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Identification and characterization of somatic rice chromosomes by imaging methods

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Summary. Somatic rice chromosomes from 30 spreads were analyzed by imaging methods. Morphological characters of each of the 12 rice chromosomes were obtained both by the imaging methods and by visual inspection. The numerical data of relative length, arm ratio, and condensation pattern (CP) were statistically analyzed. The descriptive morphological information obtained was also summarized into numbers of "key characters" or essential short sentences to characterize the traits. The fitness probability or the appearing frequencies of the key character for each of the 30 chromosomes was calculated. Altogether, 118 key characters were extracted to distinguish each rice chromosome. Furthermore, several "discriminants" or critical key characters were determined among the key characters, and a discrimination chart or flowchart to identify all the rice chromosomes was constructed using the discriminants.

Key words: Rice – Somatic chromosome – Condensation pattern – Karyotype – Discrimination chart

Introduction

The chromosome number of rice was found to be 2n = 24 in 1910 by Kuwada. Since then researchers have studied rice chromosomes and there have been several reports on the identification of rice chromosomes at the meiotic pachytene stage (Shastry et al. 1960; Kurata et al. 1981 b; Chen et al. 1982; Khush et al. 1984; Chung and Wu 1987) as well as at the mitotic metaphase stage (Nandi 1936; Hu 1958; Ishii and Mitsukuri 1960; Kurata and Omura 1978; Kurata et al. 1981 a; Wu et al. 1985; Fukui 1986 a; Chung and Wu 1987). There have been some discrepancies in the data, however, in the identification, characterization, and numbering of the chromosomes (Oka and Wu 1988), mainly because it is difficult to discriminate between each rice chromosome objectively and reproducibly. Fukui and Iijima (1991) identified all the somatic chromosomes by using an image parameter, condensation pattern (CP) (Fukui and Mukai 1988; Fukui et al. 1988; Fukui 1989), and other information obtained mainly by image analysis methods.

In this paper we describe detailed morphological characteristics of each of the 12 chromosomes. We also present an objective discrimination method for somatic rice chromosomes. Critical aspects on the discrimination of rice chromosomes are also discussed.

Materials and methods

Haploid plants of rice (*Oryza sativa* L. cv Koshihikari, 2n = 12) were used as materials. Root tips were fixed in a fixative (methanol:acetic acid, 1:1) for 1 h without any pretreatment. Chromosome specimens were prepared by enzymatic maceration (4% Cellulase Onozuka RS, 1% Pectolyase Y-23, pH 4.2, at 37 °C for 50 min) and air-drying methods. They were stained with a 4% Giemsa solution (pH 6.8) for 30 min. Good prometaphase chromosomal plates were photographed through a microscope under two exposure conditions, i.e., normal exposure and underexposure (Fukui and Iijima 1991).

For the image analysis, the normally exposed photograph was first taken by a chromosome image analyzing system, CHIAS (Fukui 1985; 1986 b; 1988), through a TV camera to extract only the contour lines of the chromosomes. The underexposed image was taken and the contour lines were superimposed onto

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the chromosomal image to produce a composite image. Sixty CPs or the density distribution at the mid-rib of 720 chromatids from 30 plates were measured for the composite images, and the numerical data of the CP were stored in floppy disks. All 12 of the chromosomes from 30 plates were identified based on their CPs, chromosomal length, arm ratio, and characters by visual inspection. Sixty CPs from the chromatids of the same chromosomes identified were averaged to obtain a standard CP of the chromosome.

Based on the data of the chromosomal length, arm ratio, CP, and characters obtained by visual inspection of the chromosome images, several critical and specific morphological traits were extracted as the "key characters." The statistical basis of the key character, i.e., the fitness probability of the key character was calculated using 30 chromosome spreads. "Discriminants" were then selected based on the most important or critical key characters among 118 key characters. A discrimination chart or flow-chart to identify each rice chromosome was also produced, based on the proper arrangement of the discriminants.

For in situ hybridization, ribosomal RNA genes with the spacer region (8.4 kbp, kindly provided by Dr. S. Sano, National Institute of Genetics, Mishima, Japan) were labeled with biotinylated dUTP (Biotin-16-deoxyuridine-triphosphate) by a random primer DNA labeling method. Rice variety "Nipponbare," a trisomic of chromosome 11 of "Nipponbare" and IR36, were used as plant materials. Rice chromosome samples were prepared by the method mentioned above. After the glass slides were dipped into 70% formamide in 2 × SSC for 2 min at 67 °C, they were washed using an ethanol series of 70, 95, and 99% for 5 min each. A reaction mixture (0.1 ng/μl biotinylate probe, 50% formamide, 10% dextran sulfate, salmon sperm DNA in 2 × SSC) was boiled for 10 min at 100 °C followed by rapid cooling to 0 °C. A 15-μl aliquot was dropped onto each glass slide and covered by a coverslip. Glass slides were kept at 37 °C for 3–6 h in an incubator. After hybridization of the probe with the chromosomes, the glass slides were subsequently washed with 2 × SSC for 5 min at room temperature, 2 × SSC for 10 min at 37 °C, 2 × SSC for 5 min at room temperature, PBS with 0.1% Triton-X for 2 min at room temperature, and PBS for 5 min at room temperature. Then, 0.6% Detek I horseradish peroxidase complex (Enzo Biochemicals, Inc., New York) was dropped onto the samples and the glass slides were incubated for 30 min at 37 °C. After rinsing with 2 × SSC for 5 min, PBS with 0.1% Triton-X for 2 min, and PBS for 5 min at room temperature, a mixture of 0.05% 3,3'-diaminobenzidine tetrahydrochloride (Dojindo Laboratories, Kumamoto, Japan) and 0.1% H_2O_2 in PBS was added for 20 min in a darkroom. The glass slides were then briefly washed and dipped in a 7% Wright solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for 5–15 min. After being washed and dried, they were observed with a phase contrast microscope.

Results and discussion

Detailed characteristics of each rice chromosome

Figure 1 shows the karyotype of six representative rice chromosome complements (a–f) arranged from the early to the late pro-metaphase stage from left to right. Chromosome numbers were assigned according to the length order (Fukui and Iijima 1991); the vertical chromosome sets show the complements of a haploid spread and the complements were arranged in the order of the total chromosome length of the complement. Each of the 12 chromosomes from six complements differed in various morphological characteristics. Six chromosomes arrayed on a horizontal line, however, showed a more or less common distribution pattern of the darkly stained or condensed regions. Chromosomes 4, 11, and 12 showed unique condensation patterns, i.e., chromosome 4 was a submetacentric chromosome, and most of the short and long arm proximal regions of similar extension were condensed heavily, appearing like a "Comet." Chromosome 12 had a similar morphology to that of chromosome 4, but was approximately two-thirds of it in size. A satellite and a heavy proximal condensation region at the long arm characterized chromosome 11. All three chromosomes were easily identifiable by visual inspection if the quality of the image was good. Conversely, the quality of the image and, furthermore, the techniques for sample preparation and photography can be evaluated by the visual appearance of these three chromosomes.

The other nine chromosomes could not be differentiated easily by visual inspection alone, although a more or less common pattern of condensation could also be observed among the chromosomes arrayed on a line. The difficulty may be due to the variation in the condensation patterns observed in, e.g., chromosomes 8 (cf. chromosomes 6a and 6d in Fig. 1) and 8 (cf. chromosomes 8a and 8d in Fig. 1), and to unstable characteristics, e.g., small condensations in the distal regions of both arms appearing in chromosome 1 (cf. chromosomes 1a and 1d in Fig. 1). The appearing frequency of the unstable characteristics differed from trait to trait. Thus, statistical analysis for the description of each character would be essential to evaluate the suitability of the characters in the identification of the chromosomes. Therefore, a comprehensive characterization of each chromosome was carried out using 30 pro-metaphase complements. Each of 60 CPs, averaged CP, averaged relative length, and averaged arm ratio with their corresponding standard deviation, along with the morphological characteristics of the chromosome checked by visual inspection, were all used to characterize the chromosome. All the important characters useful for identifying the chromosome were summarized as the key characters of each rice chromosome.

Table 1 presents a list of key characters of each chromosome. Key characters were categorized into three groups. The first group (A) included the key characters that outlined whole morphological characteristics of the chromosome. The quantitative data of a relative length and arm ratio with the standard deviation were also presented. Length of the satellite was excluded from the calculation. The second group (B) included the various key characters relating to the traits of the proximal condensed regions of both the short and the long arm (pCR and qCR). The upper threshold of the gray value (gray value = 199) for the mapping of the rice chromosomes

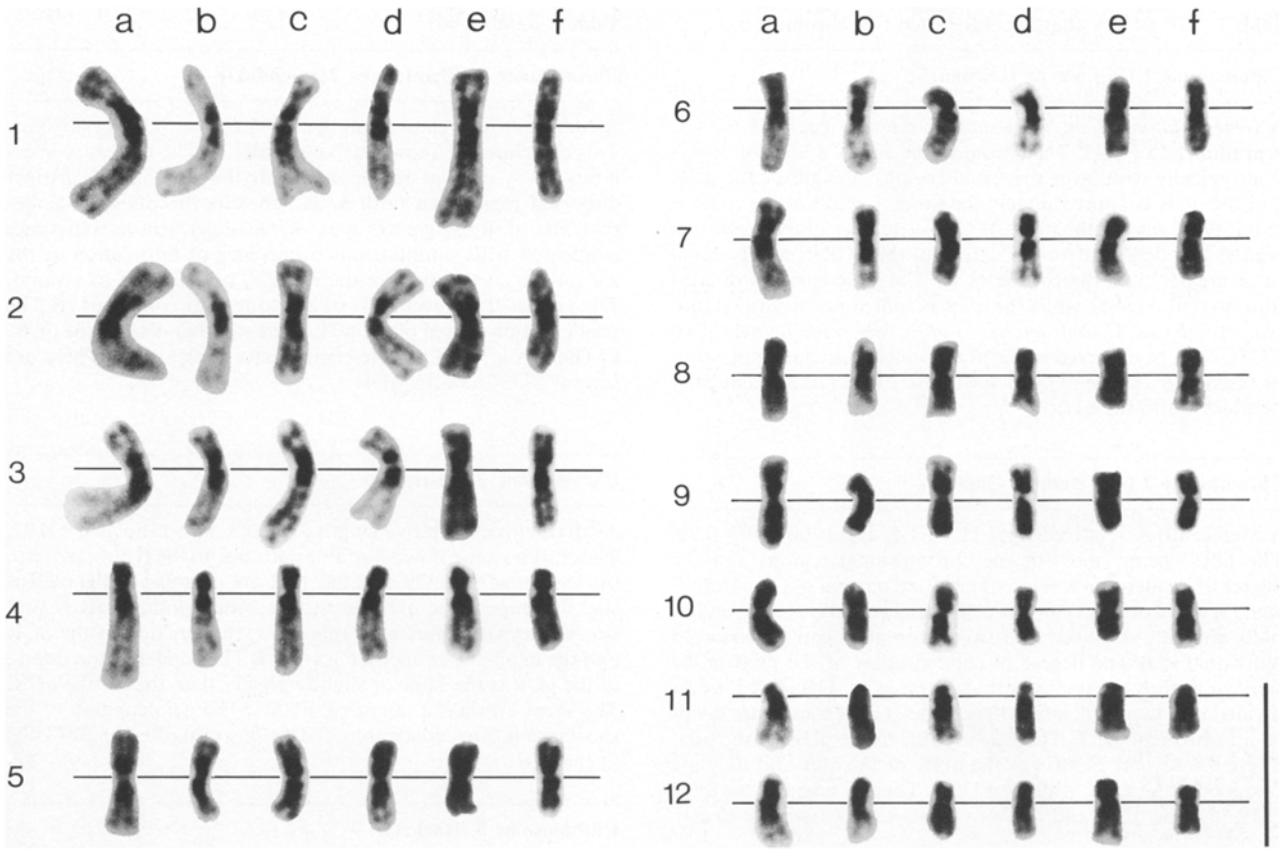

Fig. 1. Karyogram of 72 somatic rice chromosomes from six representative pro-metaphase plates. Each complement was arranged along a vertical axis. Six chromosomes of the same chromosome number were arrayed on a horizontal axis. Each complement was arranged in the order of total chromosome length from *left* to *right*. For chromosome 5, the assignment of the short and long arms was based on its length

based on the CP corresponded to the visual border of condensed and dispersed regions (Iijima and Fukui 1991). The third group (C) included the key words relating to the dispersed regions of both the short and the long arm (pDR and qDR). The various morphological characteristics of the small condensations in the distal or dispersed regions of the chromosome were collected in this group. The small condensations were abbreviated as "FUSCs" (faint, unstable, and small condensation regions), in order to differentiate them from the large condensed regions that constantly appeared in the proximal regions of both arms. The appearing frequency of each FUSC was determined using the CP.

The other morphological traits at the terminal regions (pter and qter), such as bifurcation at the telomeric regions of each chromatid observed in both arms of chromosome 2, were also summarized as the key characters. A total of 118 key characters from 12 rice chromosomes characterize the essential traits of the rice chromosomes useful for identifying each of them. Discrimination between chromosomes 6 and 7 was rather difficult because chromosome 6 did not show only one condensation pattern during the contraction from the late prophase stage to the mid-metaphase stage.

The characterization of the rice chromosomes also made it possible to analyze their contraction mechanisms. Rice chromosomes could be distinctly divided into two groups based on the CP, i.e., the 12 rice chromosomes belonged to the "heavily or early condensed" chromosome group or to the "lightly or late condensed" group. They could be visually recognized as large chromosomes and small ones. Chromosomes 1, 2, and 3 (the three largest ones) were classified into the "light" group. This grouping may represent the phenotypic expression of the differences in the packing ratio and the packing time of chromatin fibers. On the average, the gray value 123 corresponded to the boundary for the discrimination between the light and heavy groups (Fukui and Iijima 1991; Iijima and Fukui 1991).

The analysis of the averaged CP revealed various condensation patterns of rice chromosomes. Chromosomes 9, 10, and 11 exhibited the most characteristic CPs. Chromosome 9 showed two condensed regions within a long arm, which were represented by two clear concaves

Table 1. List of key characters for each rice chromosome

Chromosome 1 (Big one or Daichan)
A *General information.* Metacentrics: relative length 13.6±1.1, arm ratio 1.58±0.23. The chromosome shows a low condensation typically with large dispersed regions. Length of the pCR and the qCR is approximately the same. Length of the qDR is about twice that of the pDR. **B** *Characteristics of the condensed region.* The degree of condensation of the pCR is nearly equal to or greater than that of the qCR. The pCR is rarely divided into two subregions, while the qCR is sometimes separated into two subregions. **C** *Characteristics of the dispersed regions.* Two FUSCs can be observed in the pDR, which frequently appear to be fused into a single FUSC. One to five FUSC(s) can frequently be observed in the qDR
Chromosome 2 (Big meta or Ohmeta)
A Metacentrics: relative length 11.7±1.2, arm ratio 1.10±0.25. The DRs occupy most of the chromosomal regions and the degree of condensation is low. The visual pattern of the chromosome is more or less symmetrical. The chromatids at the end of both arms are detached and two chromatids can be observed individually. **B** The degree of condensation of the pCR is the same or slightly greater than that of the qCR. The pCR is never divided into two segments, whereas the qCR is sometimes divided into two regions. **C** There is a FUSC in the pDR or the pter. There are several FUSCs in the qDR or the qter and they are frequently fused into a single FUSC. There is a gap in both the pDR and the qDR and that of the pDR appears more clearly
Chromosome 3 (Big slug or Ohname)
A Submetacentrics: relative length 10.9±0.9, arm ratio 1.72±0.21. Both the pDR and qDR occupy a large part of the chromosome and the length of the qDR is twice that as the pDR. **B** The degree of condensation at the pCR is the same or greater than that of the qCR. **C** One telomeric FUSC often appears more distinctly at the pter than those of chromosomes 1 and 2. There are frequently one to four FUSC(s) in the qDR
Chromosome 4 (Comet or Teru)
A Submetacentrics: relative length 9.1±0.6, arm ratio 2.93±0.29. Most of the short arm is condensed. Two chromatids are not detached at the pter. The pCR and qCR show nearly the same length and degree of condensation, and the qDR is quite long and dispersed. **B** The degree of condensation in the pCR is slightly greater or the same as at the qCR. **C** There are no FUSCs at the short arm and the whole short arm is condensed uniformly. There are one to three FUSC(s) in the qDR
Chromosome 5 (Carnival)
A Metacentrics: relative length 8.3±0.6, arm ratio 1.12±0.09. The visual pattern of the chromosome is more or less symmetrical at the centromere as at the symmetrical axis. It rarely displays four concaves. The assignment of the short and long arms is completely based on the length of each arm. **B** The degree of condensation of both the pCR and qCR varies from chromosome to chromosome. **C** There is sometimes a FUSC at the pter and at the qter

Table 1 (continued)

Chromosome 6 (Phantom or Terumodoki)
A Metacentrics: relative length 7.9±0.6, arm ratio 1.68±0.21. This chromosome shows variable patterns. The typical type exhibits a very small qCR compared with the pCR and the distinct dispersed regions on both arms, whereas the pDR is smaller than the qDR. A greater part of the short arm is sometimes condensed with simultaneous occurrence of bifurcation at the end of the chromatids on the outside, tapering off to a point. The end of the chromatids of the long arm converges. **B** The condensation degree of the pCR is greater than that of the qCR. **C** There is a FUSC in the central part of the pDR. There are several FUSCs in the qDR
Chromosome 7 (Disturber)
A Metacentrics: relative length 7.6±0.5, arm ratio 1.61±0.31. The chromosome shows variable patterns. In the typical pattern, the lengths of the pCR and the qCR are identical or the qCR is slightly longer. The qDR is distinct, whereas the pDR is very small. A typical short arm appears as the junction of the pCR and the telomeric condensed region. **B** The condensation degree of the pCR is the same or slightly greater than that of the qCR. The short arm has a telomeric FUSC. The whole region of the short arm is often condensed. **C** The long arm often has a FUSC at the qDR
Chromosome 8 (Rocket)
A Metacentrics: relative length 6.6±0.4, arm ratio 1.24±0.13. The whole part of the short arm is condensed. The visual pCR is frequently longer than the qCR and the long arm has a relatively long qDR. As an alternative pattern, the pCR is divided into two segments and the pter is relatively darker than usual. Consequently, three darkly stained blocks appear, two at the short arm and one at the long arm. The qter of the chromosome is sometimes bifurcated. **B** The degree of condensation of the pCR is the same or slightly lower than that of the qCR. The pCR frequently appears longer than the qCR, although the average length determined by the CP analysis is almost the same. When a gap-like structure appears in the pCR, the CP line of the pCR becomes variable. **C** The ends of the chromatids at the pter are not separated, although they are separated at the qter
Chromosome 9 (Trimodal or Mitsuyama)
A Metacentrics: relative length 6.6±0.5, arm ratio 1.26±0.11. The greater part of the chromosome is condensed. Three condensed blocks appear or, alternatively, a slit can be observed in the center of the qCR. **B** One and two condensed regions frequently appear at the respective short and long arms. **C** There are no FUSCs at the short arm. The chromatids are not separated at the telomeric ends of the long arm
Chromosome 10 (Plump or Shimobukure)
A Metacentrics: relative length 6.1±0.4, arm ratio 1.26±0.12. The whole chromosome is condensed. **B** The degree of condensation of the pCR is usually smaller than that of the qCR. The pCR is frequently shorter than the qCR in photographic images. **C** There are no FUSCs in the pDR or in the qDR

Table 1 (continued)

Chromosome 11 (Satellite or Sat)
A Subtelocentrics: relative length 5.8 ± 0.6, arm ratio 3.90 ± 1.11. A satellite chromosome. The short arm is much smaller than the long arm. Sometimes the end of the chromatids of the long arm opens like a fan. A satellite can sometimes be found separately or attached to the short arm. The chromosome is also located at an attaching or a close position to a nucleolus. Almost all the regions of the short arm and most of the long arm are heavily condensed. It is sometimes difficult to identify the centromeric position. **B** The degree of condensation at the pCR is very low and the region is much smaller than that of the qCR. **C** There are no FUSCs at the pDR. One or two FUSCs appear frequently in the qDR
Chromosome 12 (Little comet or Koteru)
A Submetacentrics: relative length 5.8 ± 0.4, arm ratio 2.00 ± 0.37. The general morphology is very similar to that of chromosome 4, but about two-thirds in size. The relative percentage of the qDR is slightly lower than that of chromosome 4. **B** The degree of condensation of the pCR is the same or greater than that of the qCR. **C** There is frequently a FUSC in the center of the qDR. No FUSCs appear in the pDR
The pCR, qCR, pDR, and qDR correspond to the proximal condensation region at the short and long arms, and the distal dispersed regions of a short and long arm, respectively. The pter and qter correspond to the terminal or telomeric region of a short and long arm, respectively. The chromosome nomenclature is based on Levan et al. (1964)

on the CP. Chromosomes 10 and 11 displayed uneven proximal condensations between the arms. The condensation region in the long arm was much larger than that of the short arm, and this tendency was observed among the 30 pro-metaphase chromosomes studied, without any exceptions. The CP varied with time, but the pattern remained similar and reproducible for the same chromosome.

It was found, however, that chromosome 6 exhibited at least two condensation patterns. This consisted of a uniform contraction of a whole short arm, while the long arm had a dispersed region without FUSCs (cf. chromosome 6d in Fig. 1). Another pattern consisted of a larger pCR with a relatively small pDR and smaller qCR with several FUSCs (cf. chromosome 6a in Fig. 1). Chromosome 8 occasionally showed a gap-like structure in the center of the pCR (cf. chromosome 8a in Fig. 1). Although the appearing frequency of the trait was low, it was a very specific trait and a good key character in identifying the chromosome 8.

A method of discrimination of rice chromosomes

Eleven discriminants were defined based on the 118 key characters listed in Table 1, as well as on the results described above for identifying each rice chromosome. Table 2 shows the list of the discriminants. The discriminants were arranged in an orderly fashion, to identify every rice chromosome by selecting them one by one. The discrimination procedure is shown in Fig. 2 as a flowchart.

Table 2. List of the discriminants

No. 1	Is it possible to distinguish chromosomes 4, 11, and 12 by visual inspection? If not, stop further procedures
No. 2	Select chromosomes 4, 11, and 12
No. 3	Select the longest three chromosomes among the remainder. Chromosomes 1, 2, and 3 are selected
No. 4	Select the most metacentric chromosome. It is chromosome 2
No. 5	Select one chromosome with the shorter length of the short arm between the former two chromosomes. It is chromosome 3
No. 6	Is it possible to distinguish chromosomes 1, 2, and 3 completely? If not, stop further procedures
No. 7	Select one chromosome with one and two condensed blocks on a respective short and long arm. Chromosome 9 is discriminated
No. 8	Select one chromosome with a higher condensation at the long arm than the short arm among the three shortest chromosomes selected from the remaining five chromosomes. The chromosome is chromosome 10
No. 9	Discard one chromosome with the shortest long arm among the remaining four chromosomes and then omit one chromosome with the longest short arm among the three chromosomes. Then the remaining two chromosomes are either chromosome 5 or 8
No. 10	Select one chromosome with the larger relative length. The longer one is chromosome 5 and the other is chromosome 8
No. 11	Select one chromosome with a larger difference between the condensation degrees at the regions of the long and short arms. It is chromosome 6 and the other one is chromosome 7

All the discriminants have a fitness probability of more than 85% with maximum probability of 100% for nos. 3, 8, 10

The numbers in the rhombi and rectangles of Fig. 2 correspond to the discriminant number in Table 2. Discriminant nos. 1, 2, and 6 were not related to the morphology of rice chromosomes, but they served as a visual check for the quality of the chromosomal image, whether or not it is worth analyzing further. Only those images that passed these two visual inspections were subjected to further discrimination procedures. There were also minor or supplementary discriminants.

As all of the chromosome numbers in the text were settled under the haploid condition, they had to be doubled when a diploid spread was analyzed.

Discriminant nos. 1 and 2 were selected as the first a priori condition for the identification of the rice chromo-

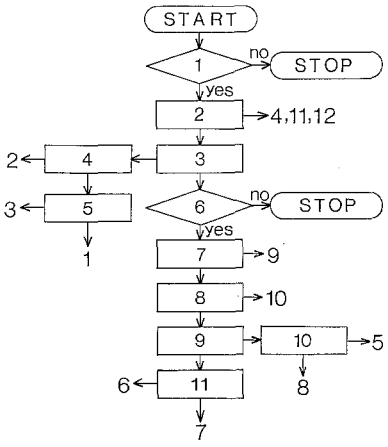

Fig. 2. Discrimination chart. Illustration of the discrimination procedure of all the rice chromosomes. Numbers in rhombi and rectangles correspond to the discriminant numbers. The other numbers correspond to chromosome numbers

somes. If it was not possible to identify all of the chromosomes 4, 11, and 12, the pro-metaphase image was discarded. Most of the chromosome images of the pro-metaphase spreads that were excluded at this step involved chromosomes that were severely distorted during the sample preparation, even though there was no overlapping or strong attachment. The fitness probability of discriminant no. 3 was 100%, i.e., all of the chromosomes 1, 2, and 3 from the 30 haploid plates were completely discerned by it. A supplementary discriminant useful for identifying the chromosomes 1, 2, and 3 in this step was that the total gray values of the CP for the selected three chromosomes were the largest ones among all chromosomes ($P=90.0\%$).

Discriminant no. 4 made it possible to discern chromosome 2 at a fitness probability of 93.3%. Discriminant no. 5 selected chromosome 3 also at a probability of 93.3%. Two supplementary discriminants of this step useful for identifying the three chromosomes were: "the short arm of chromosome 2 is longer than that of chromosome 3" and "the long arm of chromosome 1 is longer than that of chromosome 2." They had the fitness probability of 100 and 96.7%, respectively. Discriminant no. 6 was the second a priori condition that determined whether further analysis should be carried out or not. The difference in the relative length between chromosomes 2 and 3 was not statistically significant ($\alpha=0.01$). The telomeric FUSC that specifically characterized chromosome 3 could not be always observed. The difficulty in discriminating between chromosomes 2 and 3 was attributed to the morphological distortion of the chromosome spreads at the pro-metaphase. The percentage of the good plates that satisfied both the discriminant nos. 1 and 6 was ca. 97% among the total pro-metaphase photographs in the current material.

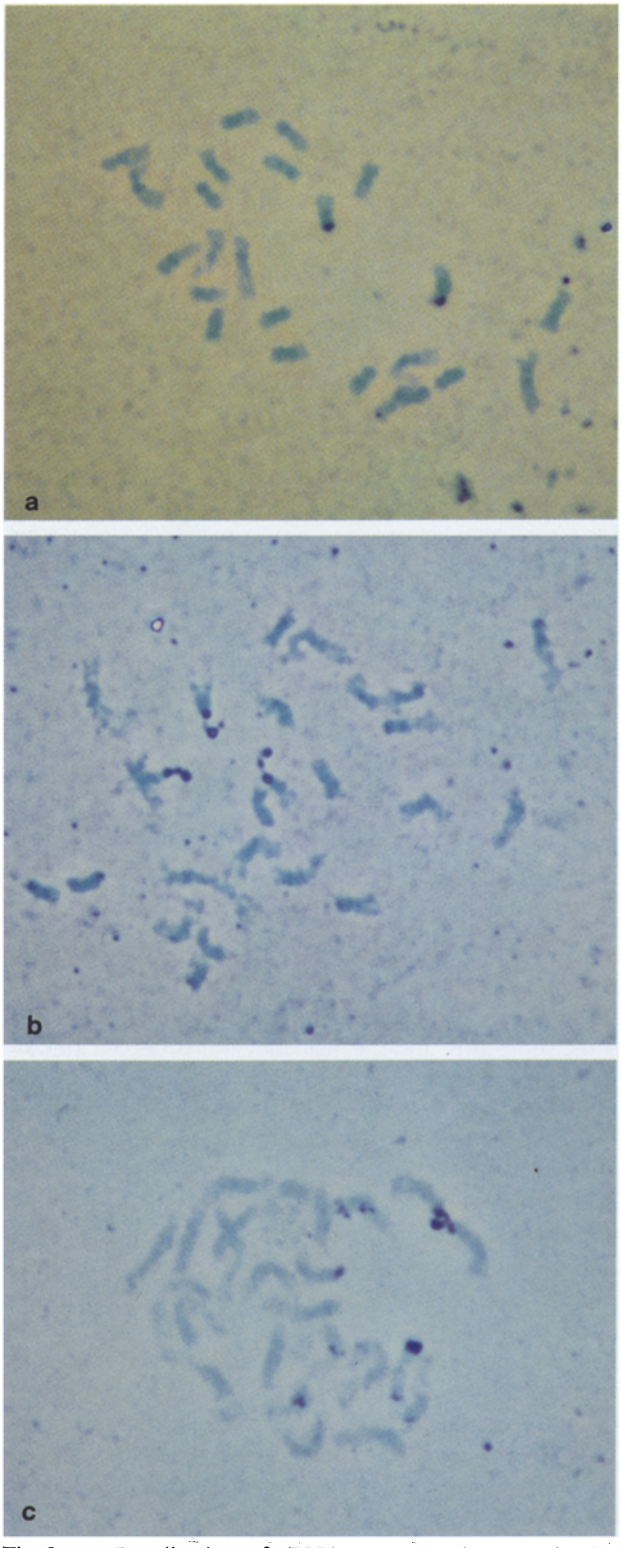

Fig. 3a–c. Localization of rRNA genes on the somatic rice chromosomes. **a** Two signals were clearly localized in the two satellite chromosome (chromosome 11) when a *japonica*-type variety "Nipponbare" was used as material. **b** In situ hybridization with the trisomic line of chromosome 11. Three signals were obviously observed. **c** Several signals were dispersed on more than two chromosomes when IR36, an *indica*-type rice, was used

The fitness probability of discriminant no. 7, which satisfied both chromatids, was 96.7%. A supplementary discriminant of this step was: "the selected chromosomes are the two smallest chromosomes, based on the average gray values of the CPs among the remainders" ($P=86.7\%$). Discriminant no. 8 selected chromosome 10 completely. The fitness probability of discriminant no. 9 to differentiate the other four chromosomes into two groups, i.e., chromosomes 5 and 8, and chromosomes 6 and 7, was 93.5%.

Discriminant no. 10 ($P=100\%$) was a complex discriminant, although only a representative one was listed in Table 2. Chromosomes 5 and 8 were able to be satisfactorily discerned by using all of the subsequent discriminants. Chromosome 5 had a larger total gray value of the CP than chromosome 8 ($P=93.3\%$). Chromosome 8 had a longer pCR than qCR ($P=73.3\%$, e.g., chromosome 8b in Fig. 1). Chromosome 5 appeared symmetrically ($P=70.0\%$, e.g., chromosome 5a in Fig. 1). The chromosome whose pCR had a faint gap within the pCR was chromosome 8 ($P=16.7\%$, e.g., chromosome 8a in Fig. 1). Discriminant no. 11 was also a complex one and the total fitness probability was 90.0%. Chromosome 6 was longer than chromosome 7 at a probability of 90.0%. Chromosome 6 was longer than chromosome 7 at a probability of 83.3%. The long arm of chromosome 6 was longer than that of chromosome 7 ($P=73.3\%$) and the value of the arm ratio was also higher ($P=70.0\%$). The telomeric region of the long arm of chromosome 6 was converged ($P=66.7\%$, e.g., chromosome 6f in Fig. 1). There was a large number of FUSCs at the qDR of chromosome 6 ($P=63.3\%$, e.g., chromosome 6c in Fig. 1). Chromosome 7 exhibited a FUSC in the telomeric part of the short arm with a probability of 80.0% (e.g., chromosome 7b in Fig. 1) and most of the short arm was relatively condensed ($P=70.0\%$, e.g., chromosome 7f in Fig. 1). When the whole short arm of chromosome 6 was condensed, the end of the chromatids bifurcated at the pter, and the tip of the chromatids appeared to be slender towards the end of the short arm ($P=20.0\%$, e.g., chromosome 6d in Fig. 1).

The chromosomes identified by the chart were confirmed one by one, using three checks based on the characters, relating not only one chromosome but also the characters relating to the several chromosomes. The ratio of complete discrimination of rice chromosomes exceeded 70% in the 30 chromosomal spreads analyzed. Detailed descriptions based on the three checks mentioned above were as follows. The first check was based on the characteristics of numerical data obtained among several chromosomes. Chromosome 1 was the largest among all the chromosomes, with a fitness probability of 86.7%. Chromosomes 2 and 5 had the smallest arm ratio among chromosomes 1, 2, 3, and 5 ($P=93.3\%$). The long arm of chromosome 8 was the shortest among those of chromosomes 5, 6, 7, and 8 ($P=90.0\%$).

The second check was based on the characteristics of the CP also relating over several chromosomes. The average gray value of the CP of chromosome 9 was the smallest or the second smallest among chromosomes 6 to 10 ($P=83.3\%$). The numbers of FUSCs occurring in both chromosomes 1 and 3 were the largest among all the rice chromosomes ($P=63.3\%$). The total gray value of the CP of chromosome 5 was larger than that of chromosome 8 ($P=93.3\%$).

The last interchromosomal check was performed using the traits based on visual inspection. There were two FUSCs in the pDR of chromosome 1 ($P=36.7\%$, e.g., chromosomes 1a and 1e in Fig. 1) and these were specific to chromosome 1 among chromsomes 1, 2, 3, and 5 ($P=70.0\%$). The short arm of chromosome 2 had a clear gap in the pDR ($P=93.3\%$, e.g., chromosome 2e in Fig. 1) and a less distinct one in the qDR ($P=53.3\%$, e.g., chromosome 2e in Fig. 1). These characters, especially at the short arm, were very specific among chromosomes 1, 2, 3, and 5 ($P=60.0\%$). Chromosomes 2 and 5 exhibited a symmetrical arrangement of the pCR and the qCR at the centromere (chromosome 2, $P=83.3\%$, e.g., chromosome 2c in Fig. 1; chromosome 5, $P=80.0\%$, e.g., chromosome 5a in Fig. 1). In the discrimination of chromosome 8 from chromosome 10, chromosome 8 had a longer pCR than the qCR ($P=83.3\%$, e.g., chromosome 8f in Fig. 1) and the reverse was noted for chromosome 10 ($P=96.7\%$, e.g., chromosome 10b in Fig. 1). A slight, gap-like structure could be observed in the qCR of chromosome 9 ($P=96.7\%$, e.g., chromosome 9a in Fig. 1) that was very specific among chromosomes 5 to 10. The telomeric region of the long arm of chromosome 9 was totally condensed ($P=86.7\%$, e.g., chromosome 9f in Fig. 1).

From 30 pro-metaphase spreads, 360 rice chromosomes were discriminated using the chart, followed by confirmation based on the above-mentioned criteria. As a result, all the somatic rice chromosomes could be identified. Although the identification ratio may be influenced by the quality of the pro-metaphase spreads, an objective identification method of rice chromosomes could be established.

The flow of the identifying methods is summarized as follows. The 12 (pairs) of rice chromosomes were first identified by the discriminants (Table 2) according to the flow of the discrimination chart (Fig. 2), with the aid of supplementary discriminants if necessary. Then the result was confirmed by the three checks mentioned above. If there was any difficulty in the identifying process, the key characters on each chromosome (Table 1) could be employed for the final confirmation.

Discrepancies among the studies on rice chromosomes

There have been several reports on the identification of somatic rice chromosomes (e.g., Hu 1958; Ishii and Mitsukuri 1960; Kurata and Omura 1978; Chung and Wu 1987). Since Kurata and Omura (1978) described the characteristics of the somatic chromosomes of the *japonica* type of rice that was used in the current study, our data were compared with those in their report. The relationship between the length order of the rice chromosomes in this report and the numbers allocated by Kurata and Omura (1978, K number) is as follows; 1 to K1, 2 to K3, 3 to K2, 4 to K4, 5 to K6, 6 to K5, 7 to K7?, 8 to K9?, 9 to K8, 10 to K11, 11 to K10, and 12 to K12. The K numbers with a question mark indicate the chromosomes for which there might be discrepancies between the photographs and description of the characteristics (Kurata and Omura 1978) and between the photographs of K7 and K9 (Kurata et al. 1981 b).

The K number was also based on the length order, and discrepancies with the number recorded in the current studies involved four pairs of chromosomes: 2 and 3, 5 and 6, 8 and 9, and 10 and 11. The value of the length of the paired chromosomes, however, was not statistically significant by the t-test ($\alpha = 0.01$). Thus, the numbering system adopted by Kurata and Omura (1978) was statistically consistent with our data. Statistical analysis based on numbers of chromosomes could thus determine the exact length order of somatic rice chromosomes, which had hitherto been controversial.

The main cause of the discrepancy in the length order may be ascribed to the fluctuations in the data associated with the small size of the samples. The difference in the varieties used could also account for the differences reported. There are several differences between the *japonica* and *indica* types of rice and also within the varieties. For example, the satellite is responsible for a great number of the morphological characteristics of a chromosome. In *japonica* rice, there is one pair of satellite chromosomes and there are two pairs in *indica* rice (Chung and Wu 1987). In situ hybridization with ribosomal RNA genes clearly revealed the presence of these satellite chromosomes (Fig. 3). In the studies of Chung and Wu (1987), Kurata and Omura (1978), and also in the current study, the respective cultivars IR36, Sekitori, and Koshihikari were used, the former belonging to the *indica* type and the latter two to *japonica*-type varieties. Thus, the CP analysis by imaging methods will be required in *indica* type rice, especially for the identification of the satellite chromosomes.

The differences in the mitotic stages as well as the differences in the pretreatment methods may account for the discrepancies observed (Kurata et al. 1981 b). As for the haploidy of the materials used in this experiment, no morphological differences were detected by imaging methods (Fukui et al. 1988).

Based on the results obtained, it was possible to characterize most of the morphological traits appearing on rice chromosomes and to identify the somatic rice chromosome objectively.

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