ワケギの起源に関する細胞遺伝学的研究 (3)

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Cytogenetic Studies on the Origin of *Allium wakegi* Araki

III. Restoration of the fertility of *A. wakegi* by doubling the chromosome complement

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Summary

Fertility was investigated in the tetraploid plants derived anew from Korean and Burmese clones of *Allium wakegi.*

These tetraploid plants were completely pollen sterile. However, they had seed sets of 9 to 15 per cent under crossing with *A. ascalonicum, A. fistulosum,* or the amphidiploid hybrid between *A. ascalonicum* and *A. fistulosum.* Many triploid or tetraploid hybrids have been obtained from these crossings.

Introduction

There seems to be no doubt that *Allium wakegi* Araki is an allodiploid plant[1~5,8,9,11]. Amphidiploidy may, therefore, offer a means of restoring fertility to this plant. One of the authors, Tashiro, has already reported on the tetraploid plants derived from a Japanese clone of *A. wakegi* in a previous paper[10]. Contrary to expectation, these tetraploid plants were completely pollen sterile and had seed sets of less than 3 per cent only under crossing with *A. ascalonicum L., A. fistulosum L.*, or the amphidiploid hybrid. On the other hand, more than 90 per cent of the pollen mother cells (PMCs) observed had 16 bivalent chromosomes which showed normal pairing. From these results it was proposed that the tetraploid plants of *A. wakegi* are amphidiploid plants but have a genic sterility. It was, however, unclear whether the genic sterility is a common phenomenon for every tetraploid plant derived from every clone of *A. wakegi.*

Following the previous report, the present paper deals with the fertility and meiotic behavior of the tetraploid plants derived anew from two other clones of *A. wakegi.*

Materials and Methods

Two clones of *A. wakegi* collected in Korea and Burma and their tetraploid plants were used for the observations. The tetraploid plants were induced by culturing the stem tips of each clone on MS basal medium[6] containing 1 g/l colchicine for 4 days[7].

The methods of fertility studies and cytological observations can be found in the previous papers[9,10].

Results and Discussion

Regardless of locality or ploidy none of the material plants examined could produce fertile pollens, and most of the pollen grains observed contained no protoplasm (Table 1). Percentages of pollen grains which contained protoplasm were somewhat lower in the tetraploid plants than in the diploid plants.

The pollen sterility prevented both the diploid plants and tetraploid plants from being selfed and crossed reciprocally with other *Allium* plants. Under crossing with *A. ascalonicum*, *A. fistulosum*, or the amphidiploid hybrid, both of the diploid plants were absolutely free from any seed setting, while both of the tetraploid plants had seed sets of 9 to 15 per cent (Table 2). Many of the seeds obtained from the tetraploid plants were able to germinate (Table 2), and the seedlings were viable. The seed set in the tetraploid plant derived from Burmese clone was somewhat higher than that in the tetraploid plant derived from Korean clone. Crossings were

Table 1. Pollen fertility of Korean and Burmese clones of *Allium wakegi* and their tetraploid plants.

Clone number	Locality	Chromosome number ($2n$)	Percentage of pollen grains: Fertile	Sterile: Contained protoplasm	Sterile: Empty
XY-14	Korea	16	0	8.0	92.0
XY-21	Burma	16	0	5.0	95.0
XXYY-14	(Derived from XY-14)	32	0	0.2	99.8
XXYY-21	(Derived from XY-21)	32	0	0	100

Table 2. Seed setting characteristics in Korean and Burmese clones of *Allium wakegi* and their tetraploid plants.

Cross combination		Number of flowers pollinated	Number of seeds produced	Percentage[a] of ovules that developed into seeds	Number of seeds that germinated	Percentage of seeds that germinated
XY-14	× *A. ascalonicum*	434	0	0	–	–
	× *A. fistulosum*	314	0	0	–	–
XY-21	× *A. ascalonicum*	420	0	0	–	–
	× *A. fistulosum*	332	0	0	–	–
XXYY-14	× *A. ascalonicum*	329	180	9.1	95	52.8
	× *A. fistulosum*	344	208	10.1	94	45.2
	× Amphidiploid hybrid *A. ascalonicum-fistulosom*	283	147	8.7	46	31.3
XXYY-21	× *A. ascalonicum*	85	76	14.9	43	56.6
	× *A. fistulosum*	87	73	14.0	60	82.2
	× Amphidiploid hybrid *A. ascalonicum-fistulosum*	84	50	9.9	22	44.0

a) Percentage of ovules that developed into seeds = $\frac{\text{Number of seeds produced}}{\text{Number of flowers pollinated} \times \text{Number of ovules per flower (6)}} \times 100$

Table 3. Chromosome configuration at metaphase-I in pollen mother cells of the tetraploid plants derived from Korean and Burmese clones of *Allium wakegi*.

Clone number	Frequency of PMCs				Total number of PMCs observed
	Chromosome configuration				
	$14_{II}+4_{I}$	$15_{II}+2_{I}$	16_{II}	$1_{IV}+14_{I}$	
XXYY-14	0	6	96	0	102
XXYY-21	2	4	93	1	100

more successful when *A. ascalonicum* and *A. fistulosum* were used as the pollen parents than when the amphidiploid hybrid was used.

Meiotic behaviors of the Korean and Burmese clones of *A. wakegi* have already been described in the previous paper[9]. These clones showed a quite irregular meiosis, particularly about the occurrences of univalents, heteromorphic bivalents and fragment chromosomes at metaphase-I, laggards and bribges at anaphase-I, and micronuclei at telophase-I. The sterility of these clones can, therefore, be ascribed to their irregular meiosis. On the other hand, meiosis appeared to be fairly normal in both of the tetraploid plants. At metaphase-I in most of the PMCs observed the chromosome configuration was 16_{II}, and in the remaining PMCs it was $15_{II}+2_{I}$, $14_{II}+4_{I}$, or $1_{IV}+14_{II}$ (Table 3). The pairing of the bivalent was quite regular, and the subsequent processes of meiosis were also normal. The chromosome pairing of the Korean and Burmese clones of *A. wakegi* has been normalized successfully by doubling the chromosome complement. Therefore, good sets of seed in the tetraploid plants can be ascribed to this.

The protoplasmic degeneration of pollen grain described in the previous paper[10] was also observed in both of the tetraploid plants examined in the present investigation. This phenomenon was surely the direct cause of the pollen sterility observed in the tetraploid plants. It is not clear from the experimental data why this phenomenon occurred in all the pollen grains. Judging from the normal meiosis, however, there is no doubt that the cause of this phenomenon is a genic one. Since the protoplasmic degeneration of pollen grain has been observed in all the tetraploid plants of *A. wakegi* so far investigated, the cause must be a very critical one, and it may be common to all the clones of *A. wakegi*. It is a subject for further study to obtain the tetraploid plant of *A. wakegi* which shows a pollen fertility.

No marked differences in meiosis and pollen fertility were noticed among the tetraploid plants of *A. wakegi* examined in the previous investigation and those in the present investigation. On the other hand, there were considerable differences in seed setting capacity among these tetraploid plants. Good sets of seed were obtained only in the present investigation. In any case, it is confirmed experimentally that the doubling of chromosome complement is an effective treatment for restoring the seed fertility of *A. wakegi*.

As in the previous investigation[10], many triploid and tetraploid hybrids between the tetraploid *A. wakegi* and other *Allium* plants have been obtained in the present investigation. The genome analysis of *A. wakegi* has already been accomplished with these hybrids. The details will be described in future reports.

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ワケギの起源に関する細胞遺伝学的研究（第3報）
染色体倍加によるワケギの稔性回復

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摘 要

韓国産およびビルマ産ワケギの染色体倍加系統を新たに作出し，これらについて稔性を調査した。

その結果，これらは花粉稔性がまったくなかったが，シャロット，ネギあるいは複2倍性雑種（シャロット×ネギ）との交雑では9～15%の種子稔性を示した．また，これらの交雑の結果，多くの3倍性あるいは4倍性雑種が得られた．