Hordeum bulbosumの受粉によるAegilopscrassa(6x)の倍数性半数体の育成

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SHORT COMMUNICATION

PRODUCTION OF A POLYHAPLOID PLANT OF *AEGILOPS CRASSA* (6X) POLLINATED BY *HORDEUM BULBOSUM*[1)]

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The genome structure of polyploid species can be considered from chromosome pairing of interspecific or intergeneric hybrids involving the species in question. It is often difficult, however, to distinguish autosyndetic pairings from allosyndetic ones. In such case polyhaploid plants provide critical information concerning the genome constitution of polyploid species (Sakamoto 1964). Recently the production of haploid plants of *Hordeum vulgare* Linn. and *Triticum aestivum* Linn. was successful by crossing them with the pollen of *Hordeum bulbosum* Linn. followed by embryo culture, during which chromosomes of the pollen parent were eliminated (Kasha and Kao 1970; Barclay 1975). With the help of this method, it became possible to induce polyhaploid plants in several genera of the tribe Triticeae which are comprised of many complex polyploid species. In order to obtain haploids in the genus *Aegilops*, several species were pollinated by *Hordeum bulbosum* and hybrid embryos obtained were cultured aseptically.

The following *Aegilops* species which have been maintained at the Plant Germ-plasm Institute, Kyoto University, were used as the female parents: *Ae. caudata* Linn. (2x), *Ae. crassa* Boiss. (4x), *Ae. crassa* (6x), *Ae. cylindrica* Host (4x), *Ae. heldreichii* Holzm. (2x), *Ae. juvenalis* Eig (6x), *Ae. longissima* Schw. et Musch. (2x), *Ae. ovata* Linn. (4x), *Ae. speltoides* Tausch (2x), *Ae. squarrosa* Linn. (2x) ssp. *strangulata* Eig, *Ae. triaristata* Willd. (4x), *Ae. triaristata* (6x), *Ae. triuncialis* Linn. (4x) and *Ae. vavilovii* Chenn. (6x). A tetraploid form of *Hr. bulbosum* collected in Turkey was used as the pollen parent. Hand-emasculated spikes of *Aegilops* enclosed in paraffin-paper bags were pollinated four days after by brushing stigma with newly broken anthers of *Hr. bulbosum*. Of 13 species used in the crossing experiment, only six set seeds as shown in Table 1. A high percentage of the seed set was observed in *Ae. crassa* (6x) (46.3%), *Ae. squarrosa* ssp. *strangulata* (30.2%) and *Ae. triaristata* (4x) (53.1%).

Since the hybrid seeds showed a sign of abortion at about ten days after pollination, the embryo culture was applied to them. The embryo of hybrid seeds was cultured with the modified medium used by Brink *et al.* (1944), adding 15 g agar, 1 g yeast extract and 1 g Vitamin B_1 per 1,000 ml of solution. After the dissection in a sterile cabinet, the embryos were planted on the surface of the agar in test tubes,

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Table 1. Seed set induced and plants obtained from intergeneric crosses between six species of *Aegilops* and *Hordeum bulbosum*

Female parents	No. of florets pollinated	No. of seeds set	Percent of seed set	No. of embryos cultured	No. of plants obtained
Aegilops crassa (6x)	320	148	46.25	85	1
Ae. ovata	75	8	10.67	8	0
Ae. squarrosa ssp. *strangulata*	232	70	30.17	58	1
Ae. triaristata (4x)	32	17	53.13	11	1
Ae. triuncialis	170	2	1.18	1	0
Ae. vavilovii	246	1	0.41	1	0

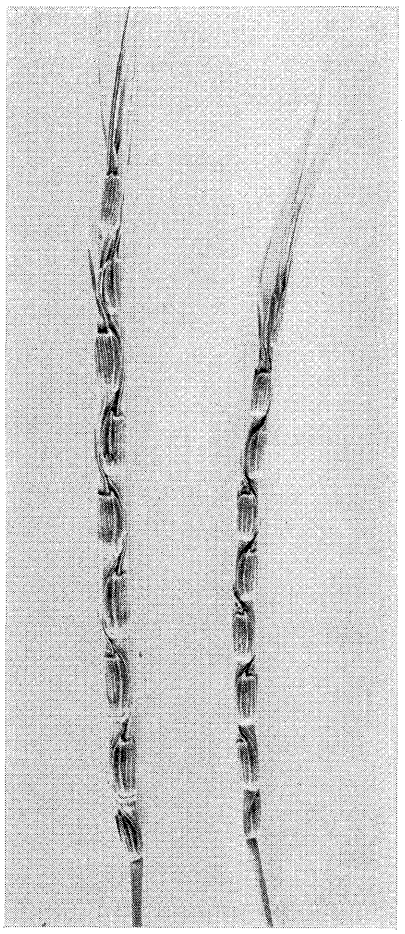

Fig. 1. Spikes of *Ae. crassa* (6x) and its polyhaploid plant (×0.85)
left: *Ae. crassa* (6x)
right: The polyhaploid plant.

and were incubated at 22°C in the dark until they germinated. Thereafter, they were grown in a growth cabinet with 20 hours of light at 25°C and four hours of darkness at 22°C. They were finally transferred to a glasshouse at about six months after pollination.

As shown in Table 1, three plants were obtained so far, but a plant from *Ae. squarrosa* ssp. *strangulata* died in the early stage of growth. Somatic chromosome numbers of the root-tips collected from the remaining two plants were examined by the aceto-carmine squash technique. A plant obtained from *Ae. crassa* (6x) had $2n=21$, indicating the polyhaploid chromosome number of this species, but the other one from *Ae. triaristata* (4x) was 2n=28. The latter plant was neither a polyhaploid nor an intergeneric hybrid, but identical with the female parent. Growth of the polyhaploid plant of *Ae. crassa* (6x) was normal and it produced a large number of tillers. However, the spikes of this plant were smaller than those of the parent as illustrated in Fig. 1. The polyhaploid plant showed complete pollen and seed sterility.

In order to examine chromosome pairing of the polyhaploid, the anthers were fixed in Farmer's solution (ethanol : glacial acetic acid=3 : 1), and stored in a refrigerator. Chromosome pairing was observed at MI of PMCs using the aceto-carmine squash method. Photomicrographs were taken from temporary preparations. Chromosome

Table 2. Chromosome pairing at MI of PMCs of the polyhaploid of *Ae. crassa* (6x)

Chromosome pairing				No. of cells observed	%
IV	III	II	I		
		1	19	2	1.1
		2	17	2	1.1
	1	1	16	1	0.5
		3	15	16	8.7
	1	2	14	1	0.5
		4	13	24	13.1
	1	3	12	8	4.4
		5	11	33	18.0
	1	4	10	18	9.8
1		3	11	1	0.5
		6	9	40	21.8
	1	5	8	11	6.0
	2	4	7	1	0.5
1		4	9	1	0.5
		7	7	13	7.1
	1	6	6	8	4.4
	2	5	5	1	0.5
1		5	7	1	0.5
		8	5	1	0.5
	1	8	2	1	0.5
Total				184	100.0

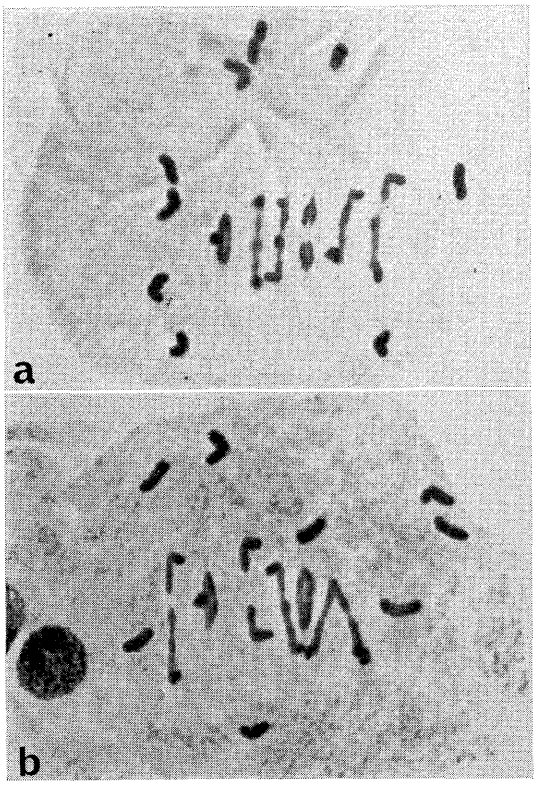

Fig. 2. Chromosome pairing at MI of PMCs of the polyhaploid of *Ae. crassa* (6x) (×880).
a: $6_{II}+9_{I}$ b: $1_{III}+5_{II}+8_{I}$

pairing at MI of PMCs of the polyhaploid is given in Table 2. Of 184 cells examined, 24, 33 and 40 cells (in total 52.9%) showed $4_{II}+13_{I}$, $5_{II}+11_{I}$ and $6_{II}+9_{I}$, respectively (Fig. 2). One or two polyvalents were observed in 53 cells (26.6% of the cells examined) (Fig. 2). The average chromosome pairing per cell was $0.02_{IV}+0.28_{III}+4.81_{II}+10.47_{I}$. Of 885 bivalents observed, 700 (79.1%) were rod-shaped associating with a terminal chiasma. The genome formula of *Ae. crassa* (6x) was designated D D D^2 D^2 M^{cr} M^{cr} by Kihara and his associates based on the results of the genome analysis of this species (Kihara 1957). This indicates that the hexaploid form of *Ae. crassa* contains duplicated D genome, D and D^2, which were derived originally from the D genome of *Ae. squarrosa* but have differentiated their genetic structures from each other. Judging from a wide range of the bivalent pairing ($1_{II}-8_{II}$) with the average of 4.81 per cell, and a rather high frequency of rod-shaped bivalents together with one or two polyvalents in the polyhaploid plant, the genome formula of *Ae. crassa* (6x) designated by the genome analysis will be supported by the present results.

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