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Chromosomal rearrangements in interspecific hybrids between *Nicotiana gossei* Domin and *N. tabacum* L., obtained by crossing with pollen exposed to helium ion beams or gamma-rays

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Abstract

It is very difficult to obtain interspecific hybrids between *Nicotiana tabacum* L. ($2n = 48$) and *N. gossei* Domin ($2n = 36$), because of strong cross incompatibility. We had already obtained interspecific hybrids between these two species, crossing *N. gossei* flower with *N. tabacum* pollen exposed to He ions or gamma-rays. Here, we analyze chromosome constitution of these hybrids by genomic in situ hybridization. In root tip cells of the two hybrids obtained with He ion exposure, most mitotic cells contained 18 chromosomes of *N. gossei* and 24 chromosomes of *N. tabacum*. However, in some cells, translocations and insertions between parental genomes were observed. On the other hand, in a hybrid obtained by gamma-ray irradiation, intergenomic rearrangements were not observed, although mitotic cells showed 19 hybridization signals with *N. gossei* DNA in 41 chromosomes. Such chromosomal changes in structure or constitution may be related to overcoming cross incompatibility between these two species.

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1. Introduction

To introduce valuable characters of wild species into cultivars, interspecific and intergeneric hybridization have been extensively carried out. However, it is generally difficult or impossible to obtain hybrid plants between distantly related species by sexual reproduction [1]. This phenomenon is called as “cross incompatibility” and includes inhibition of pollen germination and pollen

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tube growth, abortion of hybrid embryo, and hybrid inviability.

To overcome the cross incompatibility, several techniques have been exploited. One of them is pollen exposure to ionizing radiation. To date, there have been several attempts to produce mature hybrid plants using pollen exposed to low linear energy transfer (LET) radiation such as gamma-rays and X-rays [2,3]. On the other hand, ion beams have been expected to induce different biological effects, because they have a peculiar distribution of energy transfer and can deposit a lot of energy in a limited area [4]. We had already obtained interspecific hybrids between *Nicotiana gossei* Domin and *N. tabacum* L. using pollen exposed to He ion beams, and showed that He ion beams were more effective in overcoming cross incompatibility than gamma-rays [5].

Over the decade of the last century, molecular cytogenetics has been established in plants and animals. Given the ability to paint different genomes, genomic in situ hybridization (GISH) has been applied for detecting chromosomal rearrangements between two different genomes in *Nicotiana* [6,7]. In the present study, GISH was used to analyze the chromosomal constitution of interspecific hybrids between *N. gossei* and *N. tabacum*, obtained by crossing *N. gossei* flower with *N. tabacum* pollen exposed to He ions or gamma-rays.

2. Experiments

Three interspecific hybrids between *N. gossei* Domin ($2n = 36$) and *N. tabacum* L. ($2n = 48$) were used. Two of them (ion-1 and ion-2) were obtained with *N. tabacum* pollen exposed to He ion [5]. The other (gamma-1) was obtained with gamma-ray-irradiated *N. tabacum* pollen [5]. Briefly, mature pollen (about 25 μm in diameter) was collected before anthesis. After about one week in a desiccator at room temperature, pollen grains were monolayered between two sheets of kapton film (7.5 μm thickness, Toray-Dupont Co. Ltd., Japan), and exposed to 800 Gy of 6 MeV He ion beams (LET, 150 keV/ μm ; depth, 32 μm) generated by a 3 MV tandem accelerator (Taka-

saki Radiation Chemistry Research Establishment, JAERI, Gunma, Japan) [8], and to 100 Gy of ^{60}Co gamma-ray (LET, 0.2 keV/ μm) at a dose rate of 200 Gy/h (Research Institute for Advanced Science and Technology, University of Osaka Prefecture, Osaka, Japan). Exposed samples were used to pollinate the emasculated *N. gossei* flowers. In general, hybrid seedlings of *N. gossei* \times *N. tabacum* cannot survive beyond the second or third leaf stage because of hybrid inviability. Among the hybrid seedlings obtained with exposed pollen, only three showed normal growth and grew to the flowering stage. They were cultivated in a greenhouse with natural light at about 25 °C, and propagated by cutting. The parental species, *N. gossei* and *N. tabacum*, were also cultivated in a greenhouse.

The methods of probe-labeling, chromosome preparation and in situ hybridization were previously described [6]. Chromosome spreads were treated with 100 $\mu\text{g}/\text{ml}$ of RNase A, and hybridized with biotinylated total genomic DNA from *N. gossei* overnight at 37 °C. Since the hybridization signals with *N. gossei* DNA were distinctive in control preparations of *N. gossei* and *N. tabacum*, unlabeled total genomic DNA of *N. tabacum* was not applied as a blocking reagent. Biotinylated DNA was detected with avidin conjugated with fluorescein isothiocyanate (FITC), using one amplification with biotinylated anti-avidin D. Chromatin from both parents was visualized by counterstaining with 4',6-diamidino-2-phenylindole (DAPI). DAPI and FITC images were observed by fluorescence microscope (Axioplan, Zeiss) under UV- and B-excitation, respectively. Approximately 30 mitotic chromosome spreads per hybrid were examined for hybridization patterns of *N. gossei* DNA.

3. Results and discussion

In the root tip cells of the ion-1 and the ion-2, GISH was conducted using biotinylated total genomic DNA from *N. gossei* as a probe. Forty-two chromosomes were constantly observed under UV-excitation. In a large proportion of cells of both hybrids, 18 chromosomes were hybridized

with the *N. gossei* genomic probe, and visualized as a yellow–green color through a B-excitation filter. Hybridization signals were detected over the

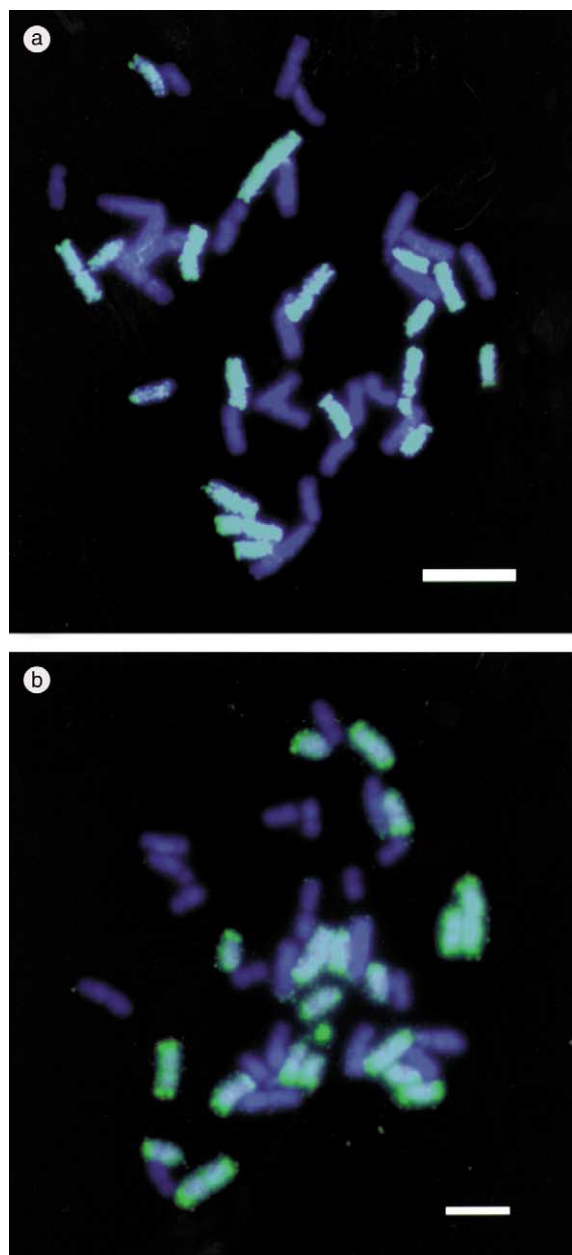


Fig. 1. Mitotic chromosome spreads of ion-1 (a) and gamma-1 (b), following GISH using biotinylated total genomic DNA from *N. gossei* as a probe. Scale bars represent 5 μ m.

entire length of those 18 chromosomes. Therefore, it was shown that the ion-1 and ion-2 had a chromosome set consisting of 18 chromosomes from *N. gossei* and 24 chromosomes from *N. tabacum* (Fig. 1(a)).

GISH was also carried out in the mitotic cells of the gamma-1. From the counterstaining with DAPI, it was found that all the cells observed had 41 chromosomes, unlike found in the ion-1 and the ion-2. The genomic probe of *N. gossei* constantly and strongly hybridized to 19 chromosomes

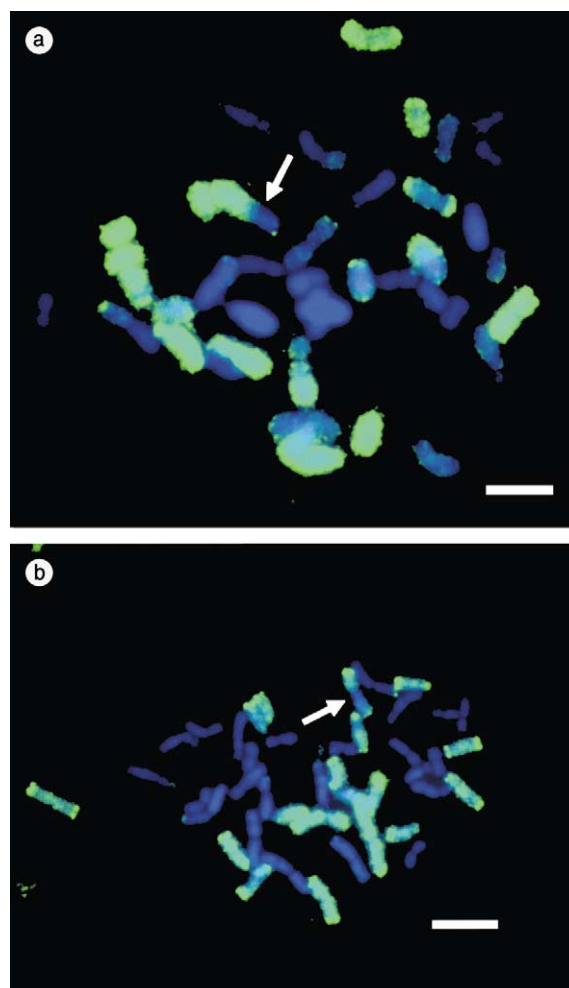


Fig. 2. Structural change of chromosomes detected in ion-1 by GISH using biotinylated total genomic DNA from *N. gossei* as a probe: (a) translocation type (arrow); (b) insertion type (arrow). Scale bars represent 5 μ m.

among the 41 chromosomes (Fig. 1(b)). Therefore, in the gamma-1, two chromosomes of *N. tabacum* were decreased and one of *N. gossei* was increased from the expected chromosome number. The elimination of two chromosomes from *N. tabacum* pollen seems to be due to the direct effect of DNA damage by the gamma-irradiation. With respect to chromosomal changes in the non-irradiated genome, chromosome breakage in the non-irradiated genome was suggested in asymmetric somatic hybrids of *Nicotiana* [9], and would be implicated in the Robertsonian rearrangement [10]. However, the additional *N. gossei* chromosome in the gamma-1 could not be explained only by a simple Robertsonian fission, because 6 pairs of (sub)metacentrics in *N. gossei* was observed in the gamma-1. This chromosome constitution might be accounted for by the unequal segregation of daughter chromosome of *N. gossei* in the early stage of embryo development of the gamma-1.

In the small part of mitotic cells of the ion-1 and ion-2, some chromosomes were partially hybridized with the *N. gossei* DNA (arrows in Fig. 2). These hybridization patterns indicate chromosomal changes between the parental genomes. Based on the signal-free location, they were classified into two types. In the first type, hybridization signal was not detected in the distal region of the chromosome (Fig. 2(a)). These hybridization patterns suggest that translocation occurred between chromosomes of *N. gossei* and *N. tabacum*, though strong hybridization signal could not be detected in 24 chromosomes derived from *N. tabacum*. In the second type, a lack of signal was observed only in the vicinity of the centromere (Fig. 2(b)). This feature seemed to result from insertion of *N. tabacum* chromatin into *N. gossei* chromatin. No insertion into the interstitial region of each chromosome arm was detected in any chromosomes. Moreover, the size of the segments translocated or inserted varied among chromosomes. In our previous study of interspecific hybrids between *N. gossei* and *N. tabacum*, obtained by ovule culture after a cross with non-irradiated pollen of *N. tabacum*, chromosomal reconstruction was not observed [6].

Throughout our observation of the ion-1 and ion-2, some chromosome spreads were found to

Table 1

Frequency of cells with intergenomic chromosomal rearrangements in interspecific hybrids between *N. gossei* and *N. tabacum*

Hybrid	No. of cells observed	No. of cells with chromosomal changes	
		Translocation-type	Insertion-type
ion-1	31	4	3
ion-2	32	2	2
gamma-1	29	0	0

possess bicolored chromosomes. In contrast, there was no bicolored chromosome in the gamma-1 (Table 1). This difference might be related to the type of radiation used for pollen treatment. However, we cannot compare these results in terms of the relation between radiation quality and chromosomal changes, because dose effects of He ions and gamma-rays on pollen used here were unclear.

The boundaries between parental chromatins in the bicolored chromosomes were mainly located either at the proximal region of the centromere (Fig. 2) or at the secondary constriction (data not shown). It is generally known that there are conserved and/or homologous DNA sequences, such as centromere-specific DNAs [11] and 45S ribosomal RNA genes [12] in those chromosomal regions. These DNA sequences may be involved in the formation of chromosomal changes between the genomes of *N. gossei* and *N. tabacum*. Since it is thought that the main pathway for the repair of DNA double strand breaks in plants is non-homologous end-joining [13], further analysis on the process of chromosome rearrangement between different genomes is necessary.

Although pollen irradiation has been conventionally performed to overcome cross incompatibility until now, chromosome constitution of the hybrids obtained has not been fully analyzed. In the present study, we detected chromosomal changes in structure or constitution in those hybrids. Such chromosomal changes may be related to overcoming the cross incompatibility between these two species. It is intriguing that those hybrids possess chromosomal changes involving the non-irradiated *N. gossei* genome.

References

- [1] G.L. Stebbins, *Adv. Genet.* 9 (1958) 147.
- [2] J.D.H. Reusch, *Nature* 178 (1956) 929.
- [3] Y. Shintaku, K. Yamamoto, T. Nakajima, *Theor. Appl. Genet.* 76 (1988) 293.
- [4] G. Kraft, M. Krämer, M. Scholz, *Radiat. Environ. Biophys.* 31 (1992) 161.
- [5] T. Yamashita, M. Inoue, A. Nakamura, H. Watanabe, A. Tanaka, *Breed. Sci.* 44 (Suppl. 1) (1994) 187.
- [6] S. Kitamura, M. Inoue, N. Ohmido, K. Fukui, *Breed. Sci.* 47 (1997) 67.
- [7] S. Kitamura, M. Inoue, N. Ohmido, K. Fukui, *Theor. Appl. Genet.* 101 (2000) 1180.
- [8] A. Tanaka, H. Watanabe, T. Shimizu, M. Inoue, M. Kikuchi, Y. Kobayashi, S. Tano, *Nucl. Instr. and Meth. B* 129 (1997) 42.
- [9] A.S. Parokonny, A.Y. Kenton, Y.Y. Gleba, M.D. Bennett, *Plant J.* 2 (1992) 863.
- [10] W.R.B. Robertson, *J. Morphol.* 27 (1916) 179.
- [11] G. Moore, *Curr. Opin. Plant Biol.* 1 (1998) 116.
- [12] K. Fukui, in: K. Fukui, S. Nakayama (Eds.), *Plant Chromosomes: Laboratory Methods*, CRC Press, Boca Raton, FL, 1996, p. 1.
- [13] V. Gorbunova, A.A. Levy, *Trends Plant Sci.* 4 (1999) 263.