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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

The accuracy evaluation of linkage map in cultivated strawberry (*Fragaria* × *ananassa* Duch.) by direct cycling-primed *in situ* hybridization technique
(直接リサイクリングPRINS法による栽培イチゴの連鎖地図における正確性の評価)

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Cultivated strawberry ($2n = 8x = 56$) is proposed to be allopolyploid with the genome composition AAA'A'BBB'B'. Hence, it had been difficult to conduct theoretical genetic analysis based on the Mendel's law of inheritance. For the purposes of conducting theoretical genetic analyses, several linkage maps (LMs) have recently been constructed using various types of DNA markers. The LMs are desirable to evaluate the accuracy. Primed *in situ* (PRINS) hybridization technique could confirm the accuracy of the LM by distinct the location of the DNA primers on the chromosome. Nevertheless, there has few studied about the verification of the LMs using PRINS hybridization in cultivated strawberry. The purpose of this studied was undertaken to; 1) to develop the PRINS hybridization for chromosome labelling in cultivated strawberry, 2) to examine the availability of a PRINS hybridization technique for chromosome mapping and, 3) evaluate the accuracy of the LM.

The sample was harvested from the Japanese cultivated strawberry 'Sachinoka'. In experiment 1, the effects of key points such as the enzymatic maceration time (20, 25, 30, and 35 minutes), percentage of acetic acid (30%, 45% and 60%), 37°C incubation time (0, 24, 72, 120, 168 hour), and the number of PCR cycles were investigated. In experiment 2, three CAPS markers (APX2, F3H3, and OLP) were labeled on the chromosome by using the appropriate C-PRINS hybridization technique. In experiment 3, the LM which had been constructed using SSR markers was verified by using two colors C-PRINS hybridization technique. A pair of SSR markers which locate near both ends of each linkage group (LG) was labeled on the chromosomes using red and green colors, generated by tetramethylrhodamine-12-dUTP and fluorescein-12-dUTP, respectively.

The results in experiment 1 showed appropriate treatment in each step. The 25-minute treatment was appropriate for the maceration to obtain many cells with 56

chromosomes. The images which obtained from 20-minute treatment showed many overlapped chromosomes that made it difficult for observation. In contrast, the results from treatments of 30 and 35 minute showed a small number of chromosomes. The 45% acetic acid was an effective treatment for obtaining clear chromosome images. The 30% acetic acid was not high enough to clear various granular substances around the chromosomes, as shown by the lack of difference in color between the chromosomes and cytoplasm. The quality of images from 45% and 60% acetic acid treatments were not significant differences. However, a high percentage in 60% acetic acid treatment might damage the chromosomal DNA. The number of chromosomes decreased after conducting PRINS hybridization. It seems like the attachment between chromosomes and glass slide was not strong enough. In this experiment, we found that the 72-hour incubation was suitable treatment to increase the attachment. This treatment also increased the percentage of hybridization. However, the chromosome damage was found in the 120 and 168 hour treatments with the ghost-like shape. Consequently, the 72-hour incubation was appropriate to use for PRINS hybridization. The hybridization and brightness of signal increased along with the number of PCR cycles.

The hybridization signals from CAPS marker were observed on the chromosomes. The chromosome images clarified that these CAPS markers had a true chromosome specificity, and the C-PRINS hybridization technique is applicable to physical chromosome mapping of the cultivated strawberry. In case that used 2 markers in same reaction, two signals located on two chromosomes were observed. Two possible reasons can be considered. One possible reason was that amplification failed in one of the two pairs. The other was that the two signals on the chromosome had overlapped because their physical distance was extremely close.

The 2 colors C-PRINS hybridization technique indicated the signals from different SSR primers by different colors (red and green). All 56 chromosomes with 16 red and 13 green signals were observed when C-PRINS labeling with two SSR markers located on LG 1A was performed. Two chromosomes in the cell showed both signals on both ends, indicating that the LG 1A was correct. By the same approach, a total of 14 LGs was confirmed as having been correctly assigned.