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ORIGINAL ARTICLE

Chromosome association and chiasma frequency of cultivated wheat (*Triticum aestivum* L.) in Bangladesh

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A B S T R A C T

Chiasma frequency estimation is usually considered a good indicator for genetic recombinations in a population, especially in polyploid organisms like hexaploid wheat (*Triticum aestivum* L.). The hexaploid wheat is functional as diploid with 21 bivalents (21 II), indicating the chromosome number 2n=6x=42. The aim of this study is to identify chromosome association and chiasma frequencies of seventeen cultivated wheat varieties in Bangladesh. Chiasma distribution and its frequencies were studied by using pollen mother cells (PMCs). Data were recorded from diakinesis and metaphase-I of meiotic-I cell division. Mean chiasma frequency per cell and per bivalent were varied in studied varieties which ranged from 40.28 to 40.87 and 1.92 to 1.95 in Sonora-64 and Barkat, respectively. The percentages of ring bivalent were extensively more than rod bivalent in all of the studied varieties. From our observation, it can be stated that chiasma distribution and its frequency varied marginally in seventeen commonly cultivated wheat varieties of Bangladesh. From this observation, it can be stated that commonly cultivated wheat varieties of Bangladesh are closely related to their primary genepool and it is possible to improve those varieties crossing with different genepool imported from other countries.

INTRODUCTION

Wheat (*Triticum aestevum* L.) is the most widely cultivated cereals due to its excellent baking quality. It has become the most important source of carbohydrate throughout the world and its use dates back 6000 years or more (Arzani and Ashraf 2017). Wheat ranks next to rice in importance as a cereal in Bangladesh. In our country, it is grown in the rabi season just after harvest of the Aman rice. At present, a large number of wheat cultivars are being cultivated in Bangladesh. They are registered by different names such as Gourab, Sourab, Akbar, Sonalika etc based on their trial performances (BARI 2012).

Now-a-days, *Triticum* has been divided as wild and advanced cultivated species (Kellogg 2001). In present time, the wild wheat species has already lost its eligibility, but the cultivated species have been distributed all over the world for their high yield potentials (FAO 2011). According to the number of chromosomes, wheat can be divided into three groups, named Einkorn, Emmer and bread wheat (Arzani 2011). Wheat species constitute an allopolyploid series, includes diploid, tetraploid and hexaploid classes with 2n=14, 28 and 42 chromosomes, respectively. The diploid wheat have a single genome designated A, which is shared by the tetraploid and hexaploid wheat. The tetraploid wheat has in addition the B genome so that their genomic composition is AABB. The hexaploid wheat has the compsition of tetraploid and extra D genome thus it has the genomic constitution AABBDD (Kerber 1964). The hexaploid wheat is functionally diploid with 21 bivalents (21 II), which indicated the genomic status 2n=6x=42 chromosomes in number.

Varietal identification is the cornerstone of seed certification and genetic purity testing. Traditional identification techniques utilise varieties that are

differentiated in the field based on plant morphological characteristics. Variety protection acts have made varietals identification testing increasingly difficult and questionable (McDonald 1988). Knowledge of genetic diversity among adapted cultivars or elite breeding materials has a significant impact on the improvement of crop plants and this information

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has been successfully used for efficient germplasm management and genotype selection for different breeding purposes. The genetic variability of a population results from the action of a series of genetically controlled factors that are also under the action of natural selection. The sites of genetic exchange are cytologically visible as 'crosses' between the arms of chromosome pairs during late prophase -I. These crosses were originally described by Janssens (1909) and termed as 'chiasmata'. The most convincing evidence supporting the chiasma type theory was provided by Tease and Jones (1978) using techniques for differential sister chromatid labelling. Chiasma frequency is usually considered a good indicator of genetic recombination in a population, especially in organisms in which genetic analysis is difficult or impossible to perform (Koszural et al. 2012).

In the last century, mean chiasma values have been determined for many species with cytologically well-defined meiotic chromosomes (Adamowski et al. 2008). Chiasma frequency is very much useful for comparing different taxa and in some cases it may be more precise parameter than the karyotype itself.

Chiasma formation reflects similarities both in genetic content and in the arrangement of gene. Crossing over, cytologically recognized by the presence of chiasmata, is also variable, both in respect to frequency and localization of the chiasmata. Therefore present study aims to identify chromosome association and chiasma frequencies of seventeen cultivated wheat varieties in Bangladesh.

METHODOLOGY

Materials

Seventeen varieties of hexaploid wheat were used as plant materials in the present study. A brief account of the materials is given in Table 1.

Methods

Fresh and dry seeds of all the wheat varieties were sown in three replicates following RCBD in the experimental field of Botany Department of Rajshahi University. Standard agronomic practices were followed (BARI 2006).

Determination of chiasma distribution and frequency

The method followed to study the chromosome association and chiasma frequencies were measured according to Jahan et al. (1992), as follows:

a) Collection of young inflorescences

Young inflorescences of suitable sizes were collected between 9.00 to 10.00 am for fixation.

b) Fixation and preservation of inflorescences

The inflorescences from each variety were fixed for 48 hours in Carnoy's solution (6 ethanol: 3 chloroform: 1 acetic acid) and then preserved in 70% ethanol and kept in a refrigerator till used.

c) Preparation of slides

Temporary slides were prepared from suitable anthers by acetocarmine smear technique as follows:

i. Young anther was placed on a clean slide and a drop of 2% acetocarmine was added.

ii. The anther wall was ruptured by curve dissecting needle and the anther wall was removed from slide.

iii. There after the pollen mother cells (PMCs) were covered by a coverslips, warmed gently over an alcohol flame and a slight pressure exerted by thump to spread out the PMCs as well as the chromosomes.

iv. Additional heating was applied with 45% acetic acid as needed until the cytoplasm became clear.

d) Photomicrography and observation

Temporary slides were used for taking photomicrographs and observed under compound microscope for recording the dates from diakinesis and metaphase-I stages.

e) Recording of data

Data on chromosome association and chiasma frequency was recorded from the desired preparations. Chromosome configurations during diakinesis and metaphase-1 were used to determine the pairing and number of bivalent. The number of univalent, trivalent and quadrivalent if present was also counted and the data were recorded. The ring and rod bivalent with position of chiasmata were also recorded.

RESULTS

Chiasma distribution and its frequency in 17 varieties of *Triticum aestivum* were studied from well prepared temporary slides as well as from photomicrographs of pollen mother cells (PMCs). Possible chromosome configuration at diakinesis of prophase-I of *Triticum aestivum* L. is presented in Figure 1 and Figure 2. Pollen mother cells containing majority of the bivalents and a few number of univalents, trivalents and quadrivalents were observed at diakinesis or metaphase-I. Their association and chiasma frequencies in different varieties of *T. aestivum* are given in Table 2 and the results are described below:

Agrahani: The percentage of ring bivalent, rod bivalent, univalent, trivalent and quadrivalent were found to be 93.37%, 6.38%, 0□09%, 0.09% and 0□17, respectively. The percentage of terminal and interstitial chiasmata were found 99.96% and 0.04%, respectively. Mean chiasma frequency per cell and per bivalent were found 40.47 and 1.93, respectively (Table 2).

Akbar: The percentage of ring and rod bivalents, were found to be 94.05%, and 5.95%, respectively. The percentage of terminal and interstitial chiasmata were 99.89% and 0.11% respectively. Mean chiasma frequency per cell and per bivalent were found 40.80 and 1.94, respectively (Table 2).

Ananda: The percentage of ring and rod bivalent were 93.79%, and 6.21%, respectively (Table 2). The percentage of terminal and interstitial chiasmata were found 99.91% and 0.09%, respectively. Mean chiasma frequency per cell and per bivalent were found to be 40.73 and 1.94, respectively.

Balaka: The percentage of ring and rod bivalent were found to be 94.05% and 5.95%, respectively. The percentage of terminal and interstitial chiasmata were 99.90% and 0.10% respectively. Mean chiasma frequency per cell and per bivalent were 40.79 and 1.94, respectively (Table 2).

Barkat: The percentage of ring and rod bivalent were 94.42%, and 5.58%, respectively. The percentage of terminal and interstitial chiasmata were found to be 99.91% and 0.04%, respectively. Mean chiasma frequency per cell and per bivalent were found to be 40.87 and 1.95, respectively (Table 2).

Gaurav: The percentage of ring and rod bivalent were found to be 92.98%, and 7.02%, respectively. The percentage of terminal and interstitial chiasmata were found to be 99.93% and 0.07%, respectively. Mean chiasma frequency per cell and per bivalent were found to be 40.55 and 1.93, respectively (Table 2).

Innia-66: The percentage of ring and rod bivalent were found to be 92.94%, and 7.06%, respectively. The percentage of terminal and interstitial chiasmata were 99.96% and 0.04%, respectively. Mean chiasma frequency per cell and per bivalent were found to be 40.53 and 1.93, respectively (Table 2).

Kanchan: The percentage of ring and rod bivalent were 93.42%,

and 6.58%, respectively. The percentage of terminal and interstitial chiasmata were found to be 99.86% and 0.14%, respectively. Mean chiasma frequency per cell and per bivalent were 40.68 and 1.94, respectively (Table 2).

Kallayansona: The percentage of ring and rod bivalent were found to be 92.84%, and 7.16%, respectively. The percentage of terminal and interstitial chiasmata were 99.94% and 0.06%, respectively. Mean chiasma frequency per cell and per bivalent were found to be 40.52 and 1.93, respectively (Table 2).

Kheri: The percentage of ring bivalent, rod bivalent, univalent, trivalent and quadrivalent were 93.21%, 6.51%, 0.08%, 0.08% and 0.12%, respectively. The percentage of terminal and interstitial chiasmata were 99.96% and 0.04%, respectively. Mean chiasma frequency per cell and per bivalent were found to be 40.48 and 1.93, respectively (Table 2).

Pavon-76: The percentage of ring bivalent, rod bivalent, univalent, trivalent and quadrivalent were found to be 93.02%, 6.59%, 0.13%, 0.13% and 0.13%, respectively. The percentage of terminal and interstitial chiasmata were found to be 99.96% and 0.04%, respectively. Mean chiasma frequency per cell and per bivalent were found to be 40.40 and 1.92, respectively (Table 2).

Protiva: The percentage of ring and rod bivalent were 92.71% and 7.29%, respectively. The percentage of terminal and interstitial chiasmata were 99.96% and 0.04%, respectively. Mean chiasma frequency per cell and per bivalent were 40.48 and 1.93, respectively (Table 2).

Satabdi: The percentage of ring and rod bivalent were found to be 92.86% and 7.14%, respectively. The percentage of terminal and interstitial chiasmata were 99.96% and 0.04%, respectively. Mean chiasma frequency per cell and per bivalent were found to be 40.52 and 1.93, respectively (Table 2).

Saurav: The percentage of ring and rod bivalent were found 92.46% and 7.54%, respectively. The percentage of terminal and interstitial chiasmata were found 99.96% and 0.04%, respectively. Mean chiasma frequency per cell and per bivalent were found to be 40.43 and 1.93, respectively (Table 2).

Seri-82: The percentage of ring bivalent, rod bivalent, univalent, trivalent and quadrivalent were found to be 93.26%, 6.50%, 0.040%, 0.040% and 0.16%, respectively. The percentage of terminal and interstitial chiasmata were found 99.96% and 0.04%, respectively. Mean chiasma frequency per cell and per bivalent were found to be 40.48 and 1.93, respectively (Table 2).

Sonalika: The percentage of ring bivalent and rod bivalent were found 93.80% and 6.20%, respectively. The percentage of terminal and interstitial chiasmata were found 99.95% and 0.05%, respectively. Mean chiasma frequency per cell and per bivalent were found to be 40.72 and 1.94, respectively (Table 2).

Sonora-64: The percentage of ring bivalent, rod bivalent, univalent, trivalent and quadrivalent were found to be 92.30%, 7.39%, 0.08%, 0.08% and 0.15%, respectively. The percentage of terminal and interstitial chiasmata were 99.94% and 0.06%, respectively. Mean chiasma frequency per cell and per bivalent were found to be 40.28 and 1.92, respectively (Table 2).

Table-1. Name, pedigree, source and type of different varieties of hexaploid wheat (*Triticum aestivum* L.)

Sl.	Varieties	Pedigree	Source
1.	Agrahani	Innia/3/Son64/P4160-E//Son64 PK6841-2A-1A-OA	Regional Wheat Research Station, Shyampur, Rajshahi
2.	Akbar	Ron/TOB's CM7705-3M-1Y-2M-2Y-0Y-OJO	
3.	Ananda	Kal/BB CM26992-30M-300Y-300M-500M-0Y-OJA	
4.	Balaka	PI 's'/HD 845 HD1981-100JA-OI	
5.	Barkat	Jun's#BB/GLL/CARP/3/PVN 's'-CM33483-C7M	
6.	Gaurav	TURACO/CH 122	
7.	Innia-66	LR64/Son64 1119008-83M-100Y-100M-100Y-100C	
8.	Kanchan	UP301/C 306 1187-I-IP-5P-55A-OJO	
9.	Kallayansona	PJ 's'×GB 55 II8165	
10.	Kheri	Primitive	
11.	Pavon-76	VCM/CNO 's'/7C3/Kal/ BB CM8399-D-4M-3Y-1M-1Y-1M-OY	
12.	Protiva	KUH Selection (LPSW-151)	
13.	Satabdi	NAC/VEE # 5	
14.	Saurav	NAC/VEE#5	
15.	Seri-82	VEERY#5 's' KVZ/BUHOY//Kal/BB	
16.	Sonalika	1154-388/An/3/YT54 NI OB/ LR64//18427-4R-1M	
17.	Sonora-64	YT 54/NI OB//2Y54 118469-2Y-6C-4C-2Y-1C	

Table 2. Chromosome association and chiasma frequency in seventeen wheat (*Triticum aestivum* L.) varieties

Varieties	Total cell studied	No. of bivalent		Uni-valent	Tri-valent	Qiadro-valent	% of chromosome association					No. of chiasmata			% of chiasmata		Chiasmata	
		Ring	Rod				Ring bivalent	Rod bivalent	Uni-valent	Tri-valent	Quadro-valent	Terminal	Inter-stitial	Total	Terminal	Inter-statial	Per cell	Per bivalent
Agrahani	110	2142	144	2	2	4	93.37	6.38	0.09	0.09	0.17	4450	2	4452	99.96	0.04	40.47	1.93
Akbar	108	2133	135	-	-	-	94.05	5.95	-	-	-	4401	5	4406	99.89	0.11	40.80	1.94
Ananda	115	2265	150	-	-	-	93.79	6.21	-	-	-	4680	4	4684	99.91	0.09	40.73	1.94
Balaka	100	1975	125	-	-	-	94.05	5.95	-	-	-	4075	4	4079	99.90	0.10	40.79	1.94
Barkat	105	2082	123	-	-	-	94.42	5.58	-	-	-	4287	4	4291	99.91	0.09	40.87	1.95
Gaurav	112	2187	165	-	-	-	92.98	7.02	-	-	-	4539	3	4542	99.93	0.07	40.55	1.93
Innia-66	120	2343	178	-	-	-	92.94	7.06	-	-	-	4862	2	4864	99.96	0.04	40.53	1.93
Kanchan	102	2001	141	-	-	-	93.42	6.58	-	-	-	4143	6	4149	99.86	0.14	40.68	1.94
Kallayansona	125	2437	188	-	-	-	92.84	7.16	-	-	-	5062	3	5065	99.94	0.06	40.52	1.93
Kheri	122	2376	166	2	2	3	93.21	6.51	0.08	0.08	0.12	4936	2	4938	99.96	0.04	40.48	1.93
Pavon-76	114	2213	157	3	3	3	93.02	6.59	0.13	0.13	0.13	4604	2	4606	99.96	0.04	40.40	1.92
Protiva	130	2531	199	-	-	-	92.71	7.29	-	-	-	5261	2	5263	99.96	0.04	40.48	1.93
Satabdi	118	2301	177	-	-	-	92.86	7.14	-	-	-	4779	2	4781	99.96	0.04	40.52	1.93
Saurav	125	2427	198	-	-	-	92.46	7.54	-	-	-	5052	2	5054	99.96	0.04	40.43	1.93
Seri-82	120	2337	163	1	1	4	93.26	6.50	0.04	0.04	0.16	4856	2	4858	99.96	0.04	40.98	1.93
Sonalika	106	2088	138	-	-	-	93.80	6.20	-	-	-	4314	2	4316	99.95	0.05	40.72	1.94
Sonora-64	127	2447	196	2	2	4	92.30	7.39	0.08	0.08	0.15	5112	3	5115	99.94	0.06	40.28	1.92

DISCUSSION

Genetic variability of a population results from the action of a series of genetical controlled factors that are also under the action of natural selections. Particularly significant among the factors is crossing over which involves chromatid exchange between homologous chromosomes with consequent intra-chromosomal recombination, giving rise to new combinations of alleles (Baptista et al., 2000).

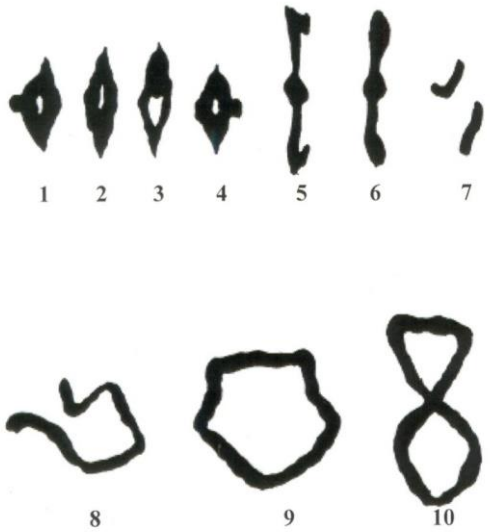

Figure 1. Showing possible meiotic configuration at diakinesis or metaphase-I (1-4. Ring bivalent; 5-6. Open bivalent; 7 univalent pair; 8-10 quadrivalent.

The chromosome association and chiasma frequencies in seventeen varieties of *T. aestivum* were determined at diakinesis and metaphase-I. Wilson and Morrison (1953) stated that constriction is near maximum and the chromosome pairs are well spread throughout the cell as through by mutual repulsion and pairs of homologous chromosomes are still held together at chiasmata but are elsewhere separated. The existence of structural differences in hexaploid wheat and their possible role in gene evolution have been given considerable attention following the works of Vega and Lacadena (1982, 1983). Kato and Yamageta (1982) reported that low temperature decreased pairing and the numbers of chiasmata were dependent on the genetic makeup of the plant. Therefore, the temperature reduced regular chromosomal behaviour at meiosis by restricting pairing and chiasmata formation with the segments. They also reported the influence of genotype-environmental interaction on chiasma frequency in plants where no structural change was involved. In present study paired chromosome at diakinesis were found to be held together finely at chiasmata. In metaphase-I, chromosomes were found to observe with maximum contraction and well spread in the cells. Thus, the data on chromosome association and chiasma frequency were recorded from diakinesis as well as from metaphase-I.

In the present investigation the frequencies of bivalent in different varieties were varied. However, the variations in the percentage of ring and rod bivalents were observed among different varieties. A few numbers of univalent, trivalent and quadrivalents were occurred in the present study. Occurrence of univalents, trivalents and quadrivalents was very low and thus, they coursed low frequency of the desynapsis in the bivalent. It indicated that the increase in frequency of univalents may also result from precocious chiasma terminalization because chiasma/cell at diakinesis may be high and most bivalents presents two chismata. The variation of percentage of ring and rod bivalent was perhaps due to inconsistency in the configuration of bivalents which might have been produced as a result of homogenesity in the chromosome complement of different varieties. Among the cultivated varieties of *T. aestivum*, most of the long chromosomes were found to form ring bivalents. Very low frequencies of rod bivalents were observed. Patil (1968) also found the similar results in seven strains of *Triticum durum* and also found very high frequencies of ring bivalents with double chiasmata and very low frequencies of rod bivalents with single chiasma. Regular bivalent formation was observed in most of the cases. Univalents, trivalents and quadrivalents were observed in few cases. In the present study, percentage of terminal and interstitial chiasmata were also found. The terminal chiasmata was found to range from 99.86 to 99.96% and the interstitial chiasmata was found to range from 0.0.4 to 0.14%. Baptista et al. (2000) found analysis of variance to shows some heterogeneity among varieties ($P<0.05$) for interstitial and terminal chiasma frequency.

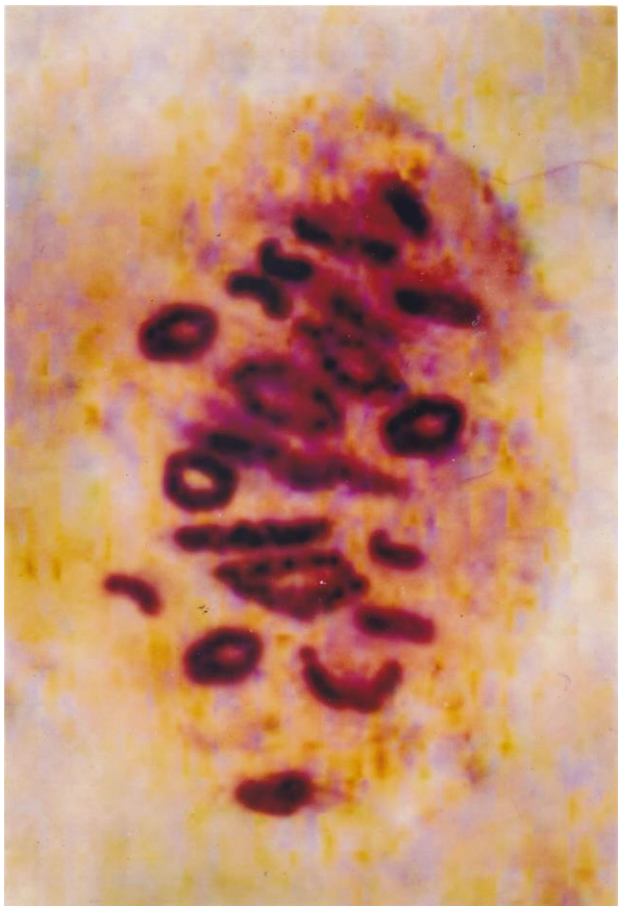

Figure 2. Representative chromosome configuration at diakinesis of prophase-1 in the pollen mother cell of *Triticum aestivum* L.

It has long been recognized that chiasma formation is under genotypic control (Rees 1961; Hazarica and Ress 1967). They also demonstrated that aging, temperature, centromere of chromosome and heterochromatin greatly affects the frequency of crossing over. However, three groups of factors that affect chromome pairing as well as chiasma formation were identified by Rees and Naylor et al. (1971) and those factors were firstly, the homology structural and chemical similarities between chromosomes; secondly, the genetic factors and thirdly, the cellular environment during meiosis, which is also influenced by the external environment.

The chiasma formation reflects similarities both in genetic content and in the arrangement of genes. Chromosome pairing at metaphase-I in intercultivar hybrids of wheat is less regular than their corresponding parents, resulting in a higher frequency of cells with univalents and a lesser number of chiasma per cell. The present findings reveal the chiasma frequencies per cell and per bivalent were slightly varied among the all varieties. The chiasma frequency per cell was found range from 40.28 to 40.87 and per bivalent was found to range from 1.92 to 1.95.

The results indicated their closeness in the range of primary gene pool. Thus, it may be expected that genes from useful varieties may be incorporated to others using conventional breeding technique.

CONCLUSIONS

Chiasma distribution and its frequency in seventeen varieties of wheat commonly cultivated in Bangladesh were studied from pollen mother cells (PMCs). Data on chromosome association and chiasama frequency were recorded from diakinesis and as well as from metaphase-I of meiotic cell division. In present study it was found that percentage of ring bivalent were extensively more than rod bivalent in all the varieties. Mean chiasma frequency per cell and per bivalent were found to vary slightly in all the varieties which ranged from 40.28 to 40.87 and 1.92 to 1.95 in Sonora-64 and Barkat, respectively. From the above observation, it can be stated that chiasma distribution and its frequency varied marginally in seventeen commonly cultivated wheat varieties of Bangladesh indicated that they are closely related to their primary genepool.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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