants with the *ddPpp* or *dddPpp* genotype could have comprised just a subgroup of these 18 plants. Instead, it seems more probable that the phenotype of *ddPpp* and *dddPpp* plants is expressed as eusporous syngamy. Some of the 25 eusporous syngamic $BC_1$ plants and some of the 18 non-parthenogenetic plants with few seeds or progeny likely had the genotype *ddPpp* or *dddPpp*. The absence of phenotypically eusporous-parthenogenetic plants in the $BC_1$ population can be explained by assuming that the presence of diplospory gene is a prerequisite for, or epistatic to, the expression of genes controlling parthenogenesis in Chinese chive. This means that the presence or absence of the *P* gene cannot be determined in eusporous offspring by the ovule-clearing method.

The apomictic diploid parent KaD2 originates from a tetraploid variety 'Kaohsiung' (Kojima and Nagato 1997) through reduced embryo sac formation followed by parthenogenesis. The 'Kaohsiung' was assumed to be heterozygous at both *D* and *P* loci. Because apomixis of Chinese chive is facultative (Kojima and Nagato 1991), reduced embryo sacs as well as unreduced ones can be formed in apomictic plants. In the $BC_1$ progeny, 28 plants showed the diplosporous parthenogenetic phenotype (Fig. 9). In these plants, not only unreduced female gametes but also reduced ones may be formed and some of the reduced female gametes except for haploid ones should have either or both apomixis genes, *D* and *P* as does 'Kaohsiung'. In contrast, in the 25 $BC_1$ plants showing the eusporous syngamic phenotype, only reduced female gametes were formed and none show parthenogenesis. As mentioned above, plants with the genotype *ddPpp* or *dddPpp* were eusporous syngamic. Therefore, it is inferred that the *D* gene has some genetic function promoting the expression of the *P* gene in female gametes in addition to inducing unreduced embryo sac formation.

The modes of inheritance of diplospory and parthenogenesis proposed here for Chinese chive are the same as those reported for *Erigeron annuus*. By analyzing a segregating population of 130 $F_1$ plants from a cross between *E. strigosus*, a eusporous syngamic diploid species and *E. annuus*, a diplosporous parthenogenetic triploid species, Noyes and Rieseberg (2000) constructed an amplified fragment length polymorphism (AFLP) map of loci controlling diplospory and parthenogenesis. They concluded that genes controlling diplospory and parthenogenesis are unlinked, and that each component is influenced mainly by a single dominant gene. In addition, the gene controlling parthenogenesis was suggested to be silent in the absence of the gene controlling diplospory. Noyes (2006) confirmed this hypothesis by reconstructing diplosporous parthenogenesis in the progenies from crosses between two highly diplosporous but non-parthenogenetic triploids lacking any of the four AFLP markers linked to parthenogenesis and a eusporous, phenotypically non-parthenogenetic hyper-diploid carrying all the four parthenogenesis-linked markers. Recently, Koltunow *et al.* (2011) investigated the role of two individual dominant loci in *Hieracium*, loss of apomeiosis (LOA) and loss of parthenogenesis (LOP), using mutants that had lost function of one or both loci. They found that loss of function in either LOA or LOP caused partial reversion to sexual reproduction, while loss of both LOA and LOP functions led to complete reversion to sexual production and concluded that sexual reproduction was the default reproductive mode, upon which apomixis was superimposed. Although mode of apomixis differs between *Allium* (diplosporous) and *Hieracium* (aposporous), apomixis might have evolved in these two species in a similar manner.

As demonstrated for the *Antennaria*-type diplosporous apomixis in *Erigeron* (Noyes and Rieseberg 2000), the segregating heteroploid population produced in the present study will be useful for genetic mapping of genes controlling diplospory and parthenogenesis for *Allium*-type diplosporous apomixis. Moreover, some hypo-triploid apomicts and segregants in this population can be employed in future efforts to reconstruct apomictic diploids free from lethal factors. Such diploids, though not yet obtained in any plant species, will provide an elegant basis for further genetic studies of apomixis.

## Acknowledgement

The present study was supported in part by funds from the Ministry of Agriculture, Forestry and Fisheries of Japan (Bio-Design Program) and also by the Grant-in-Aid for Young Scientists (B)(21780030) of Japan Society for the Promotion of Science. We are deeply grateful to Messrs. Fumiaki Hori, Hisayoshi Maruyama, Satoshi Masuji and Yasuhiro Takashi for their contributions to material plant growing. We wish to express our gratitude to Mses. Teruko Kihira, Keiko Wakita, Hiroko Tanoda and Kimiko Tanaka for their assistance to this work.

## Literature Cited

Akiyama, Y., J.A. Conner, S. Goel, D.T. Morishige, J.E. Mullet, W.W. Hanna and P. Ozias-Akins (2004) High-resolution physical mapping in *Pennisetum squamulatum* reveals extensive chromosomal heteromorphism of the genomic region associated with apomixis. Plant Physiol. 134: 1733–1741.

Asker, S.E. and L. Jerling (1992) Apomixis in plants. CRC Press, Boca Raton, Fl.

Barcaccia, G., A. Mazzucato, A. Belardinelli, M. Pezzotti, S. Lucretti and M. Falcinelli (1997) Inheritance of parental genomes in progenies of *Poa pratensis* L. From sexual and apomictic genotypes as assessed by RAPD markers and flow cytometry. Theor. Appl. Genet. 95: 516–524.

Blakey, C.A., S.L. Goldman and C.L. Dewald (2001) Apomixis in *Tripsacum*: comparative mapping of a multigene phenomenon. Genome 44: 222–230.

Carman, J.G. (1997) Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory, and polyembryony. Biol. J. Linn. Soc. 61: 51–94.

Fritsch, R.M. and N. Friesen (2002) Evolution, domestication and taxonomy. *In*: Rabinowitch, H.D. and L. Currah (eds.) Allium Crop Science: Recent Advances. CABI Publishing, Wallingford, pp. 5–30.

Goel, S., Z. Chen, Y. Akiyama, J.A. Conner, M. Basu, G. Gualtieri, W.W. Hanna and P. Ozias-Akins (2006) Comparative mapping of the apospory-specific genomic region in two apomictic grasses: *Pennisetum squamulatum* and *Cenchrus ciliaris*. Genetics 173: 389–400.

Herr, J.M. Jr. (1982) An analysis of methods for permanently mounting ovules cleared in four-and-a-half type clearing fluids. Stain Technol. 57: 161–169.

Huff, D.R. and J.M. Bara (1993) Determining genetic origins of aberrant progeny from facultative apomictic Kentucky bluegrass using a combination of flow cytometry and silver-stained RAPD markers. Theor. Appl. Genet. 87: 201–208.

Kojima, A., Y. Nagato and K. Hinata (1991) Degree of apomixis in Chinese chive (*Allium tuberosum*) estimated by esterase isozyme analysis. Jpn. J. Breed. 41: 73–83.

Kojima, A. and Y. Nagato (1992a) Diplosporous embryo-sac formation and the degree of diplospory in *Allium tuberosum*. Sex. Plant Reprod. 5: 72–78.

Kojima, A. and Y. Nagato (1992b) Pseudogamous embryogenesis and the degree of parthenogenesis in *Allium tuberosum*. Sex. Plant Reprod. 5: 79–85.

Kojima, A., T. Kozono, Y. Nagato and K. Hinata (1994) Non-parthenogenetic plants detected in Chinese chive, a facultative apomict. Breed. Sci. 44: 143–149.

Kojima, A. and Y. Nagato (1997) Discovery of highly apomictic and highly amphimictic dihaploids in *Allium tuberosum*. Sex. Plant Reprod. 10: 8–12.

Kojima, A., S. Shimazaki, T. Wako and T. Ohara (2001) Sexual diploids found among Mongolian accessions of Chinese chive. Abstracts of the Second International Apomixis Conference, April 24–28, 2001, Como, Italy: 86.

Koltunow, A.M. and U. Grossniklaus (2003) Apomixis: a developmental perspective. Annu. Rev. Plant Biol. 54: 547–574.

Koltunow, A.M., S.D. Johnson, J.C.M. Rodrigues, T. Okada, Y. Hu, T. Tsuchiya, S. Wilson, P. Fletcher, K. Ito, G. Suzuki *et al.* (2011) Sexual reproduction is the default mode in apomictic Hieracium subgenus Polosella, in which two dominant loci fuction to enable apomixis. Plant J. 66: 890–902.

Kumazawa, S. and H. Katsumata (1965) Nira (Chinese chives). *In*: Kumazawa, S. (ed.) Sosai-engei kakuron (Vegetable crops). Yokendo, Tokyo, pp. 292–294.

Kurita, M. (1962) Extreme fragmentation of meiotic chromosomes in *Allium tuberosum* Rottler. Mem. Ehime Univ. Sec. II Ser. B 4: 403–408.

Mathur, M. and S.L. Tandon (1965) Cytology of an autotetraploids *Allium tuberosum*. Indian J. Hort. 22: 382–384.

McMeniman, S. and G. Lubulwa (1997) Project development: an economic evaluation of the potential benefits of interesting apomixis into hybrid rice. Canberra Economic Evaluation Unit ACIAR 28: 1–26.

Muller, H.J. (1932) Some genetic aspects of sex. American Naturalist 66: 118–138.

Nakazawa, Y., K. Namai, A. Kojima, S. Kobayashi, K. Tasaki and M. Amagai (2006) Estimating the inheritance for parthenogenesis in tetraploid Chinese leek (*Allium ramosum*, syn. *A. tuberosum*). Breed. Res. 8: 89–98.

Nishiyama, I. (1961) Saibou Idengaku Kenkyuhou. Yokendo, Japan: 160–163.

Nogler, G.A. (1984a) Gametophytic apomixis. *In*: Johri, B.M. (ed.) Embryology of angiosperms. Springer-Verlag, Berlin, pp. 475–518.

Nogler, G.A. (1984b) Genetics of apospory in *Ranunculus auricomus* V, Conclusions. Bot. Helv. 94: 411–422.

Noyes, R.D. (2006) Apomixis via recombination of genome regions for apomeiosis (diplospory) and parthenogenesis in *Erigeron* (daisy fleabane, Asteraceae). Sex. Plant Reprod. 19: 7–18.

Noyes, R.D. (2007) Apomixis in the Asteraceae: Diamonds in the rough. Func. Plant Sci. Biotech. 1: 207–222.

Noyes, R.D. and L.H. Rieseberg (2000) Two independent loci control agamospermy (apomixis) in the triploid flowering plant *Erigeron annuus*. Genetics 155: 379–390.

Ozias-Akins, P., D. Roche and W.W. Hanna (1998) Tight clustering and hemizygosity of apomixis-linked molecular markers in *Pennisetum* implies genetic control of apospory by a divergent locus that may have no allelic form in sexual genotypes. Proc. Natl. Acad. Sci. USA 95: 5127–5132.

Roche, D., P. Cong, Z. Chen, W.W. Hanna, D.L. Gustine, R.T. Sherwood and P. Ozias-Akins (1999) An apospory specific genomic region is conserved between buffelgrass (*Cenchrus ciliaris* L.) and *Pennisetum squamulatum* Fresen. Plant J. 19: 203–208.

Savidan, Y. (1981) Genetics and utilization of apomixis for the improvement of guineagrass (*Panicum maximum* Jacq.). Proceedings of the XIV International Grasslands Congress, Lexington, Ky., pp. 182–184.

van Dijk, P.J., I.C.Q. Tas, M. Falque and T. Bakx-Schotman (1999) Crosses between sexual and apomictic dandelions (Taraxacum) II: the breakdown of apomixis. Heredity 83: 715–721.

Vijverberg, K., R.G.M. van der Hulst, P. Lindhout and P.J. van Dijk (2004) A genetic linkage map of the diplosporous chromosomal region in *Taraxacum officinale* (common dandelion; Asteraceae). Theor. Appl. Genet. 108: 725–732.