*Zoological
Studies*

Analysis and Utility of Chromosome Information 75. Semi-automatic Image Analysis for Small Plant Chromosomes

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Plant chromosomes are categorized into two groups. One group consists of large chromosomes represented by the genera *Triticum* and *Hordeum*. The other is small represented by the genera *Oryza* and *Brassica*, etc. It had been difficult to identify the small chromosomes, because of the similarity in the morphology at the *mitotic* metaphase stage. Moreover the band patterns are simple and it is even possible that there are no bands on the chromosomes after the banding treatments. The small plant chromosomes, however, show on uneven condensation pattern along the chromosomes especially at the prometaphase stage of the mitosis. The condensation pattern, CP, which appears as darkly and lightly stained regions along a chromosome at the prometaphase stage of small plant chromosomes was first defined by Fukui and Mukai (1988). All the rice chromosomes have been identified based on the condensation pattern (Fukui and Iijima 1991). Moreover it has been shown that the CP is a representative characteristic of the chromosome morphology in plants since all the rice chromosomes have been semi-automatically identified using only the information of CP (Kamisugi et al. 1993). For quantitative analysis of the CP, image analysis has been employed. Quantitative maps of small plant chromosomes have thus been obtained based on the CP. In this paper, we present new imaging methods that enable us to obtain numerical data of the CP as the grey value profile and to generate a quantitative chromosome map based on the CP.

Two photographs, under- and normal exposure, of the same chromosomal image have been employed for image analysis. The under-exposed photograph has higher resolution in grey levels at the condensed or darkly stained region of chromo-

somes (Iijima and Fukui 1991). This is used for the precise determination of the density distribution of the chromosomes. The normally exposed photograph is suitable for the determination of chromosomal areas. Thus this photograph is used to discriminate the chromosomal regions.

The imaging programs that enable us to obtain the quantitative map based on the CP are divided into the six parts. The procedures for standardization of the CP and the outlines of the programs are as follows;

1. Under-exposed chromosome images are captured into the image memories. Using the contour lines of the normally exposed chromosome images, the background of the under-exposed image is masked in preparation for the analysis.

2. Light deflection of the image is adjusted by standardization of the grey values. i.e., the minimum and maximum grey values of the chromosomal area within a plate are extended from grey value, 1 to grey value, 255. Thus all the grey values in the chromosomal area within the plate are distributed between the grey values 1 and 255.

3. Each chromosomal region is extracted from the image and each of their grey values are measured. Chromosomal constriction(s) is/are interactively marked and the grey value profiles under the mid-lib lines of the chromatids are read digitally.

4. The minimum and maximum grey values of the homologous chromosomes in the plate are adjusted to the average grey values of all the plates measured.

5. The length of each chromosome arm is adjusted to the average length of the arm of homologous chromosomes of all the plates by an affine transformation.
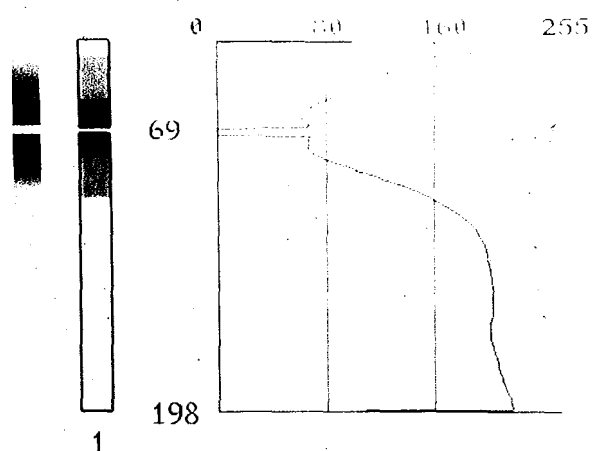
6. The standard greygram of the chromosome

Fig. 1. Standard greygram, idiogram and grey value profile of chromosome 1 of *Brassica nigra* constructed by the semi-automatic imaging programs.

is constructed from the average grey value. The darkly, intermediately and lightly stained areas of the standard greygram are decided, interactively. The quantitative idiogram is developed by setting two thresholds on the standard greygram. The standard grey value profile of each chromosomes represents well the characteristics of the condensation pattern of the chromosomes.

The image analysis and processing programs constructed are semi-automatic. Some procedures in the standardization procedures have already been automatized. For example, the

grey values of all the chromosome images within the plate have been measured and standardized automatically. The adjustments after the measurement of the grey value profile of each chromosome are also automatic.

The adjustments after measurement of the grey gram profiles are also automatized. The remaining two interactive steps in the imaging program will be automatized. One is the automatic positioning of the under exposed images to the contour of the normal exposed ones for capturing images to image memories. The other is the automatic determination of the mid-lib lines of two chromatids for measuring the grey value profiles from the chromosomes. Although the mapping of small plant chromosomes based on the CP had taken long time and required an image analysis expert, the fast and easy mapping is enabled by the semi-automatic imaging programs.

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