EFFECTS OF TEMPERATURE AND SOIL MOISTURE ON FREQUENCY OF ACCESSORY CHROMOSOMES IN RYE, *SECALE CEREALE* L.

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Synopsis. In order to find the correlation between the environmental factors and frequency of accessory chromosomes in different populations of rye, clonal plants divided from a plant to get the uniformly genotypical constitution were grown under the different temperature or soil moisture conditions. The frequency of acc. chromosomes was lower in the progeny populations derived from the plants grown under the high temperature or dry soil conditions. Such a low frequency may be due to the highly meiotic elimination of acc. chromosomes, the less occurrence of non-disjunction at pollen mitosis and the decrease of seed fertility of the parental plants under such unfavourable conditions.

The investigation on the relations between the different frequencies of plants with accessory chromosomes in different populations and the environmental factors will come to be a clue for clarifying the mechanism of ecological differentiation and/or of geographical distribution, and the evolutionary significance of accessory chromosomes.

It is generally known that the frequency of accessory chromosomes in population is governed by the genotypical constitution and the environmental conditions. Therefore, in order to find the correlation between the frequency of accessory chromosomes and environmental factors, it is necessary to analyze the effects of environmental factors on a frequency of accessory chromosomes under the uniformly genotypical constitution. On the other hand, the frequency of accessory chromosomes is decided by the manner of transmission, and if its transmission is influenced by environmental factors, its frequency in a progeny population also may vary.

Based on the point of view mentioned above, this paper deals with the variation of the frequency of accessory chromosomes in progeny populations derived from rye plants grown under the different temperature or soil moisture conditions.

Materials and Methods

The present material belongs to a strain, JNK, of a native rye, *Secale cereale* L., in Japan. In order to uniformize the genotypical constitution, a young plant with accessory chromosomes was divided into two or three clonal plants, viz., as artificial twin or triplet plants, after the stage of maximum number of tillers. Each of clonal plants was used in each plot of a set of treatments.

On the effect of temperature: Each clonal plant from 9 plants with two accessories and with four ones was transplanted in a pot respectively and set in two groups, high and low temperature plots. The former, the high temperature plot, was grown in the greenhouse at Saga University, and the latter, the low temperature plot, in the field of Mitsuze Branch, at 400 m above sea-level, of Saga Prefecture Agricultural Experiment Station. The variations of average temperatures per five days from the middle of April to the end of June, 1967, are shown in Figure 1. The difference in temperature between two plots in the whole period was an average of 9.3°C, and in the period from meiosis to fertilization, which may be most sensitive to environment, 10.1°C.

On the effect of soil moisture: Each clonal plant from 6 plants with two accessories was

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Fig. 1. Variation of average temperature per five days.

transplanted in a pot respectively and set in three groups, moist, control and dry plots. The percentage of soil moisture in three plots was 68~72, 48~52 and 28~32 to the maximum water holding capacity per pot, respectively. These percentages were kept by measuring pot-weight and pouring water

on pot frequently, from early in April to the middle of June.

As to the frequency of accessory chromosomes in the population of natural intracrossed progeny per plot in each treatment mentioned above, the number of accessory chromosomes was counted at mitotic metaphase in root-tip cells. The root-tips were pretreated with 8-oxyquinoline solution (0.003 M) for 5~6 hours at about 16°C, and then warmed to macerate in 1 N-HCl, stained and squashed with aceto-orcin.

Results

Effect of temperature

Almost all of the accessory chromosomes showed the standard type, and the other types, such as the large iso- and small iso-types, were observed rarely.

The frequency of accessory chromosomes in the progeny populations from the plants with two accessories and with four ones grown in the high and low temperature plots is shown

Table 1. Frequency of accessory chromosomes in progeny populations from plants grown under different temperature conditions

Parent popul.	Plot	No. of acc. chromosomes in progeny populations										No. of plants	Average number of acc. chr. per plant
		0	1	2	3	4	5	6	7	8			
2 acc. × 2 acc.	Low Temp.	7 (4.7)	4 (2.7)	43 (28.6)	15 (10.0)	80 (53.3)	1 (0.7)	—	—	—	150 (100.0)	3.07	
	High Temp.	9 (6.0)	10 (6.7)	62 (41.3)	17 (11.3)	52 (34.7)	—	—	—	—	150 (100.0)	2.62	
4 acc. × 4 acc.	Low Temp.	— —	— —	— —	2 (5.0)	5 (12.5)	—	24 (60.0)	2 (5.0)	7 (17.5)	40 (100.0)	6.00	
	High Temp.	— —	— —	2 (6.1)	1 (3.0)	11 (33.3)	2 (6.1)	11 (33.3)	3 (9.1)	3 (9.1)	33 (100.0)	5.21	

Table 2. Accessory chromosomes at meiosis under different temperatures

Parent popul.	Plot	Frequency* of acc. chromosomal pairing at MI					No. of micronumclei at Tetrad				
		**I	II	III	IV	No. of cells	1	2	3	4	No. of cells
2 acc.	Low T.	%	%	%	%	300	%	%	%	%	3,000
	High T.	10.0	90.0	—	—		94.5	2.7	2.5	0.3	
4 acc.	Low T.	4.8	44.8	3.6	46.8	500	95.5	2.9	1.4	0.2	4,000
	High T.	14.4	52.9	5.5	27.2		93.5	3.8	2.2	0.4	

Notes: * —The percentages were calculated from the data of accessory chromosome configurations.

** —I, II, III and IV denote univalent, bivalent, trivalent, and quadrivalent respectively.

Table 3. Seed fertility under different temperature conditions

Plot	2 acc.	4 acc.
	%	%
Low Temp.	46.3 (100)	14.2 (100)
High Temp.	28.4 (61.3)	4.1 (28.9)

in Table 1. The mode and average number of accessory chromosomes per plant in the high temperature plot were fewer than those in the low temperature plot. The difference in average number is statistically highly significant.

The behaviour of accessory chromosomes at meiosis in two plots is shown in Table 2. The bivalent association of accessory chromosomes in the plants with two accessories and the quadrivalent in the plants with four accessories in the high temperature plot were less than those in the low temperature plot. On the contrary, the number of univalent in the high temperature plot was more than that in the low temperature plot. Consequently, the number of micronuclei at pollen tetrad in the high temperature plot was more than that in the low temperature plot. The micronuclei are generally lost in the cytoplasm. The fact described above indicates that the meiotic elimination of accessory chromosomes in the high temperature plot is higher than that in the low temperature plot.

As shown in Table 1, on the other hand, that the mode of accessory chromosomes in the progeny population of plants with two accessories in the low temperature plot was

4 may be due to that the non-disjunction mechanism at pollen mitosis in the low temperature is more effective than that in the high temperature plot. In the progeny population of plants with four accessories, the same phenomenon may also arise as mentioned above, although the number of plants examined was few.

From the results observed, it is natural to consider that the accessory chromosomes in a progeny population from the plants grown in the high temperature plot generally decreases in number.

The low frequency of accessory chromosomes in the high temperature plot may also be due to the decrease of seed fertility resulted from both the injury of high temperature and the deleterious effect of accessory chromosomes (Table 3).

Effect of soil moisture

The frequency of accessory chromosomes in the progeny populations from the plants with two accessories grown in the moist, control and dry plots is shown in Table 4. That is, with the decrease of soil moisture, the mode of accessory chromosomes in the populations shifted from 4 to 2, and the accessory chromosomes per plant decreased in number.

As shown in Table 5, with the decrease of soil moisture, the bivalent of accessory chromosomes decreased in number and the univalent increased; as a result the number of micronuclei at the pollen tetrad stage increased. This agrees very well with the experimental result reported by MORI (1948). It indicates that the meiotic elimination of

Table 4. Frequency of accessory chromosomes in progeny populations from plants grown under different soil moisture conditions

Parent popul.	Plot	No. of acc. chromosomes in progeny popul.							No. of plants	Average number of acc. chr. per plant
		0	1	2	3	4	5	6		
2 acc. × 2 acc.	Moist	4 (2.7)	12 (8.0)	44 (29.3)	11 (7.3)	72 (48.0)	1 (0.7)	6 (4.0)	150 (100.0)	3.08
	Control	5 (3.3)	7 (4.7)	49 (32.7)	25 (16.7)	60 (40.0)	2 (1.3)	2 (1.3)	150 (100.0)	2.95
	Dry	9 (6.0)	9 (6.0)	57 (38.0)	12 (8.0)	56 (37.3)	2 (1.3)	5 (3.4)	150 (100.0)	2.82

Table 5. Accessory chromosomes at meiosis under different soil moisture conditions

Parent popul.	Plot	Frequency of acc. chromosomal pairing at MI			No. of micronuclei at Tetrad					
		I	II	No. of cells	0	1	2	3	4	No. of cells
2 acc.	Moist	11.7%	88.3%	300	96.8%	1.9%	1.2%	0.1%	—	3,000
	Control	13.3	86.7	300	96.3	2.6	0.9	0.2	—	3,000
	Dry	15.0	85.0	300	95.9	1.6	2.2	0.2	0.1	3,000

Table 6. Seed fertility under different soil moisture conditions

Plot	2 acc.
Moist	37.5% (95.2)
Control	39.4 (100)
Dry	31.1 (78.9)

accessory chromosomes is high under the dry soil condition. That the mode of accessory chromosomes in the populations decreased from 4 to 2 indicates that non-disjunction mechanism might be less effective under the dry soil condition.

Consequently, it seems that the number of accessory chromosomes in a progeny population decreases if parental plants are grown the dry soil condition. The other cause may be the low fertility of seed under such a condition (Table 6).

Discussion

Up to date, the frequency and geographical or ecological distributions of plants with accessory chromosomes have been extensively investigated. However, the correlation between the environmental factors and the frequency of accessory chromosomes is not always apparent.

In wild populations of *Festuca pratensis* in different regions of Sweden, BOSEMARK (1956) has found a marked positive correlation between the clay content of soil and the frequency of plants with accessory chromosomes. In *Clarkia elegans*, LEWIS (1951) has found a correlation between the adaptation to arid regions and the presence of supernumerary chromosomes. A quite similar result was obtained by FRÖST (1958) in *Centaurea scabiosa* collected from Sweden and other parts of Europe. In this species the geo-

graphical difference of frequency largely coincides with a difference in humidity, the frequency of accessory chromosomes thus being significantly higher in regions with a dry climate than in regions with a humid climate. A remarkable case of correlation between accessory chromosomes and ecology has been described by SHARMA and AIYANGAR (1961) in *Allium starcheyi*. They have indicated that the accessory chromosomes in this species predominantly occur in diploid species and in cold regions than in tetraploid and in hot regions.

In rye, studies on the frequency and geographical distribution of accessory chromosomes have been made by MÜNTZING (1954 and 1957) in the materials from Sweden, Switzerland, Spain, Turkey, Afganistan, Transbical, and Korea. A similar study was carried out by LEE and MIN (1965) in Korean rye. There were considerable differences between the different areas, but it was difficult to draw any definite conclusion as to the cause of these differences. Afterwards, LEE (1966), using rye plants collected from various localities in Korea, has analyzed the relationship between the frequency of accessory chromosomes and soil properties, and found that the frequency was higher in acidic soil than in basic soil.

The genetic property of accessory chromosomes, as WHITE (1951) pointed out, cannot be considered apart from the genotypical constitution of the individuals and populations in which they occur and the environment where those individuals and populations inhabit. Besides, it is considered that the frequency of accessory chromosomes is the function of environmental factors and genotypical constitution. No experimental evidence, however, has been presented whether

or not the several relations mentioned above reflect the differences in the genotypical constitution of plants grown in different areas.

As mentioned previously, when the relation between the frequency of accessory chromosomes and environment is discussed, one has to analyze the relation in different environmental factors under the uniformly genotypical constitution or that in different strains of genotypical constitution under the uniformly environmental condition. The present studies conformed to the former. The relations between the temperature or soil moisture and the frequency of accessory chromosomes can be summarized as follows.

The frequency of accessory chromosomes in the progeny populations from the plants grown under the high temperature or dry soil condition was lower than that under the low temperature or moist soil condition respectively. As to the causes for its low frequency under the former conditions it seems primarily that the meiotic elimination of accessory chromosomes may be higher and the non-disjunction at pollen mitosis less effective than those under the latter favourable environmental conditions. This indicates that the behaviour of accessory chromosomes in its transmission is affected by environmental conditions. This may also cause for the natural selection for accessory chromosomes under different climatic and edaphic conditions. Moreover, unfavourable environmental conditions may promote the deleterious action of accessory chromosomes, inhibiting the development of fertilized embryo. As a result the seed fertility was reduced, and it was difficult to produce the plants with many accessory chromosomes in the progeny. This may be the secondary reason for the low frequency of accessory chromosomes in the progeny populations. This also may make a positive selection for accessory chromosomes ineffective.

In view of the facts described above it seems most reasonable to conclude that the conditions of relatively low temperature and of moist soil may be more favourable for the transmission of accessory chromosomes in

Japanese rye. This indicates that when the populations with different genotypical constitutions grow in another environment, the frequency of accessory chromosomes would change correspondingly. Therefore, the adaptation of accessory chromosomes may be due to the balance among the mechanism of numerical increase, the genetical effect of accessory chromosomes, the genotypical constitution of plants, and the environment, in the process of natural selection.

Summary

The frequency in a population of accessory chromosomes is governed by the genotypical constitution and the environmental factors. Therefore, in order to find the relation between the frequency of accessory chromosomes and environmental factors, it is necessary to analyze the effects of environment on the frequency under the uniformly genotypical constitution. And, if the transmission of accessory chromosomes is influenced by environment, the frequency in a progeny population also may vary.

In order to uniformize the genotypical constitution, the young plants with accessory chromosomes were artificially divided into two or three clonal plants. Such clonal plants were respectively grown under different temperature or soil moisture conditions.

The frequency of accessory chromosomes in the progeny population derived from each plot was lower under the high temperature or dry soil condition than under the low temperature or moist soil condition. Such a low frequency may be due to the meiotic elimination of accessory chromosomes was higher and the non-disjunction less effective under the high temperature or dry soil condition, and that the seed fertility of parental plants was reduced by such unfavourable conditions in combination with the deleterious effect of accessory chromosomes.

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ライ麦における過剰染色体の頻度におよぼす温度と土壌水分の影響

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集団における過剰染色体の頻度と環境との関連を明らかにすることは、過剰染色体の地理的分布の機構ないし進化的意義を解明する手掛りとなる。一般に、集団における過剰染色体の頻度は系統の遺伝子型と環境条件によって異なる。従って、その頻度と環境要素との関連を見出す場合には、遺伝子型と一定にして環境の影響について分析しなければならない。また集団における過剰染色体の頻度はその伝達の仕方に基づくので、その伝達時の過剰染色体の行動が環境によって影響されるならば後代集団における頻度も変ってくる。

このような観点から、ライ麦を材料とし、遺伝子型を揃えるために最高分蘗期後、幼植物を株分けし、それぞれの株を異なる温度および土壌水分量下で栽培し、得られた子孫集団における過剰染色体の頻度を比較調査した。

比較的高温あるいは土壌水分量の少い区からの子孫集団における過剰染色体の頻度は、低温あるいは土壌水分量の多い区からのそれよりもかなり低下した。前者の条件下における低下は、そのような環境条件においては減数分裂期における過剰染色体の消失が著しく、また花粉粒第一分裂後期におけるその不分離が十分行われなかったことに因ると考えられる。さらに過剰染色体の増加による有害作用がそのような条件下で強調され、授精胚の発育が阻害されて種子稔性の低下をきたし、過剰染色体を多く有する個体の出現が減少したこともその一因であろう。

上の結果から、ライ麦集団における過剰染色体の頻度は、高い気温および乾燥土壌の条件下では減少することが確認された。