# CHROMOSOME STUDIES IN SOME INDIAN BARLEY—II

By Arun Kumar Sharma and Ramendra Nath Mukherji

(*Cytogenetics Laboratory, Botany Department, Calcutta University, 35, Ballygunge Circular Road, Calcutta-19*)

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## Introduction

The problem of origin of agricultural strains of the common cereals has been dealt with recently by a number of workers. With the gradual invention of newer methods for a critical study of the karyotype, such investigations are being more and more facilitated. The details of the chromosome structure including even the minute differentiated segments can now be brought out at ease with the aid of the recent techniques.

Investigations have shown that the different strains of a single crop species may differ one from the other in their karyotypes, a difference which is detectable under the microscope. They suggest possibly that not merely gene mutations, but even visible structural changes of chromosomes may provide means for the origin of different agricultural strains. Such evidences have been gathered in case of *Hordeum vulgare* by Bhaduri and Sharma (1948), by Sharma and Bhattacharjee (1956) in species of *Sorghum*, etc. In these plants proof of the structural changes of chromosome possibly acting as a means in the evolution of strains, has been gathered. The present investigation with six different agricultural strains of *Hordeum vulgare* were undertaken with the same end in view, *i.e.*, to detect the role of structural changes of chromosome, if any, in their evolution.

## MATERIALS AND METHODS

In the present investigation altogether six different strains of Barley (*Hordeum vulgare*) were taken into consideration. The different strains are as follows:

| | | | |
|---|---|---|---|
| 1. | *Hordeum vulgare* | .. | Strain B 17 |
| 2. | *H. vulgare* | .. | „ B 42 |
| 3. | *H. vulgare* | .. | „ B 122 |
| 4. | *H. vulgare* | .. | „ B 134 |
| 5. | *H. vulgare* | .. | „ B 135 |
| 6. | *H. vulgare* | .. | „ B 137 |

The above six different types had the grains markedly differing one from the other in external morphological details, *viz.*, size of the grains, shape, surface features (wrinkled or smooth, etc.), colour of endosperm, etc.

All these different strains of Barley seeds were obtained through the kind courtesy of the Head of the Division of Botany, Indian Agricultural Research Institute, New Delhi. These were all raised at the same Institution.

*Method of germination*

At first some (about 25–30) seeds of a strain were taken in a petri dish and the latter was properly labelled along with the strain number. A quantity of water was poured in the petri dish so as to plunge the seeds into water and thus to allow them to soak thoroughly. After about twenty-four hours the excess water was poured off from petri dish and the seeds were covered with a thin layer of saw dust and subsequently gently wetted by adding a few drops of water. In this way they were kept for another twenty-four hours, during which period the seeds germinated and from each individual came out a cluster of four to five roots (about 1–1·5 cm. long). The same method was followed for each and every strain investigated.

*Fixation for somatic chromosomes*

In order to study the somatic chromosomes and for better scattering and manifestation of morphological details, prior to fixation, the tissues were subjected to some pre-fixation chemical treatments.

The tip portions (about 3–4 mm.) of healthy roots were taken and were subsequently subjected to pre-fixation, fixation and staining procedure.

A number of techniques involving the use of Aesculine (Sharma and Sarkar, 1955), Coumarin (Sharma and Bal, 1953), Oxyquinoline (Tjio and Levan, 1950), Paradichlorobenzene (Sharma and Mookerjea, 1955), α-Bromo

naphthalene and Oxyquinoline (Bhaduri and Ghosh, 1954), Chromic formalin, etc., were tried. Of these Paradichlorobenzene method yielded best results. The schedule in brief is as follows:

The healthy root-tips were treated in saturated aqueous solution of Paradichlorobenzene at 10–12° C. for three hours. These were then heated gently over a flame for three or four seconds in a mixture of 2% Aceto-orcein and N HCl mixed in the proportion of 9 : 1. The root-tip was then smeared in 1% Aceto-orcein by putting uniform pressure over the cover glass and blotting the excess stain.

The figures were drawn using a Leitz compensating eyepiece of × 20 and 1·3 apochromatic objective with a condenser 1·4 N.A. at a table magnification of approximately 3,000 times.

While drawing the idiograms the chromosomes have been arranged on the basis of their types irrespective of their sizes. That is why in some cases 'B' has been followed by 'A' and not *vice versa*.

## Observations

*Strain B* 17.—The chromosomes of this strain could be classified into the following morphologically distinguishable types (Figs. 1, 1 *a*):

1. A pair of long chromosomes with submedian primary constriction and a satellite at the end of the shorter arm (A).

2. A pair of long chromosomes with nearly submedian primary constriction and a satellite at the end of the slightly longer arm (G).

3. Five pairs of medium-sized chromosomes with nearly median primary constriction (C).

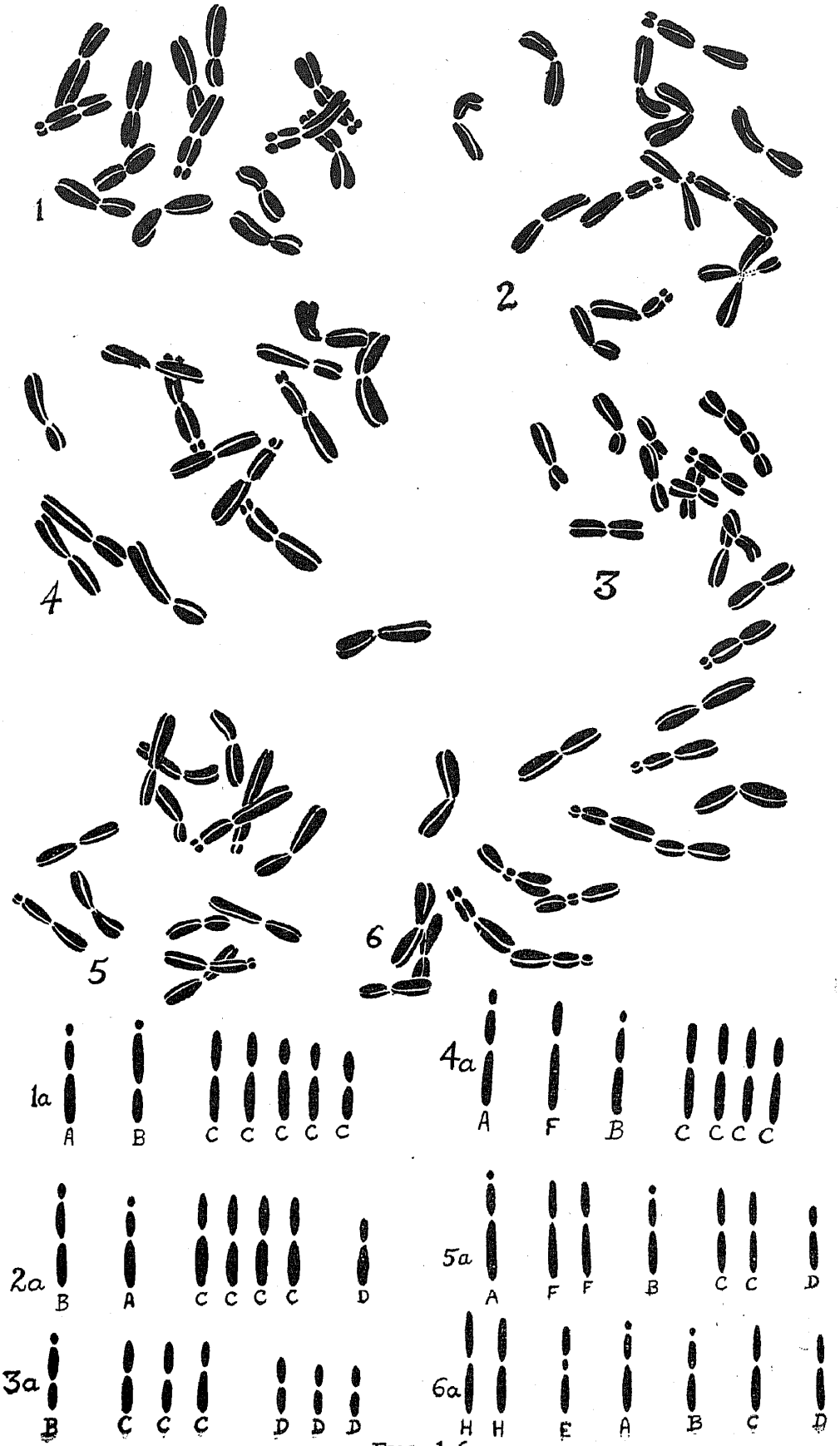
*Strain B* 42.—The chromosomes of this strain could be classified into the following morphologically distinguishable types (Figs. 2, 2 *a*):

1. A pair of long chromosomes with median primary constriction and a satellite at the end of one of the arms (B).

2. A pair of long chromosomes with submedian primary constriction and a satellite at the end of the shorter arm (A).

3. Four pairs of medium-sized chromosomes with median primary constriction in each (C).

4. A pair of short chromosomes with submedian primary constriction (D).


1
2
3
4
5
6
1a
A B C C C C C
2a
B A C C C C D
3a
B C C C D D D
4a
A F B C C C C
5a
A F F B C C D
6a
H H E A B C D

FIGS. 1-6

*Strain B* 122.—The chromosomes of this strain could be classified into the following morphologically distinguishable types (Figs. 3, 3 *a*):

1. A pair of long chromosomes with median primary constriction and a satellite at the end of one of the arms (B).

2. Three pairs of medium-sized chromosomes with nearly median primary constrictions in two and nearly submedian primary constrictions in one (C).

3. Three pairs of short chromosomes with median primary constrictions (D).

*Strain B* 134.—The chromosomes of this strain could be classified into the following morphologically distinguishable types (Figs. 4, 4 *a*):

1. A pair of long chromosomes with submedian primary constriction and a satellite at the end of the shorter arm (A).

2. A pair of long chromosomes with submedian primary constriction (F).

3. A pair of long chromosomes with median primary constriction and a satellite at the end of one of the arms (B).

4. Four pairs of medium-sized chromosomes with nearly median primary constrictions in three pairs and nearly submedian in one pair (C).

*Strain B* 135.—The chromosomes of this strain could be classified into the following morphologically distinguishable types (Figs. 5, 5 *a*):

1. A pair of long chromosomes with submedian primary constriction and a satellite at the end of the shorter arm (A).

2. Two pairs of long chromosomes with submedian primary constrictions (F).

3. A pair of long chromosomes with median primary constriction and a satellite at the end of one of the arms (B).

4. Two pairs of medium-sized chromosomes with median primary constrictions (C).

5. A pair of short chromosomes with submedian primary constriction (D).

*Strain B* 137.—The chromosomes of this strain could be classified into the following morphologically distinguishable types (Figs. 6, 6 *a*):

1. Two pairs of long chromosomes with median primary constriction (H).

2. One pair of long chromosomes with sharp median primary constriction and a secondary constriction very close to the primary one (E).

3. A pair of long chromosomes with submedian primary constriction and a satellite at the end of the comparatively shorter arm (A).

4. A pair of long chromosomes with median primary constriction and a satellite at the end of one of the arms (B).

5. A pair of medium-sized chromosomes with median primary constriction (C).

6. A pair of short chromosomes with submedian primary constriction (D).

## DISCUSSION

The genus *Hordeum* has been considered by a number of cytologists as an attractive material for investigation and a good deal of work of importance have been carried out in the genus. As the members of this genus are regarded as important cereals, having much of economic importance, the significance of such studies from a practical standpoint is not negligible. Furthermore, it is fortunate that the chromosomes of *H. vulgare* are quite long and the diploid number is quite small, *i.e.*, $2n = 14$. These two facts are possibly responsible for their being the subject of attraction of a number of workers.

Chin (1941) reported the chromosome number of *Hordeum vulgare* as $2n = 14$, which has also been confirmed by a number of other investigators. His observations, mainly involving the number of chromosomes and satellites, led him to suggest a phylogeny of the species. While drawing up the ancestry of the species he has given considerable emphasis on deMol's concept of the nucleolar number as an index of polyploidy. It needs no mention that this concept in the light of the recent findings cannot account always for the increase in the number of nucleoli and other theories have been proposed providing evidences for additional means of such an increase. It has been suggested that non-homologous translocation involving nucleolar and non-nucleolar chromosomes may result in an increase in the number of nucleoli.

Recent investigations on a number of Indian strains of Barley (Bhaduri and Sharma, 1948) have revealed the presence of a high number of secondary constrictions in the different strains. Furthermore, some of the strains have been found to differ in karyotypes significantly from that of the others. The presence of supernumerary constrictions in some of the strains have also been recorded. On the basis of available evidences, emphasis has been laid on the role of structural changes of chromosomes in the evolution of different strains.

Their regular meiotic behaviour has been claimed as due to the homozygosity for translocation they have attained during evolution. The remarkably stable behaviour of the species is thus due to their homozygous constitution. Cross-breedings between different strains has been suggested as reliable methods for the mapping of such segmentally interchanged ends of chromosomes. This would be possible as ring formation is expected in such hybrids, at least in some of the crosses, which would lead to a detection of chromosome ends. It is worthy of note that recently Hagberg and Tjio (1952) have reported translocations in Barley. These two findings are no doubt significant.

The present report, which has dealt with the six different strains of Barley, indicate certain interesting peculiarities. Though all of them show chromosome number as $2n = 14$, karyotypic differences are noticed between different strains. This is apparent at a glance at their karyotypes. For example, the chromosome type " E " of " B 137 " where primary and secondary constrictions are very near each other could not be located in any of the other strains. Furthermore, there are six chromosomes with two constrictions in each, are to be found in this particular strain in contrast to others, where the number is less. The strain " B 122 " shows one pair of chromosomes with secondary constriction whereas the rest four strains are provided with four chromosomes with two constrictions. Amongst these rest four, the karyotypes reveal minor differences.

A significant fact emerging out from the above finding is relevant in connection with the problem of origin of different strains. It needs no emphasis that a study of their karyotypes, as the present findings reveal, may serve to some extent as a criterion for their identification. The evolution of these different varieties or strains, not necessarily involve imperceptible genic mutation, as is generally believed, but rather gross chromosomal changes have also taken place. At the present state of our knowledge, it is not possible to state precisely that such chromosomal rearrangements are responsible for their evolution. These facts are yet to be proved. This much can be said that if structual changes of chromosomes can have a reflection on the expression of the phenotype, which is an accepted fact, then the morphological or physiological differences of one strain from other can possibly be accounted for, on the basis of these changes.

In any case as all these varieties owe their origin to a common ancestor, either through direct or through indirect means, segmental homology of varying extent are expected between complements of different strains. In that case cross-breeding between themselves may give evidences of segmental

interchanges, manifested in their cytological behaviour. It is clear, however, that the homozygosity has been attained by these strains during evolution and judicial selection. If intercrosses are made and concrete evidences of segmental interchanges are obtained, mapping of chromosome ends might be possible in case of *Hordeum vulgare*. It is worthy of note that recent findings in this laboratory have also revealed such karyotypic differences between strains of *Sorghum vulgare* (Sharma and Bhattacharjee, 1956). More and more work in these lines in different cereals are desirable with the aid of the refined techniques.

## SUMMARY

The present report has dealt with the study of the karyotypes of six agricultural strains of *Hordeum vulgare* raised at the Indian Agricultural Research Institute, New Delhi. The observations have been carried out following temporary squash preparations according to Paradichlorobenzene method suggested by Sharma and Mookerjea (1955). The types are:

1. *Hordeum vulgare* strain B 17
2. *H. vulgare* ,, B 42
3. *H. vulgare* ,, B 122
4. *H. vulgare* ,, B 134
5. *H. vulgare* ,, B 135
6. *H. vulgare* ,, B 137

All of them possess fourteen chromosomes, but the karyotypes differ one from the other. The number of chromosomes with two constrictions have been found to be four in strains B 17, B 42 and B 134; six chromosomes with two constrictions each, occur in strain B 137. In addition, gross differences in karyotype have been noticed in a number of them. It has been suggested that the structural changes of chromosomes provide a means for the evolution of these strains.

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## Explanation of Figures

Figs. 1–6 *a*. Figs. 1 and 1 *a*. Somatic metaphase and idiogram of *Hordeum vulgare*, Strain No. B 17. Figs. 2 and 2 *a*. Somatic metaphase and idiogram of *H. vulgare*, Strain No. B 42. Figs. 3 and 3 *a*. Somatic metaphase and idiogram of *H. vulgare*, Strain No. B 122. Figs. 4 and 4 *a*. Somatic metaphase and idiogram of *H. vulgare*, Strain No. B 134. Figs. 5 and 5 *a*. Somatic metaphase and idiogram of *H. vulgare*, Strain No. B 135. Figs. 6 and 6 *a*. Somatic metaphase and idiogram of *H. vulgare*, Strain No. B 137.