C-banded Karyotypes of Seven Cultivars of *Oryza sativa*

M. A. Hamoud¹, Y. A. Hassan¹, W. Nagl² and E. E. Selim¹

¹Botany Department, Faculty of Science, Tanta University, Tanta, Egypt

²Department of Cell Biology, Kaiserslautern University, Kaiserslautern, West Germany

Accepted December 13, 1990

Preferential Giemsa staining of heterochromatin has been used as a mean of distinguishing the somatic metaphase chromosomes of several plants. Marks and Schweizer (1974) reported considerable inter-specific variations in the karyotypes of six species of *Anemone* and in *Hepatica nobilis*, both regarding the amount and distribution of Giemsa C-bands. Filion (1974) also found a wide range in the amount of constitutive heterochromatin; as measured by the amount of banding, between three cultivars of *Tulipa*. Ramachandran *et al.* (1985) also found infraspecific variation in C-banding pattern of non-cultivated and cultivated *Cucumis sativus* accessions. In addition, Giemsa C-banding has been used in the identification of common wheat chromosomes (Endo 1986).

Giemsa C-banding has also been used as a further cytological tool to verify both generic and specific delimitation of many plants. It has also been proved valuable in providing evidence for the taxonomic arrangement of *Scilla* and *Puschkinia* (Greilhuber and Speta 1976, 1978), *Anacyclus* (Schweizer and Ehrendorfer 1976), *Nigella* (Gilot-Dehale *et al.* 1976) and *Allium* (Badr and Elkington 1977, 1984, Elgadi and Elkington 1975, Elkington *et al.* 1976, Vosa 1976a, b).

Until recently such studies were lacking in rice because of its small chromosomes which have been difficult to obtain in a good cytological preparation. Kurata (1987) investigated the morphological characteristics of rice chromosomes in mitosis and meiosis. He studied centromere position, relative chromosome length, nucleolous organizing regions as well as darkly stained chromosome segments in the strain japonica of *Oryza sativa*, *O. perennis*, *O. punctata* and *O. officinalis* by the aid of Giemsa stain. Some other cytological studies were also carried out on rice (Shastri *et al.* 1960, Wu 1967, Misra and Shastri 1967, Ranganadhacharyulu and Raj 1974, Khan 1975, Reddi and Reddi 1977, Dolores *et al.* 1977, Sato *et al.* 1980, Wu and Chung 1986). We present here the C-banding patterns in seven cultivars of *Oryza sativa* being cultivated in Egypt. Their relative chromosome lengths and arm ratios are also included.

Materials and methods

The seven cultivars used for this study from *Oryza sativa* L. were obtained as grains from the Agricultural Research Centre, Cairo, A. R. Egypt. These cultivars are: Sabene, IR 28, Reiho, Giza 159, Giza 171, Giza 172 and Giza 175.

Root tips were taken from grains germinated at room temperature (24°–28°C) on moist filter papers in glass petri dishes. The procedure used for Giemsa banding; a modification from that of Gostev and Asker (1978); can be summarized as follows:

Pretreatment: Root tips. 0.5–1.5 cm long, were pretreated with a solution of 2 mM 8-hydroxyquinoline for 3 hr at room temperature.

Fixation: Root tips were then washed with distilled water and fixed in a freshly prepared mixture of absolute alcohol/glacial acetic acid (3:1 v/v) at room temperature for 24 hr, then-

after, they were washed and stored in 70% ethanol in refrigerator until use.

Maceration: Root tips were treated with an enzyme mixture of 6% (W/V) pectinase and 2% (W/V) cellulase in 0.01 M citrate buffer, pH 4.6, for 4 hr at 37°C (The buffer was prepared from a ten times concentrated stock solution consisting of three parts of 0.1 M trisodium citrate and two parts of 0.1 M citric acid).

Squash: The meristematic parts were cut off and transferred to a small drop of 45% acetic acid on a clean slide. The contents of the meristematic piece were then pressed out to form a cell suspension, then covered with clean cover slip and squashed cautiously without heating.

Dehydration: The cover slip was separated from the slide by using a flexi-cool plate. Slides were immersed briefly in absolute alcohol and then air dried. In order to obtain good resolution of bands, slides were incubated for 1 or 2 days at 40–45°C.

Denaturation: The slides were immersed in 5% (W/V) Ba(OH)₂ for 10 min at room temperature and then washed in running tap water for 15–60 min.

Renaturation: Slides were kept in 2×SSC (Sodium Saline Citrate) at 60°C for one hr, washed in distilled water and then 1/15 M Sorensen phosphaste buffer, pH 6.8, which was prepared as a mixture of 1/15 M KH₂PO₄ and 1/15 M Na₂HPO₄·2H₂O.

Staining: Staining of slides was carried out for 3 hr in 2% Giemsa (Merck) prepared in 1/15 M Sorensen phosphate buffer (pH 6.8). Then, slides were briefly washed in buffer and air dried.

Mounting: Mounting was carried out in Euparal TM and slides were left to dry at 60°C overnight.

The slides were examined under light microscope at a magnification of 1000× using oil immersion. Well stained configurations of chromosomes were photographed using Agfa Ortho black-white film and idiograms were then constructed on tracing paper. The number and size of chromosomes as well as heterochromatic bands were also recorded.

Results

Results of chromosome relative length (RL) and arm ratios (AR) of the seven *Oryza sativa* cultivars are given in Table 1. These results show that the karyotype of *O. sativa* is constructed of five metacentric (mc); K3, K6, K7, K8 and K11; five submetacentric (sm); Kx, K2, K5, K9 and K12 and two subtelocentric (st); K4 and K10; chromosome pairs. These results are similar to those obtained by Kurata (1987) and Kurata and Omura (1978). However, they recorded one chromosome pair, K10, with nucleolar organizer.

The idiograms of Giemsa C-banded complements of different cultivars are presented in Figs. 1–7.

Sabene: Fig. 1.

This cultivar exhibits a low amount of constitutive heterochromatin in its genome. All mc and sm chromosomes have centrometric bands except K11 which is not banded and K12 which has telomeric band on the short arm. The st chromosome type K4, however, has a telomeric band on the short arm while the other st type K10 shows two telomeric bands on both arms.

IR 28: Fig. 2.

The three sm chromosome types K1, K2 and K9 show interstitial bands on their long arms while K5 and K12 exhibit centrometric bands. The only banded mc chromosome types are K3 which has two interstitial bands on its long arm, and K11 which has centromeric band. However, the other mc chromosome types K6, K7 and K8 are not banded. The at chromo-

somes K4 and K10 have two thick bands each, on both short and long arms with bands covering the whole short, and most of the long arms of the latter type.

Reiho: Fig. 3.

The sm chromosomes types K2 and K5 exhibit centrometric bands, while K1 has one interstitial band on each arm. The other two types K9 and K12, however, show no bands. The mc types K3, K7 and K11 also have interstitial band on each of their arms whereas the two types K6 and K8 are not banded. On the other hand, the two st types K4 and K10 have similar appearance of bands. Their whole short arms are banded while long arms have an intercalary band each.

Table 1. Means of chromosome relative length (RL) and arm ratio (AR) in seven cultivars of *Oryza sativa*

Chromosome Type	Sabene		IR 28		Reiho		Giza 159		Giza 171		Giza 175		Giza 172	
	RL	AR	RL	AR	RL	AR	RL	AR	RL	AR	RL	AR	RL	AR
K 1	13.90	1.75	12.40	1.88	11.80	1.80	13.30	1.95	12.60	1.78	13.90	1.78	12.00	1.85
K 2	12.50	2.15	10.10	2.10	10.70	2.16	12.60	2.30	12.00	2.33	12.50	2.30	11.50	1.91
K 3	9.70	1.33	9.30	1.33	9.60	1.36	10.10	1.40	11.00	1.50	10.80	1.30	9.40	1.25
K 4	8.30	3.67	9.30	3.75	8.80	4.28	8.40	4.33	10.30	3.88	8.40	3.67	9.40	3.70
K 5	8.30	2.10	8.70	2.16	8.30	2.05	8.40	2.20	9.00	2.00	8.40	2.00	8.10	2.00
K 6	7.60	1.17	8.20	1.13	8.30	1.11	7.70	1.08	7.30	1.14	7.00	1.08	8.10	1.20
K 7	7.30	1.30	7.60	1.40	8.30	1.25	7.00	1.22	6.60	1.40	7.00	1.25	7.70	1.40
K 8	6.90	1.25	7.60	1.20	8.00	1.15	7.00	1.18	6.60	1.11	7.00	1.10	7.70	1.20
K 9	6.90	1.75	7.60	1.83	8.00	1.80	7.00	1.78	6.60	1.75	7.00	1.75	7.30	1.77
K10	6.90	3.80	7.30	4.33	7.00	4.40	6.30	4.80	6.30	4.00	7.00	4.00	6.80	3.96
K11	5.90	1.28	6.20	1.17	5.90	1.17	6.30	1.21	6.00	1.20	5.60	1.30	6.00	1.33
K12	5.60	2.45	5.60	2.33	5.30	2.33	5.90	2.33	5.60	2.22	5.60	2.30	5.60	2.19
Total Genome (μm)	28.80		35.50		37.40		28.60		30.10		28.70		23.40	
Mean	2.40		2.96		3.12		2.38		2.51		2.39		1.95	
$\pm$ S. E.	± 0.21		± 0.19		± 0.20		± 0.20		± 0.22		± 0.22		± 0.13	

Giza 159: Fig. 4.

In this cultivar two bands are exhibited by each of the sm types K1, K2 and K5, the mc types K3 and K7, and the st type K10. The chromosome types K1, K5, K7 and K10 have their bands distributed on their two arms while K2 and K3 have the two bands on their long arms only. A single band however is exhibited by the st type K4 on telomere of the short arm and by the sm type K9 on its centromere. The other mc types, K6, K8, K11 and the sm type K12 are not banded.

Giza 171: Fig. 5.

The sm types K1 and K2 have two intercalary bands each, one on either arms of the former and the two on the long arm only of the latter. The two sm types K5 and K9 however, have single intercalary band each, on their long arms while K12 has centrometric band. The only banded mc chromosome type is K3 which has two intercalary bands on both arms while the other types K6, K7, K8 and K11 are not banded. Both the st types K4 and K10 have their short arms fully banded with the former having another two intercalary bands on its long arm.

Giza 175: Fig. 6.

The five longest chromosome types K1, K2, K3, K4 and K5 of this cultivar are similar in

their band positions to those of the previous one. The shortest chromosome type K12 is also similar to its counterpart in the previous cultivar in having centromeric band. The three mc types K6, K7 and K8 which are not banded in Giza 171 are banded in this cultivar and have single intercalary band on the short arm of the former two types (K6 and K7) and two intercalary bands on both arms of the latter type (K8). The sm type K9 which has single intercalary

Figs. 1-7. Idiograms of Giemsa C-banded karyotypes of seven cultivars of *Oryza sativa*. 1, Sabene. 2, IR 28. 3, Reiho. 4, Giza 159. 5, Giza 171. 6, Giza 175 and 7, Giza 172.

band on its long arm in Giza 171, however, is not banded in this cultivar while the st type K10 has one intercalary band on its long arm in addition to the band observed on the short arm.

Giza 172: Fig. 7.

The sm chromosome type K1 has two intercalary bands, one on each arm, whereas the other sm types K2, K5, K9 and K12 have single centromeric band each. Also the mc type K7 has two intercalary bands, one on each arm, and K3 has single centromeric band whereas the

other mc types; K6, K8 and K11; exhibit no bands. However, the two st types K4 and K10 are similar in having their short arms fully banded and having single intercalary band on their long arms.

Discussion

The present study on mitotic chromosomes of seven *Oryza sativa* cultivars shows that total genome size varies from relatively high in Reiho (37.4 μm) to relatively small in Giza 172 (23.4 μm). It ranges in the other five cultivars between 28.7 and 35.5 μm . In spite of these variations, the genome of each cultivar is identifiable into twelve distinguished chromosome types. These are designated K1 to K12 according to their length (Kurata 1987, Kurata and Omura 1978).

The results also show that the genome of *O. sativa* is considerably rich in heterochromatin with intraspecific variation in C-banding patterns. Karyotypic variation may involve linear amplification or deletion of specific repetitive sequences, translocation and inversion of chromosome segments, or unequal crossing over (Rees and Jones 1972, Hutchinson *et al.* 1977). Kurata (1987) and Kurata *et al.* (1981) observed; in rice; several bands distributed all along the chromosome which decreased in number with the advancement of stage. These bands showed the characteristics of the G-bands reported in other organisms (Yunis and Sanchez 1973, Yunis

Table 2. Collective frequency of C-bands in different chromosome types of seven cultivars of *Oryza sativa*

Chromosome Type	K1 sm	K2 sm	K3 mc	K4 st	K5 sm	K6 mc	K7 mc	K8 mc	K9 sm	K10 st	K11 mc	K12 sm
Band type												
Telomeric	—	—	—	7	1	—	—	—	—	8	—	1
Centromeric	1	3	2	—	4	1	1	1	3	—	1	4
Interstitial	11	7	10	7	3	1	7	2	2	5	2	—

et al. 1978). In the present study, however, the C-bands observed in metaphase chromosomes showed a high degree of stability. The number of bands per chromosome ranges from zero to three at the most with some variation in their size. The subtelocentric chromosomes K4 and K10 are identifiable with their wholly banded short arms in all cultivars. In Sabene, most of the chromosomes show single centromeric band and terminal bands are sometimes observed. An intercalary band is recorded in this cultivar only on the long arm of the st chromosome type K10. On the other hand, the other cultivars show less centromeric and high interstitial and terminal bands. The highest number of interstitial bands is exhibited by the chromosomes of Giza 175 (14 bands) followed by Reiho, Giza 159 and Giza 171 (10 bands each). Hsu (1973) interpreted the interstitial and terminal C-bands, and the lack of centromeric bands, as being a result of inversions or translocations. This interpretation seems to fit the case in these cultivars of *O. Sativa*.

The present study shows infraspecific differences in C-band patterns among the seven cultivars investigated of *Oryza sativa*. The highest frequency of bands, collectively in the seven cultivars, has been recorded (Table 2) in the subtelocentric chromosomes K4 and K10. These two chromosome types exhibit high frequencies of telomeric and interstitial bands with no centromeric ones. The longest three chromosome types K1, K2 and K3 display high frequencies in interstitial bands with frequent centromeric bands and no telomeric ones. The two meta-centric chromosome type K8 and K11 are often not banded in the cultivars investigated.

Summary

Seven cultivars of *Oryza sativa*, being grown in Egypt, have been cytologically investigated with the aid of Giemsa C-banding technique. These cultivars are Sabene, IR 28, Reiho, Giza 159, Giza 171, Giza 172 and Giza 175. All these cultivars have karyotypes that constructed from five meta-, five submeta- and two subtelo-centric chromosome pairs. The lowest value for total genome size; 23.4 μm ; was recorded in Giza 172 and the highest; 37.4 μm ; was recorded in Reiho. The other cultivars showed genomes that ranged between 28.6 and 30.1 μm , except IR 28 which exhibited genome size of 35.5 μm . The results also showed that the genome of *O. sativa* is considerably rich in heterochromatin with variation in C-banding pattern among the different cultivars. The frequency of telomeric and interstitial bands was very high in the subtelocentric type of chromosomes which exhibited no centromeric bands. On the other hand, interstitial bands were recorded in high numbers in the longest meta- and submeta-centric chromosomes which frequently exhibited centromeric bands.

References

- Badr, A. and Elkington, T. T. 1984. Giemsa C-banded Karyotypes and Taxonomic relationships of some North American *Allium* species and their relationships to Old World species (Liliaceae). *Pl. Syst. Evol.* **144**: 17–24.
- and — 1977. Giemsa C-band and fluorochrome banded Karyotypes and species relationships in *Allium* subgenus *Molium*. *Ibid* **128**: 23–35.
- Dolores, R. C., Chang, T. T. and Ramirez, D. A. 1979. The cytogenetics of F_1 hybrids from *Oryza nivara* Sharma et Shastry $\times$ *O. sativa* L. *Cytologia* **44**: 527–540.
- El-Gadi, A. and Elkington, T. T. 1975. Comparison of the Giemsa C-band karyotypes and the relationships of *Allium cepa*, *A. fistulosum*, and *A. galanthum*. *Chromosoma* **51**: 19–23.
- El Kington, T. T., Badr, A., Hussain, L. and White, S. 1976. Giemsa C-band and quinacrine banded karyotypes and systematic relationships in *Allium*. In Jones, K., Barndham, P. E. (eds.): *Current chromosome Research*, 214–215. North Holland Press.
- Endo, T. R. 1986. Complete identification of common wheat chromosomes by means of the C-banding technique. *Jpn. J. Genet.* **61**: 89–93.
- Filion, W. G. 1974. Differential Giemsa staining in plants I. Banding patterns in three cultivars of *Tulipa*. *Chromosoma* **49**: 51–60.
- Gilot-Delhale, J., Degraeve, N. and Moutschen, J. 1976. Cytotaxonomic investigations of the genus *Nigella* (Helleboreae) with C-banding techniques. *Caryologia* **29**: 139–154.
- Gostev, A. and Asker S. 1979. A C-banding technique for small plant chromosomes. *Hereditas* **91**: 140–143.
- Greilhuber, J. and Speta, F. 1976. C-banded Karyotypes in the *Scilla hohenackeri* group, *S. persica* and *Puschkinia* (Liliaceae). *Pl. Syst. Evol.* **126**: 149–188.
- and — 1978. Quantitative analysis of C-banded karyotypes and systematics in the cultivated species of the *Scilla siberica* group (Liliaceae). *Ibid* **129**: 63–109.
- Hsu, T. C. 1973. Longitudinal differentiation of chromosomes. *Ann. Rev. Genet.* **7**: 153–176.
- Hutchinson, J., Narayan, R. K. and Rees, H. 1977. Constraints upon the composition of supplementary DNA. *Chromosoma* **78**: 137–145.
- Khan, S. H. 1975. A technique for staining rice chromosomes. *Cytologia* **40**: 595–598.
- Kurata, N. 1987. Chromosome analysis of mitosis and meiosis in rice. *Rice Genet.*, Japan; 143–152.
- and Omura T. 1978. Karyotype analysis in rice. I. A new method for identifying all chromosome pairs. *Jpn. J. Genet.* **56**: 251–255.
- , Iwata, N. and Omura T. 1981. Karyotype analysis in rice. II-Identification of extrachromosomes in trisomic plants and banding structure on some chromosomes. *Ibid* **56**: 41–55.
- Marks, G. E. and Schweizer, D. 1974. Giemsa banding: Karyotype differences in some species of *Anemone* and *Hepatica nobilis*. *Chromosoma (Berl.)* **44**: 405–416.
- Misra, R. N. and Shastry, S. V. S. 1967. Pachytene analysis in *Oryza*. 8. Chromosome morphology and karyotypic variation in *Oryza sativa* L. *J. Genet. Pl. Breed.* **27**: 349–368.
- Ramachandran, C., Brandenburg, W. A. and den Nijs, A. P. M. 1985. Intraspecific variation in C-banded karyotype and chiasma frequency in *Cucumis sativus* (Cucurbitaceae). *Plant Syst. Evol.* **151**: 31–41.

- Ranganadhacharyulu, N. and Raj, A. Y. 1974. Pachytene analysis in an interspecific hybrid *Oryza punctata* Koschy ex steud $\times$ *O. eichingeri* A. peter. *Cytologia* **39**: 233-243.
- Reddi, V. R. and Reddi, T. V. V. S. 1977. Chromosome pairing at pachytene and meiosis in autotetraploid rice. *Cytologia* **42**: 189-196.
- Rees, H. and Jones, R. N. 1972. The origin of the wide species variation in nuclear DNA content. *Int. Rev. Cytol.* **32**: 53-92.
- Sato, S., Kinoschita, T. and Takahashi, E. M. 1980. Location of centromere and interchange breakpoints in the pachytene chromosome of rice. *Jpn. J. Breed.* **30**: 387-398.
- Schweizer, D. and Ehrendorfer, F. 1976. Giemsa banded karyotypes, systematics, and evolution in *Anaclycus* (Asteraceae-Anthemideae). *Plant syst. Evol.* **126**: 107-148.
- Shastri, S. V. S., Ranga Rao, D. R. and Misra, R. N. 1960. Pachytene analysis in *Oryza sativa*. *Indian J. Genet. Pl. Breed.* **20**: 15-21.
- Vosa, C. G. 1976a. Heterochromatic patterns in *Allium*: I. The relationship between the species of the *cepa* group and its allies. *Heredity* **36**: 383-392.
- 1976b. Heterochromatic patterns in *Allium*: II. Heterochromatin variation in species of the *paniculatum* group. *Chromosoma* **57**: 119-133.
- Yunis, J. J. and Sanchez, O. 1973. G-banding and chromosome structure. *Chromosoma (Berl.)* **44**: 15-23.
- , Sawyer, J. R. and Ball, D. W. 1978. The characterization of higher resolution G-banded chromosomes of man. *Chromosoma* **67**: 293-307.
- Wu, H. K. 1967. Note on preparing of pachytene chromosomes by double mordant. *Scientific Agric.* **15**: 40-44.
- and Chung, M. C. 1986. Rice karyotype analysis. *Proc. Int. Rice Genetic Symp.* May 1985, IRRI Manila, Philippines, pp. 135-142.
-