Karyotype analysis in rice

II. Identification of extra chromosomes in trisomic plants and banding structure on some chromosomes

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ABSTRACT

Twelve strains of primary trisomics were examined by the karyotype analysis. Four strains, B, C, E and H types were decided to have individual extra chromosomes K6, K12, K4 and K10 respectively. Between K5 and K9 and among K7, K8 and K11 chromosomes, there were some difficulties to discriminate each other. In well conditioned nuclei, however, K5, K7, K9 and K11 extra chromosomes were found to be carried by the A, D, L and F type trisomics respectively. Extra chromosome K8 was present in G, I, J and K type trisomics. Three kinds of extra chromosomes (K1, K2, K3) were not found in these 12 strains. Through the present study the modified technique for stretching the chromosomes appeared to be also promising to characterize chromosome bands.

1. INTRODUCTION

In cultivated rice, *Oryza sativa* ($2n=24$), 12 kinds of primary trisomics have been attempted from the progenies of triploid plants and phenotypically classified 12 trisomic strains were obtained (Iwata *et al.* 1970). They were examined for their extra chromosomes' correspondence with genetic linkage groups and 8 relationships were determined (Iwata and Omura 1975, 1976). Among the remaining 4 trisomics one type appeared to have the extra chromosome for the new linkage group with *su* gene (Isono *et al.* 1978). However, the cytological evidence in support of the linkage groups was not obtained, except for the correspondence of linkage groups with chromosomes obtained using the reciprocal translocation lines (Iwata and Omura 1971a, 1971b, Sato 1976).

In this study, we tried to identify the extra chromosomes of 12 trisomic strains through karyotype analysis. At present chromosomes can be identified either by the karyotype analysis of somatic prometaphase chromosomes (Kurata and Omura 1978) or by the pachytene analysis (Kurata *et al.* 1981). Since the morphology of pachytene trivalents were fairly intricate, the somatic nuclei were used for the determination of extra chromosomes in this study. Karyological informations pertinent to the band structure of chromosomes are also described and discussed.

2. MATERIALS AND METHODS

The following 12 primary trisomic types from japonica rice described by Iwata and Omura (1970) were used: A (Pale), B (Awne), C (Small grain), D (Erectoides), E (Spreading), F (Rolled leaf), G (Coarse), H (Large grain), I (Late heading), J (Spotted leaves), K (Pseudo normal) and L (Short panicle). In all strains, selfed seeds containing both diploid and trisomics were used for chromosome preparation. Trisomics were chosen for karyotype analysis. Chromosome preparations were made using the method by Kurata and Omura (1978) combined with the following pretreatment before the application of spindle inhibitor.

Germinating seeds were immersed in 2 mM deoxyadenosine (AdR: as a inhibitor of DNA synthesis) at 30°C for 15–20 h and were transferred in 0.5 mM uridine at 30°C for 3 h. These pretreated root tips were then soaked in 2 mM 8-oxyquinoline for 2 h. Then the root tips were macerated for 50–60 min at 35°C in a solution of 0.075 M KCl (pH 4.0) containing 6% pectinase (Sigma P-4625) and 6% cellulase (Onozuka R-10). After rinsing in water, a root tip was briefly fixed (about 20–30 sec) with a drop of the fixative on a slide glass till the fixative was thoroughly spread out. Then another drop of the fixative was again added, the root tip was squashed with a needle and flame dried. A part of the karyotype analysis was done using the method by Kurata and Omura (1978) without the pretreatment. The prepared slides were briefly immersed in 100% methanol and stained with Giemsa. Late prophase, prometaphase and early metaphase nuclei with clearly shaped chromosomes were photographed by a Nikon Biophoto microscope. Cells analysed in this study were assorted to three different stages according to their chromosome length. Cells with the 22–13 μm longest chromosome and the 7.5–5.5 μm shortest chromosome were assorted to the late prophase. The longest and the shortest chromosomes were 12–7 μm and 5–3.5 μm in the prometaphase and 6–4.5 μm and 3–1.5 μm in the early metaphase respectively. More than 50 nuclei were analysed in each trisomic strain. Designation of chromosomes from K1 to K12 were accorded with the analysis of Kurata and Omura (1978).

3. RESULTS AND DISCUSSION

Identification of extra chromosomes

Four types of trisomic strain, B, C, E and H, appeared to carry K6, K12, K4 and K10 as the extra chromosome respectively. Chromosomes of E and H type strains are shown in Figs. 1 and 2 respectively. The subtelocentric feature among the long chromosomes was helpful for the reliable identification of K4. K10 was easily discriminated as the nucleolar chromosome by its adhered condition to the nucleolus. Karyotypes of B and C type trisomics are shown in Figs. 3 and 4 respectively. Except K1, K2 and K3 chromosomes K6 was

Fig. 1. A part of the nucleus of E type trisomics (Spreading). Arrowed three chromosomes are K4. K4 is clearly distinguished by its subtelocentric feature.

Fig. 2. A part of the nucleus of H type (Large grain). Three K10 chromosomes (arrowed) are clearly identified by the attachment to the nucleolus.

discriminated from others by its longest short arm. However, it was sometimes mistaken for K7 chromosome due to the occasional contraction of K6 short arm. K12 was distinguished from other chromosomes by its smallness and distinct small short arm. The long arm of K12 chromosome was gradually contracted from late prophase to early metaphase, while the short arm was not so diminished during these stages. Accordingly K12 was classified as subtelocentrics and submetacentrics at different stages as shown in Figs. 9(b) and 9(a) respectively, though K12 was classified as submetacentrics in the previous work (Kurata and Omura 1978). The identification of these four kinds of extra chromosome was constant in many repeated analyses.

In the pretreated nuclei of L and A type trisomics, it was difficult to deter-

Fig. 3. The late prophase karyotype of B type trisomics (Awned) which appears as trisomy of K6 chromosome. Bar represents 10 μ m.

Fig. 4. The prometaphase karyotype of C type (Small grain) which appears a trisomy of K12 chromosome. Bar: 10 μ m.

mine whether they were trisomic for K5 or K9 chromosome. The difference of long arm length was observed to be more constant in the non-pretreated compact chromosomes than in the pretreated stretched chromosomes. In the non-pretreated compact chromosomes the whole length was a little longer in K5 than in K9 and the proximal deeply stained region on the long arm appeared slightly longer in K9 than in K5 as shown in Fig. 5. However, in the pretreated nucleus it was difficult to detect the difference in the amount of small deeply stained segment between K5 and K9, because the chromosome was stretched or diffused more than in non-pretreated compact nucleus. Due to the same reason, the difference of whole long arm length between the two

Fig. 5. The prometaphase karyotype of A type (Pale) which appears as a trisomy of K5 chromosome. Bar: 10 μ m.

Fig. 6. The early metaphase karyotype of L type (Short panicle) which is suggested to carry a trisomy for K9 chromosome. Chromosomes were prepared without the pretreatment of AdR and uridine. Bar: 10 μ m.

Fig. 7. The prometaphase karyotype of D type (Erectoides). In this figure K7 trisomy is confirmed by the coincidence of band structure in three chromosomes. Bar: 10 μ m.

Fig. 8. The early metaphase karyotype of F type (Rolled leaf). K11 chromosomes are distinguished from K7 and K8 by the feature of the shortest long arm. Bar: 10 μ m.

Fig. 9. (a): The early metaphase karyotype of J type (Spotted leaves) which is prepared without the pretreatment of AdR and uridine. Three K8 chromosomes are different from K7 and from K11 in the distal end of the long arm and in the whole length respectively.
 (b): The late prophase karyotype of K type (Pseudo normal) which represents a trisomy for K8 chromosome. K8 chromosomes are discriminated from K7 and K11 by their heterochromatic feature of the distal end of the long arm. Bar: 10 μ m.

Table 1. Relationship among extra chromosomes, trisomics, genetic linkage groups and marker genes

Extra chromosome	Type of trisomics	Linkage group*	Marker gene
K 4	E (Spreading)	II	<i>d₁₁, lg, Ph, Pl</i>
K 5	A (Pale)	—	<i>d_B, nal₂ rl₁, spl₁</i>
K 6	B (Awned)	I	<i>Cl, dp₁, ws, wx</i>
K 7	D (Erectoides)	—	<i>su</i>
K 8	G (Coarse)	VIII	<i>la, sp, z₁, z₂</i>
	I (Late heading)		
	J (Spotted leaves)		
	K (Pseudo normal)		
K 9	L (Short panicle)	VI, IX, XII	<i>d₁, nl₁, ri; gl</i>
K10	H (Large grain)	VII, V	<i>Bp, Dn, dp₂; I-Bf</i>
K11	F (Rolled leaf)	IV	<i>ds, g, Rc, spl₅</i>
K12	C (Small grain)	—	<i>fl, pgl, rk₂</i>

* Nagao and Takahashi (1963)

became smaller in the well stretched chromosomes. As a result, to differentiate between K5 and K9 the method without the pretreatment with AdR and uridine appeared most appropriate. In most cases the differences detected in the non-pretreated nuclei represented that A type was a trisomic for K5 and L type for K9. Typical karyotypes of A and L types are shown in Figs. 5 and 6 respectively.

D, F, G, I, J and K type trisomics appeared to carry one of the trisomics for K7, K8 or K11 chromosome. K7, K8 and K11 were difficult to distinguish from each other. In most cases they were similar to each other but in well conditioned nuclei they could be identified by their morphological differences. As described by Kurata and Omura (1978), K7 and K11 had similar arm ratio but were different slightly for relative length. In the present work, however, it was found in earlier stages that K7 and K11 had a difference in the amount of lightly stained chromatin on the long arm. For example, in Figs. 3 and 9(b) the deeply stained proximal segment is larger in K11 than in K7. With stage advancing, the lightly stained segment on the long arm contracted up and condensed earlier in K11 than K7. Consequently, in prometaphase the long arm of K7 possessed a small lightly stained segment, while K11 was all stained deeply. These differences made it possible to correspond K7 and K11 trisomics with D and F types respectively as shown in Figs. 7 and 8. G, I, J and K types were suggested to be only one kind of trisomics for K8 chromosome. Fig. 9 represents the karyotype of J and K type trisomics. K7 and K8 were often mistaken each other due to the similarities in length and arm ratio, however, the difference in staining intensity and/or chromatid separability of the distal end on the long arm enabled the distinction. The distal end of K8 long arm

Fig. 10. Both (a) and (b) are prometaphase nuclei with banded chromosomes. Not all but most chromosomes are differentially stained with Giemsa.

Fig. 11. K2 (a) and K4 (b) chromosomes with band structure. Late prophase, prometaphase and early metaphase chromosomes are arranged from left to right. Arrows represent the chromosome bands which appear to decrease in number as the stage advances.

was heterochromatic and two chromatids usually were joined together. In contrast, K7 was stained lighter than K8 at the distal end of the long arm and was split into two chromatids in many cases (Figs. 4, 5, 7, 8 and 9). More than a half of the analyses failed to show the differences between K8 and K11 chromosomes. K8 was in most cases discriminated from K11 only by the difference of the long arm length as shown in Figs. 8 and 9(a), but in very early stages (cf. Fig. 9(b)) they were distinguishable by the presence or absence of the lightly stained segment on the distal end of the long arms. The linkage analysis by the authors have shown that G and K types had the same extra chromosome (Unpublished). Recently, in addition, the possibility that G, I, J and K were trisomies for the same chromosome was suggested by the cross

with the reciprocal translocation lines (Unpublished). This supports the present result that G, I, J and K types were all the same trisomics for K8 chromosome.

Trisomics having one of the three longest chromosomes K1, K2 and K3 as the extra chromosome have never been found in the strains examined in the present work. It is possible that the above three trisomics could not be distinguished phenotypically from the diploid and/or the other trisomic plants. Another possibility is that the trisomics for K1, K2 and K3 are difficult to obtain from the progenies of triploid plants. The latter reasoning appears more likely because the twelve kinds of tertiary trisomics derived from the progenies of translocated chromosomes are formed distinct phenotypes. In addition, nine kinds of primary trisomics carrying one of K4, K5, K6, K7, K8, K9, K10, K11 and K12 as the extra chromosome formed more or less similar phenotypes to their respective tertiary trisomics (Iwata 1970). So, the primary trisomics for K1, K2 and K3 are supposed to form the distinct phenotypes. It appears that the gametes and/or zygotes carrying large extra chromosomes with many facultative genes may be inviable due to their genic imbalance. The correspondences among trisomic strains, extra chromosomes, linkage groups and marker genes are shown in Table 1.

Informations on chromosome banding

The pretreatment of cells with AdR and uridine produced not only many metaphases but also well-spread nuclei and well stretched chromosomes. Therefore, it was possible to analyse long stretched chromosomes even in late prophase as shown in Figs. 3 and 9(b). In addition, the method brought about considerably clear chromosome bands in some chromosomes. On K7 chromosome (Fig. 7), three bands in the short arm and three or four bands in the long arm could be recognized. In some nuclei many clearly or faintly emerged bands were detected. Prometaphase chromosomes with many detectable bands are shown in Fig. 10. Though the banding patterns were not always observed, detectable bands appeared to decrease in number with the advance of mitotic stage as shown in Fig. 11. On K2 chromosome at late prophase, prometaphase and early metaphase in Fig. 11(a), twelve, nine and seven bands were counted respectively. The chromosome bands observed here seem to show the characteristics of the G-band reported in other organisms (Yunis *et al.* 1978), because the bands observed in the present study were distributed all along the chromosome length and that they decreased with the advance of the stage. And these banding patterns are supposed to correspond to the patterns of pachytene chromosomes (Okada and Comings 1974). When the method to characterize the stable chromosome bands is established, more strict chromosome analysis will be possible.

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