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Genetic control and mechanism of chromosome elimination in the hybrids between *Hordeum bulbosum* (4X) and *H. vulgare* (4X)

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ABSTRACT

Two strains of *Hordeum bulbosum* (4X), ♯191 and ♯487, were reciprocally crossed to a strain of *H. vulgare* (4X), D8/55, and their hybrid embryos were cytologically examined in order to know time, pattern and genetic control of somatic chromosome elimination occurred in the tetraploid hybrids between these species.

Fourty hybrid embryos from each cross were sampled at 6 different times from 3 to 13 days after pollination. In all of the crosses, chromosome elimination occurred very frequently by about 9 days after pollination and a number of dihaploid cells were observed. The maximum rate of chromosome elimination was observed in the period of 3 to 5 days after pollination. It was cleary indicated that degree of chromosome elimination or dihaploid frequencies were quite different between the crosses with ♯191 and ♯487 but not different between reciprocals. This strongly suggests that the chromosome elimination is mainly controlled by nuclear gene(s) involved in *bulbosum* parents, but affected little by cytoplasms of these two species.

1. INTRODUCTION

The interspecific cross between *Hordeum bulbosum* L. (2n=2X=14) and *H. vulgare* L. (2n=2X=14) results in a haploid barley (2n=X=7) by the preferential elimination of *bulbosum* chromosomes during somatic cell division (Kao and Kasha 1969; Kasha and Kao 1970; Kasha and Sadasivaiah 1971; Lange 1971a, b; Symko 1969). This haploidization has also been confirmed to occur in the hybrid between tetraploids of these two species.

Barclay *et al.* (1972), Ho and Kasha (1975) and Kasha *et al.* (1972) suggested that the chromosome elimination was controlled by three or more genetic factors located on *vulgare* chromosomes 2 and 3. On the other hand, Fukuyama and Kurozumi (1977) and Fukuyama and Takahashi (1976) showed that the frequency of *vulgare*-like dihaploid plants (2n=14) in the crosses between tetraploid forms was largely affected by the parental genotypes of *H. bulbosum* to be mated, but not by those of *H. vulgare*: When a number of *vulgare* strains were crossed each to two strains of *bulbosum*, ♯191 and ♯487, the crosses

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with #191 generally gave 70% or higher frequency of dihaploid plants in their F_1 generations, whereas only about 20% of dihaploids were found in F_1's of the crosses with #487. The results indicate that a certain genetic factor(s) of *bulbosum* strains controls the chromosome elimination. However, it remains to be clarified whether the genetic factor(s) is in either nucleus or cytoplasm because *bulbosum* strains were used as the female parent in the crosses.

Time and rate of *bulbosum* chromosome elimination were extensively studied by Bennett *et al.* (1976) and Subrahmanyam and Kasha (1973) using mostly diploid hybrids between these species. According to them, elimination occurred 3 to 5 days (Bennett *et al.*) or 3 to 9 days (Subrahmanyam and Kasha) after pollination. However, there is scanty knowledge of time and rate of chromosome elimination in the tetraploid hybrid in which twice number of chromosomes are involved. This study was planned to know the situation and the mechanism, if possible, of chromosome elimination in the tetraploid hybrids and also the presence or absence of the reciprocal differences in elimination pattern.

2. MATERIALS AND METHODS

Two tetraploid *bulbosum* strains, #191 and #487, and an induced tetraploid strain of *H. vulgare*, D8/55, were used as the materials. Strains of #191 and #487 were grown in a glasshouse, and D8/55 was reared in the field, and four crosses, #191(♀)×D8/55, #487(♀)×D8/55 and their reciprocals were made. For simplicity, the hybrids of #191×D8/55 and #487×D8/55 will be called the #191-hybrid and #487-hybrid, respectively, hereafter. Time of pollination differed with crosses because #191 headed 10 to 15 days later than #487 and D8/55 (Table 1).

Fourty caryopses from each cross were taken 3, 5, 7, 9, 11 and 13 days after pollination, and immediately stored in cold water (0°C) for 24 hours. Then, they were fixed with 1:3 acetic alcohol for 24 hours, stained with aceto-carmine, and placed in a drop of 45% acetic acid. The embryo was carefully taken out from each caryopsis with a sharp-pointed needle under a dissecting microscope, and after measuring its length with the aid of micrometer, the embryo was squashed under coverslip and counted chromosome number of its cells.

3. RESULTS

Table 1 shows the seed sets in the four crosses. When crosses were made with *bulbosum* as the female parent, seed set was about 16% lower than those of the corresponding reciprocal crosses. Further, the seed set in the crosses with the strain #191 was always lower than those with #487, irrespective of the cross direction. The cause of these differences may not be genetical but

Table 1. *Seed sets in reciprocal crosses between 4X H. bulbosum (#191 and #487) and 4X H. vulgare (D8/55)*

Cross combination	Date of pollination (May)	No. of florets pollinated	No. of seeds induced	Seed set (%)
#191×D8/55	24 - 28th	1746	450	25.8
Reciprocal	16 - 22nd	2629	1095	41.7
#487×D8/55	7 - 15th	2671	857	32.1
Reciprocal	5 - 6th	3291	1582	48.1

Table 2. *Number of embryos (a) and cells (b) subjected to the examination of chromosome number*

Cross combination		Days after pollination					
		3	5	7	9	11	13
#191(♀)×D8/55(♂)	a*	26	33	35	37	31	32
	b**	5.4	7.3	8.2	21.9	9.0	6.2
Reciprocal	a	25	35	31	31	32	36
	b	4.3	9.1	9.2	14.4	11.2	11.0
#487(♀)×D8/55(♂)	a	29	25	29	30	33	23
	b	4.9	6.8	5.3	16.9	10.4	18.3
Reciprocal	a	23	28	29	35	32	33
	b	4.1	9.3	5.0	8.8	11.6	14.3

* Fourty caryopses were taken on each sampling date.
** Average number of examined cells per embryo.

environmental ones. Because, D8/55 grew vigorously in the field, while #191 and #487 were somewhat feeble as they were grown in pot in a glasshouse. Also, the *bulbosum* strain #191 headed later and exposed to rather higher temperature than #487.

Table 2 shows the number of embryos examined their chromosome number at each sampling date and the number of dividing cells per embryo. Twenty three to 37 out of 40 caryopses sampled at each date could be counted their chromosome number. Average number of dividing cells per embryo varied from 4.1 to 21.9, increasing to some extent with the lapse of sampling dates.

Since almost all of these hybrid embryos showed mixoploid condition, the number of chromosomes was recorded in each cell of hybrid embryos. Fig. 1 shows the frequency distribution of the cells having chromosomes from 14 to 28 at different sampling date after pollination in the four crosses.

Chromosome elimination was already recognized in three day-old embryos of the #191-hybrid: most of the cells showed the variation of chromosome numbers from 21 to 27, and the cells with 28 chromosomes were only 9-22%. Within the next two days, chromosome elimination abruptly progressed in the hybrid embryos. The cells with 14 chromosomes increased up to 42-49% and

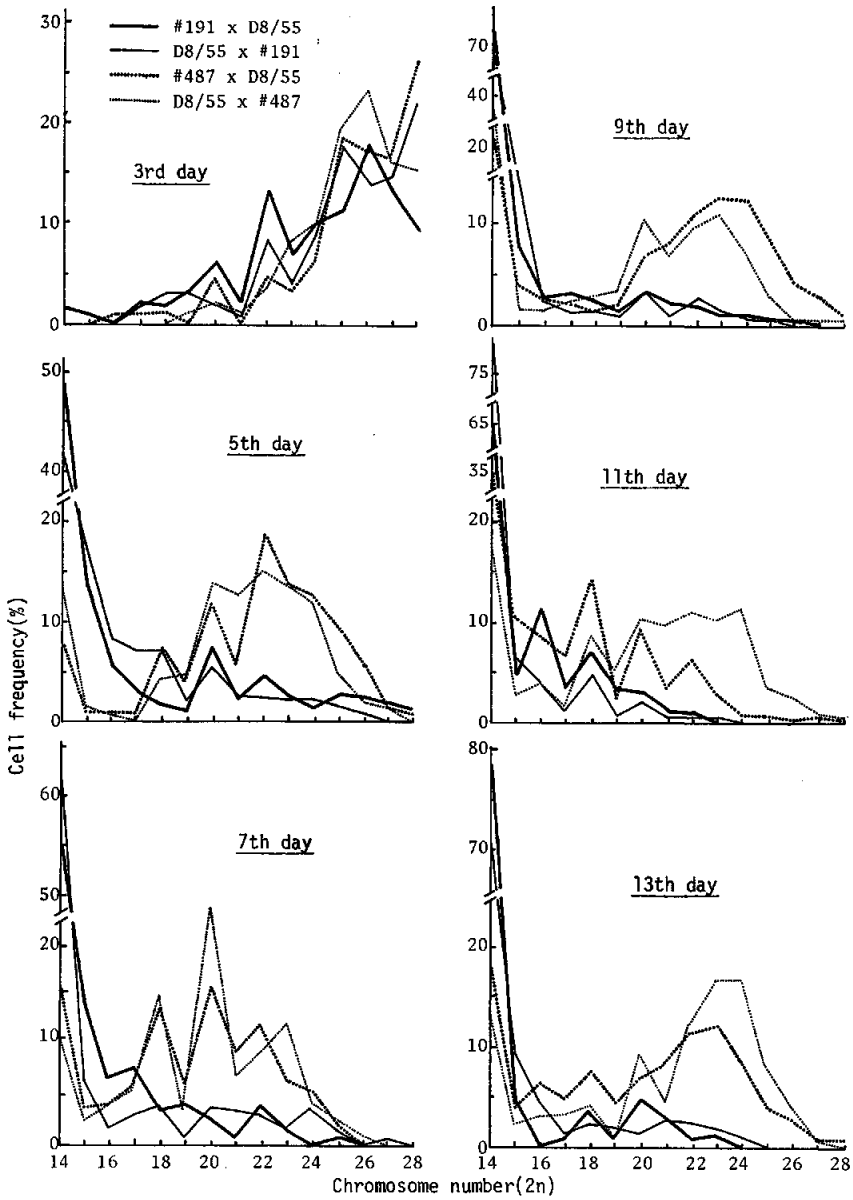

Fig. 1. Frequency distribution of the cells with chromosomes from 14 to 28 in the ♯191- and ♯487-hybrids at six different dates after pollination.

those with more than 21 chromosomes became very few. Thereafter, chromosome elimination occurred very gradually and the shape of variation curves was not much changed. In 13 day-old embryos, the cells with 14 chromosomes amounted to 71–79% of the cases and the remaining cells showed the varia-

tion of chromosome numbers from 15 to 24 with no appreciable peak.

In the case of the ♯487-hybrid, quite different situation of chromosome elimination was found as compared with those of the ♯191-hybrid. Most parts of the cells showed rather gradual chromosome elimination for a period of 3–5 days and the chromosome numbers of 5 day-old embryos varied from 18 to 27 with a peak at 2n=22. The remaining cells showed an abrupt elimination in this period as seen in the ♯191-hybrid. Thus, the cells of the ♯487-hybrid presented a bimodal distribution with peaks at 2n=14 and 22. A similar trend of variation of cell frequency was also seen in 7 or more day-old embryos. In 13 day-old embryos, the cells with 14 chromosomes was only 14–18% and the chromosome number of the remaining cells varied from 15 to 28 with a peak at 23 or 24.

On the other hand, no appreciable difference between reciprocals was recognized in the cell frequency as to chromosome numbers all through the sampling dates in both of the ♯191- and ♯487-hybrids.

It will be pointed out in Fig. 1 showing the distribution pattern of the cells in the ♯487-hybrid that the cells with chromosomes of even numbers such as 18, 20 or 22 were more frequent than those with odd numbers 19–23 at almost all sampling dates, although its cause is unknown.

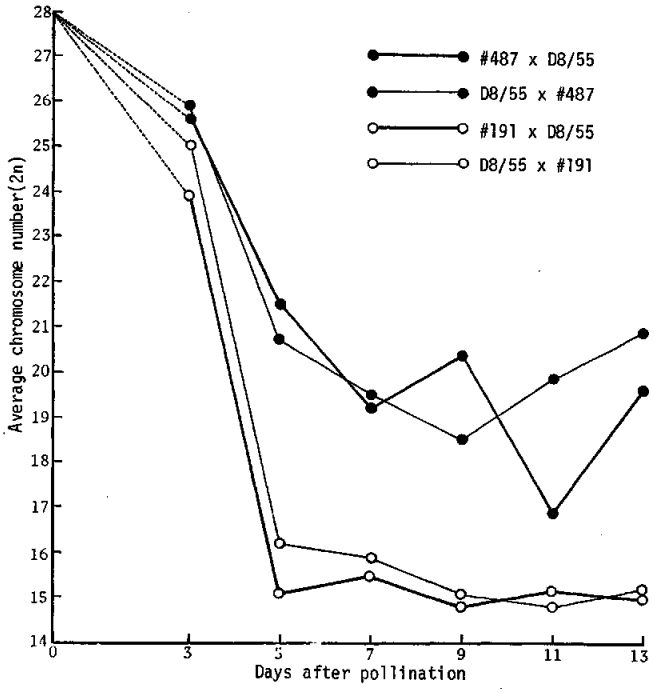

Fig. 2. Changes of average chromosome number in the cells of ♯191- and ♯487-hybrids.

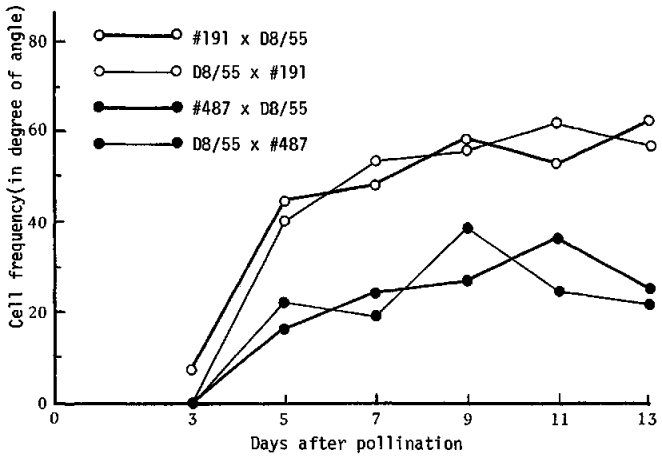

Fig. 3. Increment of the cells with 14 chromosomes in hybrid embryos.

Fig. 2 shows the change of an average chromosome number of the hybrid embryos in the four crosses for a period of 13 days after pollination. Generally speaking, both of the ♯191- and ♯487-hybrids showed more or less sigmoidal changes of the chromosome numbers with the lapse of the sampling date. In the case of the ♯191-hybrid, the average chromosome number was 24-25 on third day, but during the following 2 days (3-5 days) an abrupt chromosome elimination occurred, the rate of elimination per day being as large as 4.5. From 5 to 9 days after pollination, chromosome elimination became slowly, and thereafter no change in chromosome number was recognized. The ♯487-hybrid also showed a similar trend of chromosome elimination, but the rate of elimination was much lower than the case of the ♯191-hybrid. It is mentioned here that no change of the chromosome number was recognized after 9 days in both of the ♯191- and ♯487-hybrids. No notable difference between reciprocals of the ♯191-hybrid was seen in the change of an average chromosome number, while the ♯487-hybrid showed some variation between reciprocals, which may be due to the sampling error.

Next, the change of frequencies of the 14-chromosome cells after pollination is shown in Fig. 3, where the percentage of frequency was transformed into the degree of angle. The increase of 14-chromosome cells was very marked during the period of 3-5 days after pollination, attaining up to 40-45 degrees (40-50%) of the total number of cells, but soon after 5 days it became slow down and ceased almost competely after 9 days. Almost similar change of 14-chromosome cells was seen in the reciprocal crosses with ♯487, though the rate of increase was much less than the case of ♯191-hybrid.

Finally, the relation of the chromosome elimination with the development

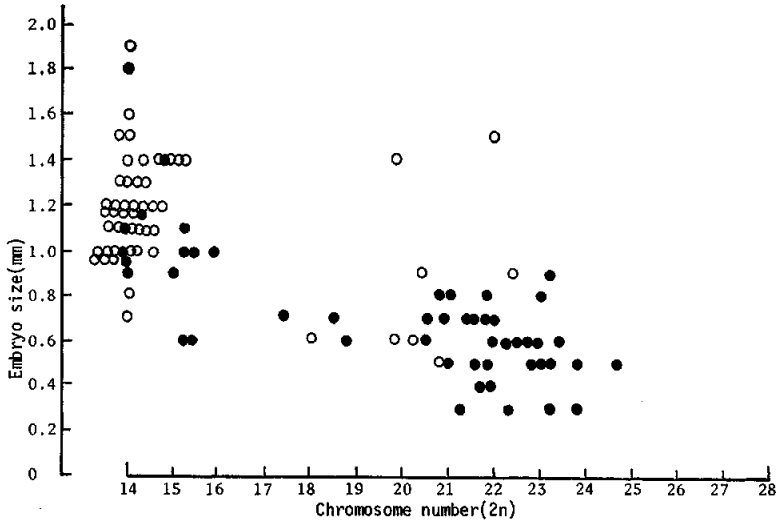

Fig. 4. Scattered diagram showing the relation between average chromosome number and embryo length of the 13 days old hybrids between ♯191 and D8/55 (open circle) and between ♯487 and D8/55 (closed circle).

of embryo was studied. Fig. 4 shows a part of the results obtained. According to this figure, the embryos of both ♯191- and ♯487-hybrids can roughly be classified into the following two groups: one is those having smaller number of chromosomes (14–17) and bigger in embryo size (1.2mm in average length). The majority parts of the ♯191-hybrids were included in this group. Another one is consisted of those having larger number of chromosomes (17 or more) and smaller embryo size (0.6mm in average). The ♯487-hybrid embryos were mostly of this group. The difference of mean embryo lengths of these two groups was statistically significant ($P<0.001$). Two exceptions were found in the ♯191-hybrids, both of which were as large in embryo size as those of the first group in spite of their larger chromosome numbers, 20 or 22. Excepting these two, the above stated results suggest that the growth of the hybrid embryos was clearly retarded by the presence of two or more additional chromosomes.

4. DISCUSSION

The present study, made with the use of hybrid embryos from tetraploid crosses between *H. bulbosum* and *H. vulgare*, has indicated that chromosome elimination occurred very frequently by about 9 days after pollination, resulting in a number of dihaploid cells with 14 chromosomes. The maximum rate of chromosome elimination was observed during the period from 3 to 5 days after pollination in both of the ♯191- and ♯487-hybrids (Figs. 2 and 3). And,

the eliminated chromosomes are supposed to be of *bulbosum* parent. Although there is no definite evidence to support this, a number of papers gave evidence that the *bulbosum* chromosomes were preferentially eliminated in the hybrids of this kind (see Kasha 1974). Fukuyama and Takaqashi (1976) also confirmed that the cross of *bulbosum* strain #191 with a *vulgare* strain D8/55 resulted in many dihaploid plants which were highly fertile and morphologically very similar to the *vulgare* parent, D8/55.

According to Bennett *et al.* (1976), diploid hybrids between *H. bulbosum* (2X) and *H. vulgare* (2X) formed haploid embryos by 5 days after pollination and the maximum rate of elimination was observed in 3 day-old embryos. On the other hand, the data in diploid hybrids by Subrahmanyam and Kasha (1973) showed that chromosome elimination occurred for the period of 3–9 days after pollination and its maximum rate was seen during 5–8 days. These observations and the results obtained in the present study lead to the following conclusion that the time of chromosome elimination in the tetraploid hybrid is almst similar to or somewhat later than the case of diploid hybrid. This implys that the number of chromosomes eliminated per a single cell division in tetraploid hybrid is larger than that of diploid hybrid.

Chromosome elimination occurred in all of the crosses used in this experiment, but degree of elimination was markedly different between cross combinations: in the #191-hybrids, most of the embryos showed dihaploid condition, while in the #487-hybrids, resultant dihaploid embryos were rather few and most of the embryos had 20–24 chromosomes. And, these situation for dihaploid embryo frequencies was consistent with the frequency of the *vulgare*-like dihaploid F_1 plants when #191 and #487 were crossed by D8/55 (Fukuyama and Takahashi 1976). Therefore, it can be said that the period of chromosome elimination in the hybrids is restricted to very early developmental stage of the embryo and thereafter the hybrid embryo become stable as to chromosome number all through the growth period until the ripening of F_1 plant.

According to Fig. 3, the frequencies of dihaploid cells in both of the #191- and #487-hybrids reached a maximum on the same embryo stage (9 days after pollination) although their frequencies were quite different. This suggests that the rapidity of chromosome elimination was different between two kinds of the crosses. Two possibilities may be considered as the cause of the difference: First, the cell generation time is different between these two *bulbosum* strains. This possibility, however, seems unlikely because the normal tetraploid hybrid plant was produced by the cross between #191 and #487 (unpublished data). If the cell generation time was different between them, the chromosome instability should be observed in their hybrid. Second possibility is that number of chromosome loss per cell division is different between these two *bulbosum* strains. The more the chromosomes are lost in a single mitosis, the more frequently dihaploid embryos are resulted within a certain period.

Bennett *et al.* (1976) observed that usually 0 to 3 chromosomes were eliminated at each mitotic division of the diploid hybrid. However, there is few report showing that number of chromosome loss per cell division is different with the parental genotypes to be mated. More detailed observations will be needed to confirm this problem.

The present results definitely indicated that the rate of chromosome elimination was quite different between the parental *bulbosum* strains, #191 and #487, but not different between reciprocal crosses, which led to a conclusion that the chromosome elimination was controlled by the genetic factor(s) present in the nucleus of *bulbosum* but not those in the cytoplasm of these species. It should be mentioned here that both of the strains #191 and #487 resulted in not only dihaploid but also hyperdiploid hybrid embryos in the crosses with 4X D8/55. Fukuyama and Takahashi (1976) demonstrated that the frequency of *vulgare*-like dihaploid plant in F_1 generation continuously varied among the genotypes of many *bulbosum* strains, including #191 and #487 used in the present study, when they were pollinated by 4X *vulgare* strains. In the case of diploid hybrid between these two species, Simpson *et al.* (1980) reported the different frequencies of haploid plants, varying from 30 to 100% of F_1's, among the crosses using 13 diploid *bulbosum* strains and 2 to 12 diploid *vulgare* lines. These results mentioned above may suggest that the genetic factor(s) involved in *bulbosum* nucleus for chromosome elimination are consisted of multiple genes.

On the other hand, Barclay *et al.* (1972), Ho and Kasha (1976) and Kasha *et al.* (1972) crossed tetraploid *bulbosum* to a series of barley trisomics and obtained a result showing that at least three genetic factors on *vulgare* chromosomes 2 and 3 controlled chromosome elimination in the hybrids. However, their results do not always mean that the genetic factors locate on '*vulgare*' chromosomes: those can be explained by another assumption that these genetic factors locate on not '*vulgare*' but the 'homoeologous *bulbosum*' chromosomes, and they control the chromosome elimination. This assumption may be supported by the fact that there is wide variation in *bulbosum* genotypes for the rate of chromosome elimination, as shown in this study and also shown before by Fukuyama and Kurozumi (1977), Fukuyama and Takahashi (1976) and Simpson *et al.* (1980), while there is few report as to the genetic variation among *vulgare* genotypes responsible for the rate of chromosome elimination. Then, we would conclude that most part of the difference in the rate of chromosome elimination occurred in the hybrid between *bulbosum* and *vulgare* are explained by the difference among the *bulbosum* nuclear gene(s).

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