Method of chromosome observation in the dioecious plant *Silene latifolia*†

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Silene latifolia is a dioecious plants, which is considered to be evolved from monoecious plants to develop heteromorphic sex chromosomes.[1)] The sizes of X and Y chromosomes are approximately 400 Mb and 570 Mb, respectively, such that they can be observed under a microscope. The architecture of these sex chromosomes has been investigated by using cytological techniques, *e.g.* fluorescence *in situ* hybridization analysis.[2)]

We previously irradiated heavy-ion beams to seeds and pollens of *S. latifolia* and obtained hermaphroditic and asexual mutants, which possessed deletions on the Y chromosome.[3)] Subsequently, a Y-chromosome map was constructed by conducting PCR-based deletion mapping. To further investigate the size of the deletions after the heavy-ion irradiation and confirm the accuracy of the deletion map, observation of chromosomes in *S. latifolia* is required. However, an efficient method for the chromosome specimen preparation has not been fully developed so far.

In many plants, root tip portions are typically used for preparing chromosome specimens because they are highly enriched in rapidly cycling cells with a steady state of growth. Synchronization of cell cycle is typically achieved by using DNA synthesis inhibitors. In *Silene latifolia*, these methods were applied to prepare the chromosome specimens. However, the timing for chromosome preparation after initiating root tip growth was not to be optimized. We hypothesized that the cell cycle was roughly synchronized during the first few days after rooting. Therefore, we investigated an appropriate incubation period after germination, during which the roots possessed many metaphase chromosomes. Moreover, we investigated variations of telocentric chromosomes by changing the duration of the ice-cold treatment (8, 16, and 32 h).

Every six hours after the germination, root lengths of thegerminating seeds were measured. The percentage of roots with a length of 2 mm that were amenable to enzyme treatment reached a maximum after 60 h of incubation starting (HAIS) at 23°C. The rough observation of metaphase cells in root tips from 48 to 60 HAIS by the Feulgen method[4)] showed a high frequency of metaphase cells during 51 to 56 HAIS. Therefore, root tip samples collected every hour from 51 to 56 HAIS were closely examined. The number of metaphase cells in the root tips reached a peak at 54 HAIS (Fig. 1).

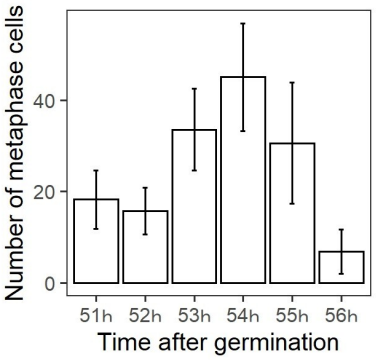

Fig. 1. Number of metaphase cells 51–56 hours after germination. Bars represent standard deviations.

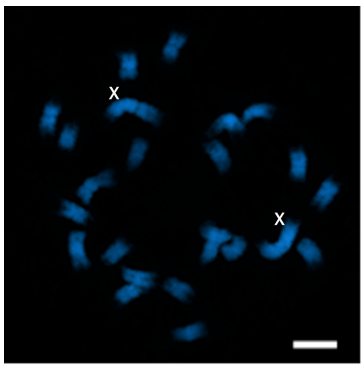

Fig. 2. Photograph of metaphase chromosomes in a female plant (Bar = 5 μm).

DNA synthesis inhibition for metaphase chromosome preparation is typically achieved by using aphidicolin. The aphidicolin treatment was initiated 24 h before 51–56 HAIS and was terminated 9 h before 51–56 HAIS. Consequently, a peak in the number of metaphase cells was observed at 54 HAIS. The mean value of the metaphase cells at 54 HAIS with aphidicolin was fourteen times higher than that at 56 HAIS without aphidicolin (Wilcoxon rank sun test, $p < 0.01$). This result indicates that DNA synthesis inhibition at the appropriate time helps increase the number of metaphase cells, which facilitates an effective chromosome observation. With this sample, *S. latifolia* chromosomes were easily observed using confocal microscopy (Fig. 2). These techniques can be applied to observe the chromosomes of heavy-ion induced partial Y-deletion mutants.

References
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