Heredity (1983), **51** (2), 429–433

NO MALE STERILITY GENE ON *B* CHROMOSOMES IN *PLANTAGO CORONOPUS*

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Received 12.i.83

SUMMARY

An investigation was carried out for two successive years to study a possible correlation between *B* chromosomes and male sterility in a strain of *Plantago coronopus*, obtained from France. Data based on a study of 40 plants (26 *B* chromosome carriers and 14 non-carriers) clearly showed a lack of any relationship between *B* chromosome and male sterility.

1. INTRODUCTION

B chromosomes have been known since 1927, when Longley reported them in maize. Detailed investigations on different aspects of *B* chromosomes have since been carried out in various plant genera, such as maize, rye, *Trigonella* etc. The most important aspect of *B* chromosomes, is their possible effect on endo- and exo-phenotypes of the plant. It is worth noting that the *B* chromosomes have mostly been thought of as inert, having no effect of their own. When present in large numbers, the *B* chromosomes have been shown clearly to affect the vigour and fertility (Randolph, 1941; Muntzing, 1949; Hakansson, 1957). Rees and Hutchinson (1973) suggested possible associations between certain ecological conditions and *B* chromosomes in rye. Roman (1948) reported differential fertilisation by non-carrier and carrier pollen grains in maize. This was also found in *Impatiens balsamina* at our laboratory (Raghuvanshi and Singh, 1976). Their effect on genetic recombination has been clearly demonstrated in rye (Jones and Rees, 1967), *Trigonella foenum-graecum* (Pant and Raghuvanshi, 1980) and *Impatiens balsamina* (Raghuvanshi and Singh, 1976 and Raghuvanshi and Mahajan, 1982). However, in exo-phenotype, evidence presented so far has frequently been unconvincing. This was the case in the report by Paliwal and Hyde (1959) concerning an association between the presence of a single *B* chromosome and male sterility in *Platago coronopus*.

We studied *B* chromosomes from different angles, and also investigated the relationship of *B* chromosomes with possible location of male sterility gene on the *B* chromosome of *Plantago coronopus*, as reported by Paliwal and Hyde (1959). They originally obtained their material from IARI, Delhi, but inspite of all possible efforts, the material we obtained from the same source did not germinate. Efforts to obtain seeds the following year also failed. Therefore, our material was obtained from Jardin Botanique de L' Université Louis Pasteur de Strasbourg, France, from a natural population.

2. Material and methods

The seeds of *Plantago coronopus*, were grown to raise a population. One generation required about three months to flower. The young spikes were fixed in acetic–alcohol (1 : 3) containing iron for 24 hrs, for cytological analysis. The squashes were made in acetocarmine stain. An ethanol–butanol schedule was used to make permanent slides.

For pollen infertility studies, mature anthers were squashed in a single staining solution (Alexender, 1969). The staining solution was made by compounding it in the following order (dyes were from British Drug Houses): ethanol, 10 ml; 1 per cent malachite green in 95 per cent ethanol, 1 ml; distilled water, 50 ml; glycerol, 25 ml; phenol, 5 gm; chloral hydrate, 5 gm; acid fusion 1 per cent in water, 5 ml; orange G, 1 per cent in water, 0·5 ml; and glacial acetic acid, 1–4 ml.

To obtain the best results, the staining solution was acidified with glacial acetic acid in differentiation, giving green pollen walls and red protoplasm. The staining was hastened by flaming the slide. In a typical stain, fertile pollen grains were red and sterile ones green.

3. Results

The number of chromosome in this species was found to be $n = 5$, confirming the report by earlier workers (McCullagh, 1934; Love and Love, 1944; Hyde, 1953). When our population was screened, it was found to consist of *B* chromosome carrier and non-carrier plants. In non-carrier plants, the five homologous chromosomes paired normally (fig. 1). The rest of their meiotic stages were also normal (fig. 3). In carrier plants, besides 5 pairs of homologous chromosomes, a small sized euchromatic *B* chromosome (fig. 2) which stains similarly to *A* chromosomes, was also present. During the prophase stages, it undergoes normal condensation, along with other chromosomes. It is frequently not oriented at the equator of spindle and may occupy a position near the metaphase plate and lie at random anywhere. At Anaphase I, it may be oriented between the two poles, does not divide and may move to either of the poles undivided (fig. 4). In comparison to the other chromosomes, it is late moving, but is able to reach the poles and be included in telophase I. This *B* chromosome appears to divide at anaphase II and is passed on to the gametes.

At metaphase I, the averages of chiasma frequency, for 26 carrier and 14 non-carrier plants, showed that the carriers and non-carriers had the chiasma frequency values $0{\cdot}655 \pm 0{\cdot}029$ and $0{\cdot}678 \pm 0{\cdot}032$ respectively. The average chiasma frequency of carriers was lower than that of non-carriers. Within carrier plants, carrier PMCs had a lower chiasma frequency, *i. e.*, $0{\cdot}614 \pm 0{\cdot}049$ than non-carrier PMCs, *i. e.*, $0{\cdot}662 \pm 0{\cdot}031$. The non-carrier PMCs of carrier plants had a lower frequency than PMCs of the non-carrier plants. Statistically, this was not significant. There was no correlation between the frequency of cells having *B* chromosomes and chiasma frequency in carrier plants. Chiasma frequency ranged from $0{\cdot}531 \pm 0{\cdot}025$ to $0{\cdot}733 \pm 0{\cdot}021$ and $0{\cdot}577 \pm 0{\cdot}018$ to $0{\cdot}765 \pm 0{\cdot}029$ in carrier and non-carrier plants respectively. Although the minimum and maximum values of chiasma frequency were lower in carrier plants than in non-carrier plants, statistically the differences were not significant. There was a significant variance ratio between nuclei and within nuclei.

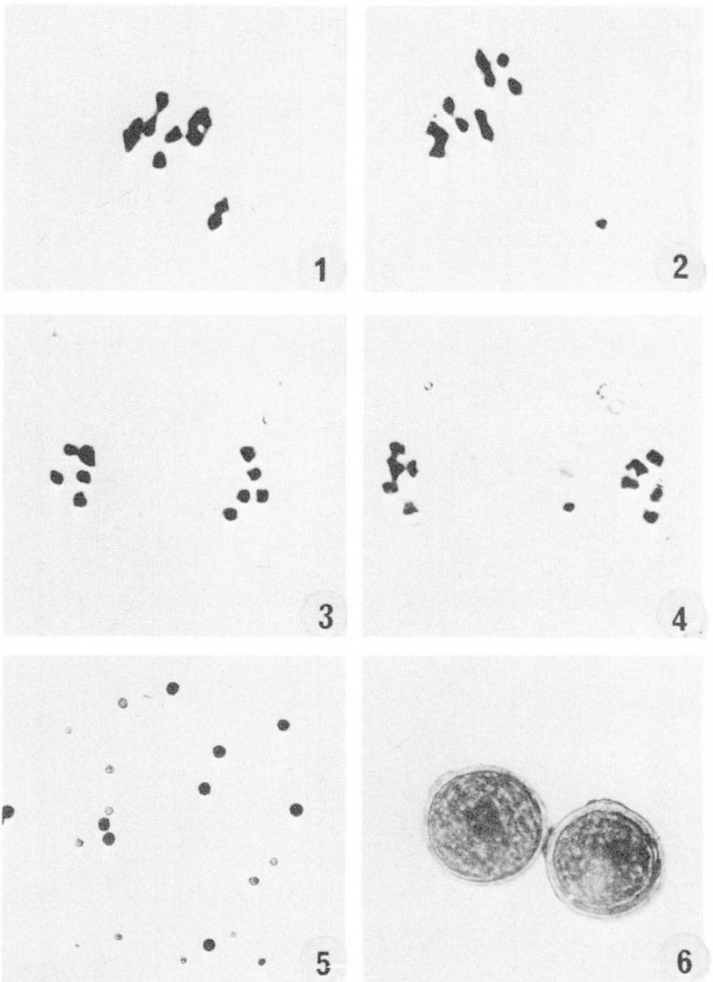

FIG. 1. Non-carrier metaphase I, showing five bivalents.

FIG. 2. Carrier metaphase I, showing five bivalents with an extra chromosome.

FIG. 3. Non-carrier anaphase I, 5–5 separation.

FIG. 4. Carrier anaphase I, *B* chromosome, moving towards one pole.

FIG. 5. Fertile and sterile pollen grains of a carrier plant ×95.

FIG. 6. Fertile pollen grains of a carrier plant ×950.

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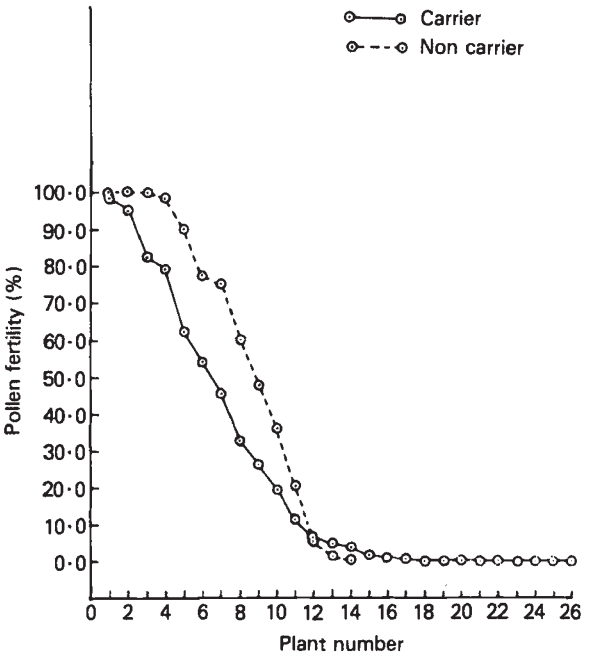

FIG. 7. Per cent pollen fertility per plant in 26 carrier and 14 non-carrier plants.

These results cover the investigations spread over two seasons (1980–81 and 1981–82.) In the first season, of 20 plants screeened, 16 were *B* chromosome carriers. The second season's progeny were grown from mixed seeds, obtained from the previous year's population. Of 20 plants screened from this population, only 10 were carriers. Thus, these observations cover investigation of 40 plants, 26 of which were carriers. It may be mentioned that the studies of behaviour of *B* chromosomes and pollen fertility over two years were similar, so the data has been pooled for drawing conclusions.

The *B* chromosome carrier plants are phenotypically indistinguishable from non-carriers. It is worth noting that out of 26 *B* chromosome carrier plants investigated, the pollen fertility (fig. 5) ranged from 0·0 per cent to 98·9 per cent (*i. e.*, the pollen sterility from 100 per cent to 1·1 per cent). A similar range of fertility from 0·0 per cent to 100 per cent (*i.e.*, pollen sterility from 100 per cent to 0·0 per cent) was displayed by the non-carriers. It is apparent from fig. 7 that though non-carriers show slightly better pollen fertility (*i. e.*, lower pollen sterility) than carriers, but the distribution of different levels of sterility does not appear to show any clear correlation with the presence or absence of *B* chromosomes. The *B* chromosome carrier plants show up to 98·9 per cent fertility. This is in sharp contrast to the observation of Paliwal and Hyde (1959). In their material all the *B* chromosome carrier plants were totally sterile. The seed setting in plants displaying higher pollen sterility was extremely poor. The *B* chromosome frequency in PMCs of different carrier plants varied from 34·4 per cent to 2·7 per cent. It has been plotted in decreasing order on the x-axis of fig. 8. The percentage of pollen fertility of these plants has been plotted on

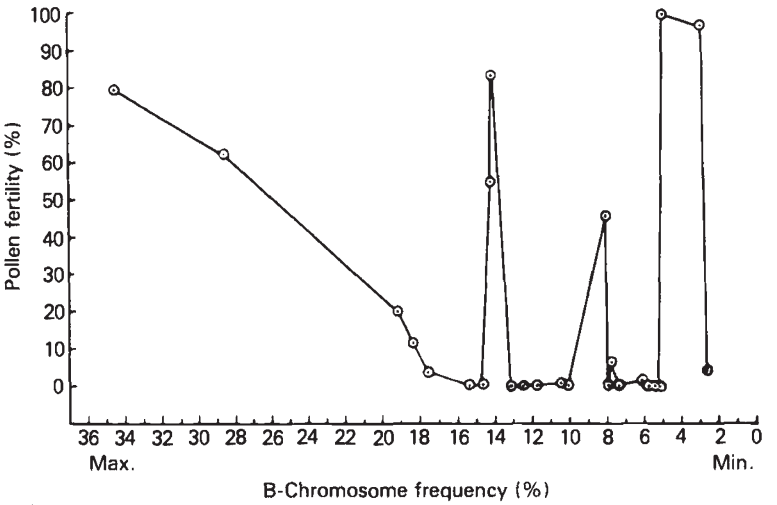

FIG. 8. Relation between the per cent pollen fertility and per cent *B* chromosome frequency in 26 carrier plants. Per cent *B* chromosome frequency arranged in decreasing order.

the y-axis. There is some indication of higher pollen sterility in plants having higher carrier PMCs, but in general there is no correlation.

4. DISCUSSION

Regarding genetic activity of *B* chromosomes, generally they produce no marked phenotypic effect. Jones (1975) reported two exceptional examples, both from plants; *Haplopapus gracilis* (Jackson and Newmark, 1960), in which the colour of achenes changed from brown red to dark purple by the presence of *B* chromosomes, *Plantago coronopus*, in which Paliwal and Hyde (1959) showed that *B* chromosomes induced complete male sterility in the carrier plants.

Working on breeding systems of different species of *Plantago*, Ross (1970) obtained data which was difficult to reconcile with the association between *B* chromosome and male sterility. It may be mentioned that Ross obtained seeds of *Plantago coronopus* from Dr Hyde.

Our studies conclusively proved that the presence of *B* chromosomes were not responsible for male sterility in our sample of *Plantago coronopus*. These studies were initially carried out using 20 plants in one generation and a further 20 plants in the second generation. Results of the two-year study have not shown any relationship between presence or absence of *B* chromosomes and male sterility. Male sterility in both carrier and non-carrier plants is manifested equally. Furthermore, there is no correlation between male sterility and percentage of carrier PMCs in the carrier plants.

Acknowledgments. We are thankful to Prof B. S. Trivedi, Head, Botany Department, Lucknow University, for providing the necessary facilities.

5. REFERENCES

ALEXENDER, M. P. 1969. Differential staining of aborted and nonaborted pollen. *Stain Tech.*, *44(3)*, 117–122.

HAKANSSON, A. 1957. Meiosis and pollen mitosis in rye plants with many accessory chromosomes. *Hereditas, 43,* 603–620.

HYDE, B. B. 1953. Differentiated chromosomes in *Plantago ovata. Amer. Jour. Bot., 40,* 809–815.

JACKSON, R. C. AND NEWMARK, P. 1960. Effect of supernumerary chromosomes in production of pigments in *Haplopapus gracilis. Science, 132,* 1316–1317.

JONES, R. N. 1975. *B* chromosome systems in flowering plants and animal species. *Int. Rev. Cytol., 40,* 1–100.

JONES, R. N. AND REES, H. 1967. Genotypic control of chromosome behaviour in rye-XI. The influence of *B* chromosomes on meiosis. *Heredity, 22,* 333.

LONGLEY, A. E. 1927. Supernumerary chromosomes in Zea mays. *Jour. Agric. Res., 35,* 769–784.

LOVE, A. AND LOVE, D. 1944. Cytotaxonomical studies on boreal plants III. *Ark. f. Bot., 31A,* 1–22.

MCCULLAGH, D. 1934. Chromosome and chromosome morphology in Plantaginaceae I. *Genetica, 16,* 1–44.

MUNTZING, A. 1949. Acessory chromosomes in *Secale and Poa. Proc. VIII Int. Congr. Genetics. (Hereditas suppl. Vol.),* 402–411.

PALIWAL, R. L. AND HYDE, B. B. 1959. Association of a single *B* chromosome with complete male sterility in *Plantago coronopus. Amer. Jour. Bot.,46(6),* 460–466.

PANT, M. AND RAGHUVANSHI, S. S. 1980. Chromosomal association in C_1, C_2 and C_8 autotetraploid B carrier *Trigonella foenum-graecum* L. *The Journal of Cytology and Genetics, 15(1),* 93–98.

RAGHUVANSHI, S. S. AND MAHAJAN, S. 1982. Chromosomal association in *B* carrier and noncarrier diploid, tetraploid and octoploid *Impatiens balsamina* L. *Ind. Nat. Sci. Acad. B48,, No. 1,* 147–151.

RAGHUVANSHI, S. S. AND SINGH, D. N. 1975. Extra chromosomes, their genetic activity, inheritance and transmission to non-carrier genotype in *Impatiens balsamina* L. Presented in seminar on chromosomes in Evolution. Oct. 5–7, Calcutta.

RANDOLPH, L. R. 1941. Genetic characteristics of the *B* chromosome in maize. *Genetics, 26,* 608–631.

REES, H. AND HUTCHINSON, J. 1973. Nuclear DNA variation due to B chromosomes. *Cold Spring Harbor Symp. Quant. Biol., 38,* 175.

ROMAN, H. 1948. Directed fertilization in maize. *Proc. National Acad. Sci., 34,* 36–42.

ROSS, M. D. 1970. Breeding systems in Plantago. *Heredity, 25,* 129–133.