Theoretical and Applied Genetics 47, 41-43 (1976)

Greater Ability of Pollen Tube Growth in Rye Plants with 2B Chromosomes

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Summary. To contribute to knowledge on the significance of B chromosomes in rye populations, a component of fertility has been estimated by a comparative study of germination and growth of pollen tubes in plants with and without B chromosomes. The highest percentage germination of pollen and mean speed of pollen tube growth was shown by 14 + 2B plants, followed by normal plants.

Observations of first pollen mitosis indicated that in most cases the chromatid constitution of the tube nucleus was the same in 14, 14 + 1 and 14 + 2B plants. In contrast, the mean growth rate of pollen tubes differed significantly among the three cases. From these data it seems that the determination of germination ability and pollen tube growth depends on the sporophyte and is related to the number of Bs carried by the plant.

Introduction

Rye B chromosomes have no qualitative influence on the phenotype, while the presence of 3 or more Bs reduces vigour and fertility (Müntzing 1963; Kishikawa 1965; Moss 1966). One might assume that B chromosomes would tend to be eliminated by natural selection but, in spite of their deleterious effects, Bs can be found in the oldest rye populations (Müntzing 1950) and are widely distributed. Their frequency varies among populations, the maximum being 90% in Korea, while they are absent from some Swedish varieties submitted to strong selection for fertility (Müntzing 1957, 1963).

The persistence of B chromosomes could be explained by their special cytological mechanism of transmission, which consists of non-disjunction and preferential distribution to the generative pole during first pollen mitosis (Müntzing 1946). This phenomenon leads to the duplication of B chromosome number in the gametes. It seems that equilibrium could be reached between the tendency towards B chromosome multiplication and the reduction of vigour and fertility occurring when the number of Bs is too high.

The existence of such peculiar mechanisms leads one to question whether B chromosomes are only subproducts of caryotypic rearrangements, or if they play an active role in rye evolution.

To contribute to knowledge on the significance of B chromosomes in rye populations, a component of fertility has been estimated by a comparative study of germination and growth of pollen tubes in plants with and without B chromosomes. Possible differences in the behaviour of pollen tubes can be considered as a reflection of competitive ability in natural pollination.

Material and Methods

Rye (*Secale cereale* L.) of the Japanese variety JNK was used. Pollen grains from plants with a chromosome constitution of 2n = 14, 14 + 1, 14 + 2, 14 + 3 and 14 + 4 Bs were examined.

Following the modified Ahloowalia's method (1973), the spikes were cut approximately one hour before anthesis was supposed to occur. They were immersed in tap water and exposed to the light of a 60 W bulb kept at about 30 cm. When anthesis occurred, pollen grains were allowed to set on a slide covered with the culture medium. The medium consisted of 30% sucrose (w/w), 1% agar (w/w), and 10 ppm each of calcium chloride, boric acid and tetrasodium salt of EDTA, pH6.3.

After sowing, preparations were kept for 5, 20 or 40 minutes and then fixed with several drops of acetic-ethanol solution (1:3).

Pollen grains chosen at random from different slides and different plants of each chromosome constitution were scored. Pollen tubes were measured immediately after fixation (Fig. 1).

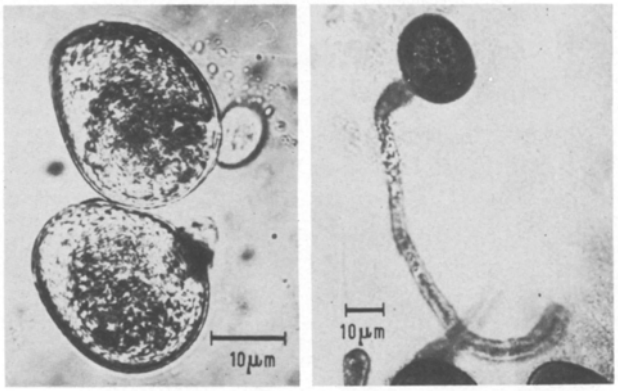

Fig. 1. Pollen from 14 + 2B plants: a) 5 min after sowing, b) 20 min after sowing

Results

Results of pollen germination 5 min after sowing are shown in Table 1. It can be observed that the highest percentage of germination corresponds to pollen from 2n = 14 + 2B plants. It is followed by pollen from plants 2n = 14 + 1, 14, 14 + 3 and 14 + 4, in that order.

Table 2 shows mean pollen tube lengths 20 and 40 min after sowing. Pollen tubes from 14 + 2 plants were the longest. These were followed by pollen from normal plants. Mean lengths differed significantly both at 20 and 40 min. With these data, the average speed of pollen tube growth was calculated, being 2.87 μ/min for normal pollen and 4.25 μ/min for plants with 2B.

Measurements of pollen tubes from 14 + 3 plants 20 min after sowing and 14 + 4, 40 min after sowing, could not be made because of the low fertility of these individuals.

Table 1. Pollen grain germination 5 minutes after sowing

Chromosome constitution of plants	14	14 + 1	14 + 2	14 + 3	14 + 4
Number of pollen grains observed	326	100	500	123	360
Germination (%)	30.67	43.00	48.00	20.32	18.91

By observing first pollen mitosis, the chromatid constitution of pollen nuclei can be estimated. In 14 + 2 plants at the stage of first pollen mitosis, some anthers were excised from the same spikes which were going to be used for pollen culture. At metaphase, pollen with 7 chromosomes (1%), 7 + 1 (98%) and 7 + 2B (1%) were observed. 78.5% of anaphases showed non-disjunction. These results indicate that meiosis was very regular in this material. That is, at Anaphase I chromosome segregation was mainly 7 + 1 : 7 + 1 to each pole. They also indicate that non-disjunction took place with high frequency; in other words, 80% of sperm nuclei carried 2Bs, while gametes lacking Bs were very rare.

Discussion

From the progeny obtained by natural pollination of crosses involving rye plants with a chromosome constitution of 14, 14 + 1 and 14 + 2B, the competitive ability of pollen carrying B chromosomes was deduced (Puertas and Lacadena, 1974). The results obtained indicated firstly that when competition between pollen from 14 + 1 and 14 plants is established, certation is totally favourable for normal pollen (the progeny had 14 chromosomes in every case). Secondly, when pollen from 14 + 2B plants competes with pollen from normal plants, pollen carrying B chromosomes fertilizes more frequently than does normal pollen.

The above data clearly coincide with those obtained in the present work. It was found that pollen grains from 14 + 2 plants showed a significantly higher germination percentage and mean growth rate of pollen tubes than did pollen from both 14 and 14 + 1 plants.

The special behaviour of B chromosomes during microsporogenesis allows the formation of gametes with either 7, 7 + 1, 7 + 2, 7 + 3 or 7 + 4 Bs in 14 + 2 plants. Consequently, the tube nucleus can also carry a variable number of Bs. To estimate the frequency of each type of gametophyte, first pollen mitosis was observed in anthers excised from the same spikes which were going to be used for pollen culture. The results indicated that almost 80% of pollen grains had their sperm nuclei with 2B, while the tube nucleus had none. This also means that in 80% of the cases the tube nucleus had the same chromatid constitution in both 14 and 14 + 2B plants.

The results of pollen culture in 14 + 1 plants showed that the pollen germinated with quite high frequency but pollen tube growth was very slow, even slower than that of 14 + 4 plants. On the other hand, it should be realized that where no meiotic elimination of the B-univalent occurred, 50% of the pollen grains would lack B chromosomes. The loss of the B-univalent has been demonstrated by Kishikawa (1965) in the same material. He found that at metaphase of first pollen mitosis there were 7 chromosomes in 60% of the cases.

Therefore, the chromatid constitution of sperm nuclei is always 7 in 2n = 14 plants, generally (80%) 7 + 2 in 2n = 14 + 2 plants, and 7, 7 + 1 or 7 + 2 (frequencies of each type of gamete are not known) in 2n = 14 + 1 plants. However, in most cases, the chromatid constitution of the tube nucleus is the same (7) in 14, 14 + 1 and 14 + 2 plants.

In spite of the similar constitution of the tube nucleus in 0, 1 or 2B plants, pollen germination and

Table 2. Pollen tube length (μ) at 20 and 40 minutes after sowing

minutes after sowing	Chromosome constitution of plants: 14		14 + 1		14 + 2		14 + 3		14 + 4	
	mean	range	mean	range	mean	range	mean	range	mean	range
20	59.18 ±2.45	17.5-210	22.68 ±0.35	17.5-52.5	100.80 ±3.15	17.5-245	-	-	35.31 ±2.10	17.5-70
40	114.90 ±3.15	17.5-227	70.77 ±4.55	17.5-280	169.99 ±4.90	35-402	10.29 ±0.00	8.5-35	-	-

pollen tube growth differed significantly for the three cases. Because of the extreme condensation of chromatin in sperm nuclei, it does not seem possible to cite gene action of sperm nuclei as the cause of such differences. On the contrary, it seems that the genetic determination of germination ability and pollen tube growth depends on the sporophyte, and is evidently related to the number of Bs carried by the plant.

If pollen tubes of pollen carrying Bs grow faster than normal pollen, it seems that gametes with Bs will fertilize the egg cell with a higher frequency; in other words, the existence of preferential fertilization for these gametes can be interpreted in terms of a selective advantage for plants carrying 2Bs.

Deleterious effects of B chromosomes on vigour and fertility have been demonstrated by a number of authors (Müntzing 1963; Kishikawa 1965; Moss 1966). However, B chromosomes have cytological mechanisms for B multiplication (non-disjunction and preferential distribution during first pollen anaphase). Kimura and Kayano (1961) proposed that the concept of distortional load (or load due to meiotic drive) could be applied to the maintenance of B chromosomes of *Lilium callosum*. The results obtained in the present work suggest that equilibrium in rye populations could be reached taking into consideration at least three different factors: first, decrease in vigour and fertility when 3 or more Bs are present; second, the distortional load due to the special behaviour of Bs during gametogenesis; and third, the selective advantage of individuals with 2B chromosomes due to preferential fertilization of their pollen.

Literature

Ahloowalia, B.S.: Germination in vitro of ryegrass pollen grains. Euphytica 22, 575-581 (1973)

Kimura, M.; Kayano, H.: The maintenance of supernumerary chromosomes in wild populations of *Lilium callosum* by preferential segregation. Genetics 46, 1699-1712 (1961)

Kishikawa, H.: Cytogenetics studies of B chromosomes in rye, *Secale cereale* L., in Japan, Agr. Bull. of Saga Univ. 21, 1-81 (1965)

Moss, J.P.: The adaptative significance of B chromosomes in rye. Chromosomes today 1, 15-25 (1966)

Müntzing, A.: Cytological studies of extrafragments chromosomes in rye. III. The mechanism of non disjunction at the pollen mitosis. Hereditas 32, 97-119 (1946)

Müntzing, A.: Accessory chromosomes in rye populations from Turkey and Afghanistan. Hereditas 36, 507-509 (1950)

Müntzing, A.: Frequency of accessory chromosomes in rye strains from Iran and Korea. Hereditas 43, 682-685 (1957)

Müntzing, A.: Effects of accessory chromosomes in diploid and tetraploid rye. Hereditas 49, 361-426 (1963)

Puertas, M.J.; Lacadena, J.R.: The transmission of rye B chromosomes in natural pollination. Theoret. Appl. Genetics 45, 197-204 (1974)

Received June 9, 1975
Communicated by F. Mechelke

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