CYTOLOGICAL STUDIES ON RYE PLANTS WITH SEVEN AND EIGHT ACCESSORY CHROMOSOMES

HIDETOSHI KISHIKAWA

Laboratory of Plant Breeding, Faculty of Agriculture, Saga University, Saga

Received October 30, 1967

So far the accessory chromosomes in *Secale cereale* L. have been extensively studied by Müntzing and his co-operators. The great majority of their investigations have dealt with the accessory chromosomes in the highbred Swedish rye variety, but the study of accessory chromosomes in the primitive Asiatic rye varieties is very scanty. To enable us to form a firm idea of the significance of accessory chromosomes in rye plant, further comparative studies in a large number of rye varieties are needed.

Thus, a series of studies were designed to clarify the characteristics of accessory chromosomes in the native rye of Japan (Kishikawa 1962, 1963, 1965 and 1966). The present paper deals with the meiotic behavior of accessory chromosomes in rye plants with seven and eight accessories.

MATERIALS AND METHODS

The present materials belong to the same strain, JNK, that the author have hitherto used for the investigations. The rye plants with seven and eight accessory chromosomes were found in the intercrossed progenies between plants with four accessories.

For somatic chromosome counts the root tips were pretreated with 8-oxyquinoline solution (0.003M) for 5~6 hours at about 16°C. They were then warmed to macerate in 1N-HCl, stained and squashed with aceto-orcein. For the study of meiosis the spikes were fixed in acetic alcohol (1 : 3) for about 12~15 hours at 6~8°C, after that they were transferred to 75 per cent ethyl alcohol and kept at 6~8°C until observation. The preparations were made by acetate-carmin squash method.

RESULTS

At mitotic metaphase in root-tip, seven and eight accessory chromosomes were observed, as shown in Figs. 1 and 2, respectively. All of them were the accessory chromosome of the so-called standard type.

The vegetative growth of these plants, as compared with that of plants with a small number of accessory chromosomes, was very poor, and their heading time delayed remarkably. The seed fertility was completely sterile.

1. The plant with seven accessory chromosomes (7 acc. plant)

At meiotic metaphase I the seven accessory chromosomes do not pair with any of the normal chromosome complements, but do very well with each other and form many

Figs. 1 and 2. Somatic chromosomes in root tip cell. $\times 1,700$. Fig. 1. $2n=14+7$ accessories. Fig. 2. $2n=14+8$ accessories. (Arrow marks: Accessory chromosomes).

multivalents. The shape of multivalents is radial. Furthermore, in the heptavalent of accessory chromosomes two types were observed (Figs. 3 and 4). In the quadrivalents and hexavalents symmetrical and asymmetrical types were distinguished. The chromosome configurations and their frequencies were given in Table 1. Some examples of these configurations are shown in Figures 3~8. The modal configuration was found in $1_{IV}+1_I+1_I$ (Fig. 6) followed by 3_I+1_I (Fig. 8).

Table 1. Association of seven acc. chromosomes at MI in the 7 acc. plant

Configurations of acc. chromosomes	No. of cells observed	%	Configurations of acc. chromosomes	No. of cells observed	%
1 VII	6	4.0	2 III + 1 I	2	1.3
1 VI + 1 I	10	6.7	1 III + 1 I	6	4.0
1 V + 1 II	7	4.7	1 III + 1 II + 2 I	13	8.7
1 V + 2 I	3	2.0	1 III + 4 I	6	4.0
1 IV + 1 III	5	3.3	3 II + 1 I	18	12.0
1 IV + 1 II + 1 I	39	26.0	2 II + 3 I	13	8.7
1 IV + 3 I	11	7.3	1 II + 5 I	11	7.3
Total number of cells observed			150		

At anaphase I the disjunctions of accessory chromosomes are summarized in Table 2. The accessory chromosomes separated to 3—4 in most of cells (Fig. 9) and to 2—5 in a few ones (Fig. 10). In the remaining cells one or two univalents of accessory chromosomes lagged on the equator plate, and the laggards divided to two chromatids and they usually passed to the poles to be included in the daughter nuclei.

Table 2. Disjunction of seven acc. chromosomes at AI in the 7 acc. plant

Distributions of acc. chromosomes	No. of cells observed	%
4 ————— 3	56	70.0
5 ————— 2	5	6.3
3 ——— 1 ——— 3	17	21.3
4 ——— 1 ——— 2	1	1.2
3 ——— 2 ——— 2	1	1.2
Total number of cells	80	

The number of micronuclei in the pollen tetrad was very few, as shown in Table 5.
2. The plant with eight accessory chromosomes (8 acc. plant)

At meiotic metaphase I the eight accessory chromosomes associate radially with each other and form many multivalents. In the radial multivalents, such as octavalent, hexavalent and quadrivalent, two types were also distinguished, the one was symmetrical and the other asymmetrical. These configurations will reflect on subsequent anaphase I separation. The various types of accessory chromosome configurations and their frequencies were summarized in Table 3. Some examples of these configurations are shown in Figures 12~17. The mode of configurations was $1_{IV} + 2_I$ followed by 1_{VII} .

Table 3. Association of eight acc. chromosomes at MI in the 8 acc. plant

Configurations of acc. chromosomes	No. of cells observed	%	Configurations of acc. chromosomes	No. of cells observed	%
1 VII	31	14.1	1 IV + 1 II + 2 I	16	7.3
1 VII + 1 I	7	3.2	1 IV + 4 I	2	0.9
1 VI + 1 II	28	12.7	2 III + 1 II	2	0.9
1 VI + 2 I	4	1.8	1 III + 2 II + 1 I	7	3.2
1 V + 1 II + 1 I	8	3.7	4 II	13	5.9
2 IV	16	7.3	3 II + 2 I	10	4.5
1 IV + 1 III + 1 I	4	1.8	2 II + 4 I	2	0.9
1 IV + 2 II	70	31.8	Total no. of cells	220	

At anaphase I the disjunctions of accessory chromosomes were given in Table 4. In most of cells 4—4 distribution was observed (Fig. 18). In the remaining cells 5—3 distribution (Fig. 19) and one or two of lagging accessory chromosomes were observed. The behavior of laggards is the same manner as mentioned above.

The number of micronuclei in the pollen tetrad was a few, as summarized in Table 5.

Table 4. Disjunction of eight acc. chromosomes at AI in the 8 acc. plant

Distributions of acc. chromosomes	No. of cells observed	%
4 ————— 4	89	74.1
5 ————— 3	14	11.7
4 ——— 1 ——— 3	15	12.5
3 ——— 2 ——— 3	2	1.7
Total number of cells	120	

Table 5. Frequencies of micronuclei in pollen tetrad

Plants \ No. of micronuclei					No. of tetrads observed
	0-0-0-0	1-0-0-0	1-1-0-0	2-0-0-0	
7 acc.	95.6%	4.4	—	—	500
8 acc.	96.0%	3.6	0.2	0.2	500

3. The comparisons between the plants with seven and eight accessory chromosomes

The frequencies of accessory chromosomal pairing at metaphase I are summarized in Table 6. In both of the plants the bivalent formation is most frequent. The 7 acc. plant has far larger number of univalent than the 8 acc. plant, while the higher association than bivalent in the 7 acc. plant is remarkably lower than in the 8 acc. plant. In the 8 acc. plant, most of the multivalent of accessory chromosomes are of even valency and an uneven valency is very meagre. This is generally applicable to the plant with even number of accessory chromosomes (cf. Kishikawa 1965).

Table 6. Frequencies of acc. chromosome pairing

Plants \ Valencies								
	I	II	III	IV	V	VI	VII	VIII
7 acc.	24.0%	30.8	9.7	21.0	4.8	5.7	4.0	—
8 acc.	5.8%	33.4	2.6	28.2	2.3	10.9	2.7	14.1

The disjunction of accessory chromosomes at anaphase I is quite normal. The cells with lagging accessory chromosomes in the 7 acc. plant were more frequently observed than those in the 8 acc. plant (Tables 2 and 4). In spite of this difference, the number of micronuclei at tetrad stage in the 7 acc. plant is about the same as one in the 8 acc. plant.

At any rate, notwithstanding the fact that the present plants have a large number of accessory chromosomes, the meiosis in PMC is normal and the meiotic elimination of accessory chromosomes is very low.

Figs. 3~11. Meiosis of PMCs in the plant with 7 accessories. $\times 1,100$. (Arrow marks: Accessory chromosomes). Figs. 3~8. Accessory chromosomes at metaphase I. Figs. 3 and 4. 1_{II} . Fig. 5. $1_{II} + 1_I$. Fig. 6. $1_{II} + 1_{II} + 1_I$. Fig. 7. $1_{II} + 1_{II} + 2_I$. Fig. 8. $3_{II} + 1_I$. Figs. 9~11. Accessory chromosomes at anaphase I. Fig. 9. 4—3 distribution. Fig. 10. 5—2 distribution. Fig. 11. 3—1—3 distribution.

Figs. 12~20. Meiosis of PMCs in the plant with 8 accessories. $\times 1,100$. (Arrow marks: Accessory chromosomes). Figs. 12~17. Accessory chromosomes at metaphase I. Fig. 12. Symmetrical 1_{VII} . Fig. 13. Asymmetrical 1_{VII} . Fig. 14. $1_{\text{VII}} + 1_{\text{I}}$. Fig. 15. $1_{\text{VI}} + 1_{\text{I}}$. Fig. 16. $1_{\text{IV}} + 2_{\text{I}}$. Fig. 17. $1_{\text{II}} + 2_{\text{I}} + 1_{\text{I}}$. Figs. 18~20. Accessory chromosomes at anaphase I. Fig. 18. 4—4 distribution. Fig. 19. 5—3 distribution. Fig. 20. $4-\frac{2}{2}-3$ ($\frac{2}{2}$: two accessory chromatids) distribution.

DISCUSSION

Although the literature contains considerable number of references to the cytology on smaller number of accessory chromosomes than four, the informations on the cytological behavior of larger number of accessory chromosomes than five are yet scanty.

In *Festuca pratensis* (Bosemark 1954a) with seven to sixteen accessory chromosomes, at metaphase I higher configurations than bivalent were rarely observed, but a larger number of univalents being observed. At anaphase I the univalent of accessory chromosomes divide, and the dividing univalents sometimes form so many bridges that a separation of the daughter nuclei is not possible and a restitution nucleus is formed. Consequently, at tetrad stage the triads, dyads and monads besides normal tetrads were formed. Even if a large number of univalents dividing at anaphase are present, the number of micronuclei at the tetrad stage was a very few, so that it seems that the meiotic elimination of accessory chromosomes is suprisingly low. In the plants with five and seven standard accessory chromosomes of *Phleum phleoides* (Bosemark 1956), the high associations were observed at diakinesis. In the plant with seven accessory chromosomes, for instance, 13 cells out of 35 showed associations of five or more accessory chromosomes. At metaphase I, however, this plant had a very higher frequency of univalents, and higher configurations than bivalents were seen only in a few cases and even these were not quite indisputable. In spite of this, micronuclei were seldom found at telophase I or at the tetrad stage.

For the meiotic behavior of many accessory chromosomes in rye, *Secale cereale*, there is only one report by Håkansson (1957). In meiosis of the rye plants (variety Wasa II) with eight accessory chromosomes he rarely observed multivalent association of accessory chromosomes. The number of univalent accessory chromosomes varies from nil to eight. At anaphase I the univalents divide usually and sometimes lag. Anaphase was occasionally faulty and may result in the forming of a restitution nucleus or in rare cases of three telophase nuclei. The elimination of accessory chromosomes is much less frequent than laggard frequency in this division.

As mentioned above, in the plants with a large number of accessory chromosomes it is generally known that the multivalent formation of accessory chromosomes is rare and the segregation is unusual. On the contrary, the accessory chromosomes in the present rye plants belonging to variety JNK form easily many multivalents, which are characterized by radial association; and the segregation is comparatively normal. This difference from Håkansson's result may be ascribed to difference in structural feature of accessory chromosomes in two materials as demonstrated by Lima-de-Faria (1963), who studied the accessory chromosomes at pachytene stage in rye varieties from Sweden, Transbaicali, Afganistan, Turkey and Korea. Accordingly, alterations in the behavior of accessory chromosomes at meiosis may essentially be considered to result from genotypic constitution.

There is a balance between the tendency to numerical increase and the unfavourable genetical effects of accessory chromosomes. Therefore, it seems that there is a limit to the number of accessory chromosomes contained within a plant, though it varies among different species. For example, the maximum number of accessory chromosomes hitherto

observed is 19 in *Festuca pratensis* (Bosemark 1954b), 22 in *Centaurea scabiosa* (Fröst 1957) and 34 in *Zea mays* (Randolph 1941), etc. In rye plants, by intercrosses between diploid Wasa II plants with six accessory chromosomes Mützing (1954) found the plant with ten accessories, and Håkansson (1957) also one plant with nine accessories. Sarvella (1959) found the plant with twelve accessories in the artificial tetraploid plant from Transbaical rye. The tetraploids appeared to tolerate more accessory chromosomes in a plant than the diploids. In the present materials, JNK, the author found one plant with nine accessories as shown in Figure 21, in which the vegetative growth was very poor and withered before heading. On the basis of these data, one might conclude that the highest numerical limit of accessory chromosomes per plant in rye is nine or ten.

Fig. 21. Somatic chromosomes in the plant with nine accessory chromosomes. $2n = 14 + 9$ accessories. $\times 1,700$.

As mentioned above, when the number of accessory chromosomes exceeds a limit, it may be almost impossible that the plant grows usually and completes the life cycle, because of the deleterious effect of accessory chromosomes. On the other hand, accessory chromosomes are usually but not always heterochromatic. It is known that heterochromatic segment is inferior to euchromatic one in the genic activity. Hence, it seems that the more heterochromatic the accessory chromosomes are, the more number per plant contains. For example, the accessory chromosome in the species with a large number of accessory chromosomes mentioned above is heterochromatic, while in the species with euchromatic accessory chromosomes, as in *Allium*, *Clarkia* and *Lilium*, etc. (cf. Battaglia 1964), the number may be comparatively low. The accessory chromosomes in rye is intermediate in the degree of heterochromatin between two types (Lima-de-Faria 1952). On the basis of the above data, it seems reasonable to assume that the numerical limit of accessory chromosomes contained per plant may be essentially related to the extent of heterochromatic segment. To generalize this supposition further studies are necessary.

SUMMARY

The cytological behavior of accessory chromosomes in the plants with seven and eight accessories was investigated in meiosis. The results are summarized as follows:

1. Notwithstanding the fact that both of the plants have a large number of accessory chromosomes, the accessory chromosomes pair very well with each other and form many radial multivalents. In the radial multivalent of even valency two types were distinguished; that is, the one is symmetrical and the other asymmetrical. The mode of configurations in the 7 acc. plant was $1_{\text{V}} + 1_{\text{I}} + 1_{\text{I}}$ followed by $3_{\text{I}} + 1_{\text{I}}$; and in the 8 acc. plant $1_{\text{V}} + 2_{\text{I}}$ followed by 1_{V} .
2. The disjunction of accessory chromosomes at anaphase I was not unusual, being determined by the type of metaphase I configuration. That is, in most of cells 3—4 and 5—2 distributions were observed in the 7 acc. plant; and 4—4 and 5—3 in the 8 acc. plant. The cells with one or two laggards of accessory chromosomes were considerably observed, but the number of micronuclei in tetrads was a very few. This indicates that the meiotic elimination of accessory chromosomes is surprisingly low.
3. The cytological behavior in different species with many accessory chromosomes and the numerical limit of accessory chromosomes contained per plant were discussed.

ACKNOWLEDGMENTS

The author wishes to express his indebtedness to Prof. T. Nagamatsu, Kyushu University, for constant guidance in the course of the work, and Prof. U. Yamakawa, Saga University, and Mr. E. S. Kurup, for their advices in the preparation of this manuscript.

LITERATURE CITED

- Battaglia, E., 1964 Cytogenetics of B-chromosomes. *Caryologia* **17**: 245-299.
- Bosemark, N. O., 1954a On accessory chromosomes in *Festuca pratensis*. I. Cytological investigations. *Hereditas* **40**: 346-376.
- Bosemark, N. O., 1954b On accessory chromosomes in *Festuca pratensis*. II. Inheritances of the standard type of accessory chromosomes. *Hereditas* **40**: 425-437.
- Bosemark, N. O., 1956 Cytogenetics of accessory chromosomes in *Phleum phleoides*. *Hereditas* **42**: 443-466.
- Fröst, S., 1957 The inheritance of the accessory chromosomes in *Centaurea scabiosa*. *Hereditas* **43**: 403-422.
- Håkansson, A., 1957 Meiosis and pollen mitosis in rye plants with many accessory chromosomes. *Hereditas* **43**: 603-620.
- Kishikawa, H., 1962 Multivalent configuration of six accessory chromosomes in rye *Secale cereale* L. *Chrom. Inform. Serv.* **3**: 25-26.
- Kishikawa, H., 1963 Meiotic pairing between standard- and large iso-accessory chromosome in rye, *Secale cereale* L. *Chrom. Inform. Serv.* **4**: 10-12.
- Kishikawa, H., 1965 Cytogenetic studies of B chromosomes in rye, *Secale cereale* L., in Japan. (Japanese with English summary). *Agri. Bull. Saga Univ.* **21**: 1-81.
- Kishikawa, H., 1966 Cytological study on triploid rye with four accessory chromosomes. *Japan. J. Genetics* **41**: 427-437.

- Lima-De-Faria, A., 1952 Chromomere analysis of the chromosome complement of rye. *Chromosoma* **5**: 1-68.
- Lima-De-Faria, A., 1963 The evolution of the structural pattern in a rye B chromosomes. *Evolution* **17**: 289-295.
- Müntzing, A., 1954 Cytogenetics of accessory chromosomes (B-chromosomes). *Caryologia* **6**, Suppl.: 289-301.
- Randolph, L. F., 1941 Genetic characteristics of the B chromosomes in maize. *Genetics* **26**: 608-631.
- Sarvella, P., 1959 The behavior of accessory chromosomes in tetraploid rye. *Hereditas* **45**: 505-563.