CHROMOSOME STUDIES IN SOME INDIAN BARLEY. I

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INTRODUCTION

The genus *Hordeum* has attracted the attention of cytologists from time to time, and a good deal of work has been carried out regarding the chromosome counts and the meiotic behaviour in different species of the genus. Different authors, viz. Kihara (1924), Kagawa (1929), Ghimpu (1929) and Tsuchiya (1952), reported the diploid chromosome number as fourteen in case of *H. vulgare* Linn. Lewitsky (1931) observed two pairs of chromosomes with satellites in the body cell of all cultivated diploid species of *Hordeum.* Chin (1941), too, corroborated his observation after a study of the morphology of the chromosomes in all cultivated diploid forms. In case of wild diploids and tetraploids, he recorded the occurrence of one pair of satellited chromosomes in the former and two pairs in the latter. Attempts towards the induction of higher polyploids of barley have also been met with marked success (Chen *et al.*, 1945). The meiotic behaviour of chromosomes of all such raised types has also been thoroughly studied. Critical study to some extent in the cereals has been carried out by Oinuma (1952).

The common Indian barley, *Hordeum vulgare* Linn., is cultivated throughout the drier parts of India and different agricultural strains have been raised in the Indian Agricultural Research Institute, New Delhi. Though much of genetical work has been done on the species, no attempt has yet been made to make a critical investigation into the cytogenetics of the different strains of barley as well as *H. murinuum* L. and *H. distichon* L., the two allied species of *H. vulgare.*

The fact that the genus *Hordeum* represents plants of much economic importance and that its species and varieties, having chromosomes quite small in number and large in size, provide good material for a critical study of their morphology, an aspect of study quite unattempted till now, it was thought desirable to carry out a critical investigation into the cytogenetics of its different species and strains with the aid of the recently improved technique. With this end in view, the present investigation was undertaken. The present paper deals with the karyotype analysis and the meiotic behaviour of different strains of barley, obtained from the Indian Agricultural Research Institute, New Delhi.

Materials and Methods

The following strains of barley have been used in the present investigation: (1) IP: 1, (2) IP: 9, (3) IP: 13, (4) IP: 14, (5) IP: 20, and (6) IP: 24.*

For the study of somatic chromosomes, root tips were fixed from seeds germinated in sawdust in the laboratory. After a strenuous trial in different fixing fluids, it became apparent that the metaphase chromosomes of these strains, though properly fixed, presented much difficulty in the interpretation of their morphology due to their considerable length as well as due to the presence of much foreshortening of their arms. For the purpose of straightening the chromosome arms, simultaneously with the exaggeration of the constrictions present, it was thought desirable to treat the root tips, before fixing, in cold temperature in dilute aqueous solution of colchicine. The application of this colchicine technique for the purpose of chromosome straightening has already been met with marked success in case of *Vicia faba* L. by Bhaduri (1939). After a series of trials, best results were, however, obtained by fixing the root tips between 12 noon and 1 p.m. in a solution of 1% aq. platinic chloride and 10% formalin in the proportion of 1: 4, after a pre-treatment in 0·5% colchicine solution for one hour and then subsequently washing in water for the same period. It was observed that a treatment in slightly lower dilution of colchicine for a longer period and in slightly higher dilution for a shorter period yielded more or less the same results. In some of the cells, however, tetraploid number of chromosomes were observed, formed as a result of direct effect of colchicine. For the study of meiosis, flower buds were collected from the plants grown in the Calcutta University Botanic Gardens, and fixed between 10 a.m. and 11 a.m. in Nawaschin's fixative after a pretreatment in Carnoy's fluid.

Sections were cut at a thickness of 18μ both in case of flower buds and root tips and stained in the usual procedure of Newton's crystal violet-iodine technique. In case of root tips, however, hydrolysis in N. HCl for fifteen minutes at 60°C. and then premordanting in 1% chromic acid for overnight became necessary to bring out best results. For the study of nucleoli, Feulgen-Light green technique of Semmens and Bhaduri (1941) was followed.

The figures were drawn at a table magnification of 3,600 times approximately using a compensating eyepiece ×18 and a 1·3 N.A. apochromatic objective.

Observations

The chromosome numbers of all the strains studied were found to be fourteen, thus corroborating the previous observations (Lewitsky, 1931; Chin, 1941, etc.). The karyotype analysis of all these strains revealed much difference in chromosome morphology in at least some of them, though the presence of *supernumerary constrictions in the* chromosome is a constant feature of all the strains studied so far. The karyotype of the strains fourteen, twenty and twenty-four were found to be identical with each other, whereas the other three strains, viz. one, nine and thirteen, showed differences in chromosome morphology among themselves as well as from the common karyotype of the strains fourteen, twenty and twenty-four (Fig. 11). The complements in all of them are characterized by having fairly long chromosomes, the size difference between themselves being not very marked. The lengths of the chromosomes were found to vary from 10·5μ to 8·3μ.

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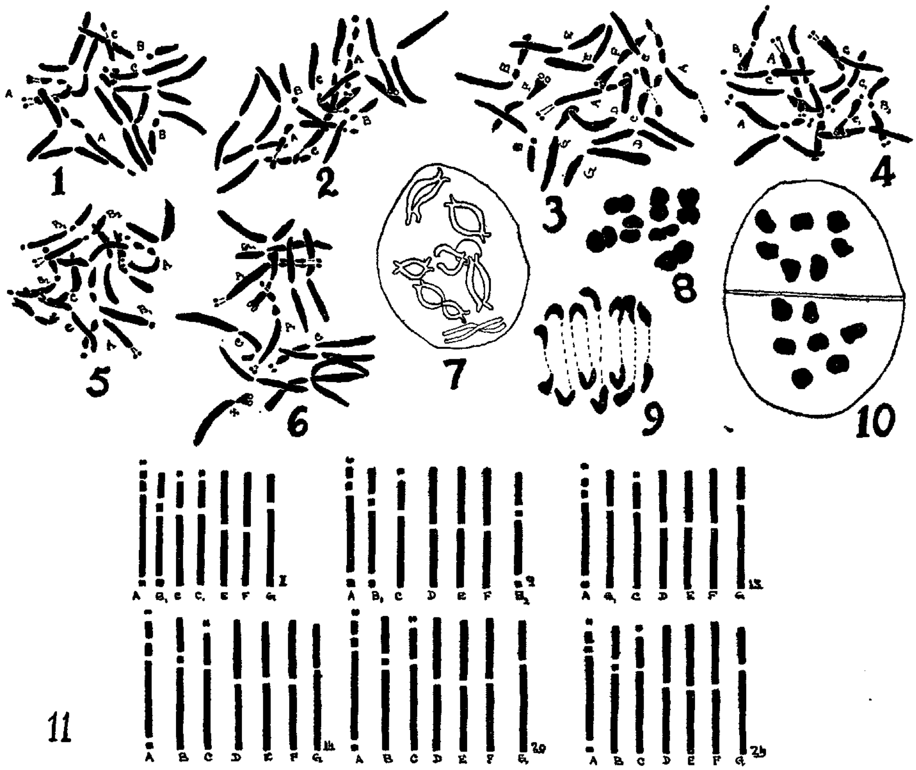
* IP = Old Pusa or Imperial Pusa.

I. *Karyotype of the strains IP: 14, IP: 20 and IP: 24* (*Figs. 1, 2, 3 and 11*)

The seven pairs of chromosomes could be classified into five distinct types which are as follows (lengths of the chromosomes vary from 10·5μ to 9·8μ):



FIGS. 1 TO 10—Somatic metaphase plates of the strains IP—14, 20, 24, 1, 9, 13 and meiotic stages of IP—24 respectively.

FIG. 11—Karyotypes of the strains IP—1, 9, 13, 14, 20 and 24 respectively.

Chromosomes with secondary constrictions:

(1) A pair of long chromosomes with nearly submedian primary constrictions and a satellite at the long arm. In the short arm, however, one median secondary constriction and a satellite at the end are also present, thus making the total number of constrictions, excluding the primary one, present in the pair as six (AA).

(2) A pair of medium sized chromosomes, with nearly submedian primary constrictions and a secondary constriction very near the primary one, located on the shorter arm (BB).

(3) A pair of medium sized chromosomes with nearly submedian primary constrictions and a satellite at the end of the short arm (CC).

The total number of constrictions, excluding the primary ones, located in these three pairs is, therefore, ten.

Chromosomes not with secondary constrictions :

(4) Three pairs of medium sized chromosomes with median primary constrictions (DD, EE, FF).
(5) One pair of medium sized chromosomes with submedian primary constrictions (GG).

II. *Karyotype of the strain IP : 1 (Figs. 4 and 11)*

The karyotype of this strain may be classified into the following distinct types (lengths of the chromosomes vary from 9·4μ to 8·3μ) :

Chromosomes with secondary constrictions :

(1) A pair of long chromosomes with nearly submedian primary constrictions and a satellite at the end of the long arm. In the short arm, a median secondary constriction and a satellite at the end are also present, thus making the total number of constrictions, excluding the primary one, present in the pair as six (AA).
(2) A pair of medium sized chromosomes, each with a submedian primary constriction and a secondary constriction very near the primary one towards the shorter arm. A satellite is also present at the end of the long arm, thus making the total number of constrictions, excluding the primary one, present in the pair as four (B_1B_1).
(3) Two pairs of medium sized chromosomes, each with a submedian primary constriction and a satellite at the end of the short arm (CC, C_1C_1).

The total number of constrictions excluding the primary ones present in these four pairs is, therefore, fourteen.

Chromosomes not with secondary constrictions :

(4) Two pairs of medium sized chromosomes with nearly median primary constrictions (EE, FF).
(5) A pair of medium sized chromosomes with nearly submedian primary constrictions (GG).

III. *Karyotype of the strain IP: 9 (Figs. 5 and 11)*

The karyotype of this strain may be classified into the following distinct types (lengths of the chromosomes vary from 9·8μ to 8·6μ) :

Chromosomes with secondary constrictions :

(1) A pair of long chromosomes, each with a submedian primary constriction and a satellite at the end of the long arm. The short arm is provided with a median secondary constriction and a satellite at the end, thus making the total number of constrictions, excluding the primary ones, present in the pair as six (AA).
(2) Two pairs of medium sized chromosomes, each with nearly submedian primary constriction, a secondary constriction very near the primary one towards the short arm, and a satellite at the end of the long arm. The total number of constrictions, excluding the primary ones, present in these two pairs is, therefore, eight (B_1B_1, B_2B_2).
(3) A pair of medium sized chromosomes, each with nearly submedian primary constriction and a satellite at the end of the short arm (CC).

The total number of constrictions, excluding the primary ones, present in these four pairs is, therefore, sixteen.

Chromosomes not with secondary constrictions:

(4) Three pairs of medium sized chromosomes with nearly median primary constrictions (DD, EE, FF).

IV. *Karyotype of the strain IP : 13 (Figs. 6 and 11)*

The karyotype of this strain can be classified into the following distinct types (lengths of the chromosomes vary from 9·4μ to 8·9μ):

Chromosomes with secondary constrictions :

(1) A pair of long chromosomes, each with submedian primary constriction and a satellite at the end of the long arm. The short arm also possesses a median secondary constriction, and a satellite at the end, thus making the total number of constrictions, excluding the primary ones, present in the pair as six (AA).
(2) A pair of medium sized chromosomes, each with submedian primary constriction and a satellite at the end of the short arm (CC).

The total number of constrictions, excluding the primary ones, located in these two pairs is, therefore, eight.

Chromosomes not with secondary constrictions :

(3) Three pairs of medium sized chromosomes with nearly median primary constrictions (DD, EE, FF).
(4) Two pairs of medium sized chromosomes with nearly submedian primary constrictions (GG, G_1G_1).

All these strains showed the presence of a high number of nucleoli, and as many as ten in case of strain IP: 9, in the telophase stage of root tip cells, showing that all the constrictions, excluding the primary ones, are *probably* nucleolar in nature. This aspect of study involving chromosome-nucleolus relationship has not, however, been carried out yet. In any case, the nature of these constrictions, whether nucleolar or non-nucleolar, is yet to be ascertained.

Meiosis

Meiotic behaviour of chromosomes so far studied proved to be more or less similar in nature in all the strains examined, being fairly regular in nature. During diplotene stages, both interstitial and terminal chiasmata were observed and homologous chromosomes held together at three chiasma points were also not of very infrequent occurrence (Fig. 7). Open bivalents in contrast to closed ones were observed to be very few in number. A study of the chiasma frequency and terminalization coefficient in all the strains is expected to yield significant data. Distinct seven bivalents were clearly observed during diakinesis as well as metaphase stages of first meiotic division (Fig. 8). Anaphasic segregation though was found to be more or less normal (Fig. 9), rare occurrence of inversion bridges resulting in a dicentric chromosome and a fragment were noted in the strains IP: 1, IP: 9 and IP: 14. No case of lagging or non-disjunction could be encountered in any of them.

In the second division, too, the chromosomes were found to behave in a fairly regular manner. Clear seven and seven chromosomes could be counted in the two spindles of the second meiotic metaphase (Fig. 10). Their segregation, too, was found to be normal, resulting in the regular formation of tetrads. Counts of morphologically abortive pollen grains showed the sterility to be 2-3%.

Difference in karyotypes of the strains studied:

It is clear from the above that the karyotypes of these different strains, although similar in the gross morphology of the chromosomes, differ in minute structural details from one another, particularly with respect to chromosomes with secondary constrictions. It has already been pointed out that the number of secondary constrictions in case of the strains IP: 14, IP: 20 and IP: 24 is ten, in IP: 1 fourteen, in IP: 9 sixteen and in IP: 13 eight.

For the sake of convenience in comparison, the chromosome types A, B, C, D, E, F and G of the strains IP: 14, IP: 20 and IP: 24 have been taken to be the standard ones and the chromosome types of the other strains in relation to their difference with these standard ones have been discussed below (Fig. 11).

The type 'A' with three secondary constrictions is present in all the strains studied.

The type 'B' with one secondary constriction is absent in the strain IP: 13, where it has been replaced by a chromosome pair with submedian primary constrictions G_1G_1 similar to the type G. In the strains IP: 1 and IP: 8, it has been replaced by B_1 type, a pair of chromosomes having morphology more or less similar to the type B, but differing in having an extra constriction at the end of the long arm.

The type 'C' with one secondary constriction is present in all the strains studied.

The D, E and F pairs of chromosomes belonging to one type are present in the strains studied, excepting the strain IP: 1, where one of the pairs, say, for instance, D, is replaced by G pair type, a pair of chromosomes having morphology similar to the type C, with submedian primary constriction and having a satellite at the end of the short arm.

The 'G' type of chromosomes is present in all the strains studied, excepting the strain IP: 9, where it has been replaced by B_2 type, a pair of chromosomes having morphology similar to the type B_1, with submedian primary constriction, a secondary constriction very near the primary one towards the shorter arm and a satellite at the end of the long arm.

Discussion

The chromosome counts of different species of *Hordeum*, as reported by previous authors, show a basic number of seven. Different polyploid species have been found, both in nature as well as in cultivated forms. Chin's (1941) observation on the meiotic behaviour of different species of the genus shows that both auto- and allopolyploidy have played an important rôle in the evolutionary process of these species. Tetraploid forms of *H. spontanum* Koch., *H. jubatum* L., *H. gussonaeanum* Parl., and *H. murinum* L., as studied by him, showed regular occurrence of bivalents during meiosis, indicating their possible allopolyploid nature. On the other hand, frequent formation of multivalents in the tetraploid form of *H. bulbosum* L. suggests its origin, possibly through autopolyploidy.

Lewitsky (1931) reported that all cultivated diploid species of *Hordeum* possess two pairs of chromosomes with satellites. Chin (1941) also corroborated his observation. The occurrence of one pair of satellited chromosomes in the wild diploid, and two pairs in the wild tetraploids, and the presence of two pairs of satellited chromosomes in the cultivated diploids, led the latter author to suggest that the phylogenetic relationship between the wild and cultivated species might not be close. Such a suggestion, however, in the light of our present-day knowledge of cytogenetics seems no longer tenable. The fact has now become well established that, apart from polyploidy, there are other ways by means of which an increase in the number of nucleoli and satellites in a species may take place.

Oinuma (1952) has recently shown that cultivated barleys are derived from wild ones and the structural changes in the chromosomes involved have also been worked out. Quite a large amount of literature (Bhaduri, 1942*a*) has accumulated in the last few years showing that interchanges involving nucleolar and non-nucleolar chromosomes may result in an increase in the number of constrictions and nucleoli of a species. Recent works of Bhaduri and Bose (1947) on different members of the family *Cucurbitaceae* and of Chakravarty (1948) on different species and genera of *Scitamineae* have brought about important evidences in support of the fact that fragmentation of chromosomes also plays an important rôle in bringing about a high number of nucleoli and satellites of the living nuclei. All these recently accumulated data suggest that the earlier assumption of Chin, regarding the phylogenetic relationship between the wild and cultivated species of *Hordeum*, may not necessarily be valid.

In the present investigation, although in one of the strains, viz. IP: 13, two pairs of chromosomes with secondary constrictions have been noted, additional supernumerary constrictions are present, making the total number of secondary constrictions and satellites to be eight. In the strain IP: 9, number of constrictions excluding the primary ones are sixteen, present in four pairs of chromosomes. In the strain IP: 1, fourteen secondary constrictions and satellites are distributed in three pairs of chromosomes. In the other strains, ten constrictions are distributed in three pairs of chromosomes. It is, therefore, apparent that the number of secondary constrictions and satellites, present in seven pairs of chromosomes in diploid species of *Hordeum*, is much higher than that noted by previous workers. The possibility of all these constrictions being nucleolar in nature is not precluded, because of the presence of a high number of nucleoli, as many as ten, in the somatic cells of at least one of the strains. In any case, this aspect of study, which has not been dealt with, is yet to be done.

Examination of wild varieties of barley as done to some extent by Oinuma (1952), employing critical technique from which the cultivated strains have evolved, may reveal whether these high numbers of constrictions were originally present in the wild complement or their evolution has taken place through structural changes of chromosome. A thorough search in this direction is highly desirable.

As has been pointed out in the text, definite evidences have been obtained to show that many of the strains of cultivated barley differ from each other with respect to their karyotypes. It is not unlikely that structural interchanges are directly responsible for the different karyotypes amongst the different cultivated strains which differ from each other in minor morphological characters only. It is interesting to note that Oinuma (1952) has obtained intervarietal hybrids in which the presence of interchanges or rings in meiosis has been detected.

The absence of any ring formation and the occurrence of regular bivalents during meiosis in these strains are probably due to their homozygous and stable nature produced as a result of continuous cultivation and judicial selection. Rare occurrence of inversion bridges, too, points towards the possibility of structural changes of chromosomes playing an important part in the evolution of these strains. In other strains, where such bridges have not been encountered, it is not at all unlikely that the absence of bridges is not due to the absence of any inverted segment in the chromosome, but due to the homozygous nature of the strain concerned, where such inversions are present in both the homologues.

If the above conclusion is correct, then the occurrence of rings of chromosome, instead of regular formation of bivalents during meiosis, is expected in intervarietal hybrids of *H. vulgare* L., where the homologous segments in otherwise non-homologous chromosomes will have the chance to meet each other. The report of Oinuma (1952) already mentioned is noteworthy in this regard. Once this theory is established, extensive hybridization of different strains of *Hordeum vulgare* would provide good materials for the study of identification and classification of chromosome ends of barley as has been done in case of *Datura* by Bergner *et al.* (1933), *Zea* by McClintock (1934), *Tradescantia* and *Rhoeo* by Bhaduri (1942*a*, 1942*b*). It is worth noting that Hagberg and Tjio (1950) have recently succeeded in getting artificial mutants of barley showing translocations.

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Summary

1. A karyotype analysis of six different strains of barley, raised in the Indian Agricultural Research Institute, New Delhi, has been carried out. It has been shown that all the strains, though having the same number of chromosomes, i.e. fourteen, more or less similar in gross morphology, differ with respect to the number of nucleolar constrictions present in the complement.

2. It has been found that the presence of a high number of supernumerary constrictions in the chromosome complement is a constant feature of all the strains studied. A high number of nucleoli has been observed in root tip cells of some of the strains. This is in strong contradiction with the report of earlier workers who reported only two pairs of satellited chromosomes in the cultivated diploid species of *H. vulgare* L. All these details in chromosome morphology could only be brought about by the application of special technique, viz. platinic chloride and formalin mixture with an increased proportion of formalin as the fixing fluid and a prefixation treatment in colchicine solution.

3. Meiotic behaviour of all the strains studied showed more or less regular behaviour excepting the rare occurrence of inversion bridges in some of the strains.

4. It has been suggested that a critical investigation with the application of the improved technique, as adopted here, should be made of the wild diploid species of *Hordeum* to find out whether these high number of constrictions were present in the original diploid set or the evolution has taken place through structural interchanges from the original set.

5. The regular behaviour during meiosis has been claimed to be due to their stable homozygous nature, produced as a result of continuous cultivation and judicial selection. In view of the fact that formation of rings during meiosis are expected in intervarietal hybrids where homologous segments in otherwise non-homologous chromosomes would get the chance of meeting each other, it has been suggested that extensive hybridization of different varieties of *H. vulgare* L. is necessary to have good materials for the study of classification and identification of chromosome ends in barley.

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