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Cytogenetics of Triticale: Chromosomal Anomalies And Intergeneric Chromosome Combination

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CYTOGENETICS OF TRITICALE: CHROMOSOMAL ANOMALIES AND INTERGENERIC CHROMOSOME COMBINATION

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This dissertation is
Dedicated
To
My Beloved Husband
Sumsir Jang Elahi Haider
And
My Two Sons
Masuk Elahi Haider
&
Marjuk Elahi Haider

University of Rajshahi

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CERTIFICATE

I have the pleasure in certifying the thesis entitled **"Cytogenetics of Triticale: Chromosomal Anomalies and Intergeneric Chromosome Combination"** Submitted by **Mst. Shahana Huq** to the University of Rajshahi for the degree of Doctor of Philosophy in Botany.

I do hereby certify that /) the candidate has fulfilled the residential requirement. //) the works embodied in the thesis were carried out by the candidate and ///) to the best of my knowledge the data are genuine and original. No part of work has been submitted in substance for any degree.

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The Authoress

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ABSTRACT

Three triticale lines and two *Triticum* species were used for the present study. The triticale lines are triticale BAT-1 (2n=56), triticale BAT-2 (2n=56), and triticale WRF (2n=42) and two *Triticum* species are *Triticum aestivum* (2n=42) and *Triticum durum* (2n=28).

Objective of this study was to determine the chromosome combination in triticale derives from the respective parents *Secale cereale* and *Triticum* species in addition to find out the influence of meiotic anomalies in semisterility of triticale lines. In present study, that has been determined following some important cytological events.

Interphase nuclear phenotype of triticale lines and *Triticum* species were studied. Nuclear volume (NV) and Interphase nuclear volume (ICV) were found to be statistically different and higher than that of *Triticum* species which are their one of the parents.

The homogeneity of the distribution of chromosomal morphology in three cells of triticale BAT-1, triticale BAT-2, triticale WRF, *Triticum aestivum* and *Triticum durum* were tested by the use of contingency table in corporating chromosome length and arm ratio classes. Total haploid complement lengths and chromosome distribution of triticale and *Triticum* species were determined. Highest CV% was 2.18 in triticale BAT-1 and the lowest value was 0.612 in *Triticum aestivum*. Other CV% values were 0.96 (triticale BAT-2) 1.94 (triticale WRF) and 1.36 (*Triticum durum*). χ^2-values were also determined, where highest value was 10.081 in triticale BAT-1 and lowest value was 0.044 in triticale BAT-2. Other χ^2 values were 0.13 (triticale WRF), 16.60 (*Triticum aestivum*) and 0.095 (*Triticum durum*).

Chromosome association and chaisma frequency in different genotypes in the present investigation were found to vary. Frequency of normal bivalent was very low and that of univalent was very high in all triticale lines. Trivalent and quadrivalent were also found in triticale. *Triticum durum* was found to show mainly quadrivalent. *Triticum aestivum* normally exhibited bivalent. The different meiotic irregularities were found mostly in triticale lines. Laggard, fragment, bridges and unequal distribution of chromosome were found frequently in all triticale lines but rarely in *Triticum* species.

Pollen grain abnormality and pollen sterility were also observed in all triticale lines. In triticale lines pollen sterility were found to vary from be 30.25% (triticale BAT-1) to 37.80% (triticale BAT-2). The highest percentage for pollen gram in abnormality was 40.00 (triticale BAT-2) and lowest was 37.25 (triticale BAT-1).

Several crosses were made among triticale lines and *Triticum* species reciprocally, but the results were found to be very poor. The F_1s of the crosses were not found to survive.

However, from crossing program and karotypic study it may be said that triticale lines are semisterile. The chromosomes of *Secale cereale* and *Triticum* species are present in triticale, but they cannot combine during meiosis and thus normal gametes are not formed.

INTRODUCTION

Cytogenetical studies in triticale are of great interest because of its partial sterile nature. It is an amphidiploid hybrid bearing the genome of wheat (*Triticum* sp.) and rye (*Secale cereale* sp.). Triticale according to fine definition was first bred by Rimpau in 1888. More than 45 years later, Landscheu and Ochler (1935) and Muntzing (1935, 1936) reported somatic cells of triticale with 56 chromosomes.

Chromosome number in triticale should be 56, twice the total gametic sum of parents. The cells at meiosis would have 28 pairs and each gamete would contain 28 different chromosomes of which 21 would be from wheat and 7 would be from rye. But this is not perfect and univalents do occur in meiosis of this allopolyploid. The number is found to be different for different triticale lines and thus, the gametes after meiosis might have chance to show anomalous chromosome number. Most of the male gametes that function are euploid or nearly so, owing to the strong selection in favour of euploid pollen, but on the contrary the female gametes can function with much anomaly and are not subject to competition. Therefore, the numbers other than 56 are very much possible and may be found frequently.

Univalent may be a source of anomalies other than that which is simply numerical. Example of such anomalies are the duplication and deficiency of different chromosomes. These two types of anomalies may occur in triticale by the occurrence of quadrivalents and trivalents, and also occurs as a result of irregular meiosis with univalent chromosome and there is possibility of iso-chromosome formation. Such division of univalents has been reported in many species as reported by Darlington (1940), Sears (1944) and O'Mara (1951). The allopolyploid is not regular meiotically and thus, the anomalies which occur at the first meiotic division after chromosome doubling before any misdivision products or other anomalous chromosomes could occur in the meiotic cells (O'Mara 1953).

By technical-methods Pieritz (1966, 1970) successfully to distinguished the morphological differences between wheat and rye chromosomes. As pointed out by Muntzing (1957) indicated by the fact that, certain strains or hybrid populations with many univalents at meiosis have a more or less pronounced tendency to revert to hexaploid wheat. In rare cases this tendency is to pronounce that the triticale constitution can only be upheld by a continuous selection typical triticale individual.

Muntzing (1957) studied the meiotic behaviour of F_1 hybrids between some hexaploid triticale. At diakinesis a regular and complete pairing of the homologous chromosomes indicated that the previous stages of meiosis had not the parental strains as well as the F_1 combinations. At first metaphase, on the contrary, chromosome pairing was much disturbed as indicated by the numerous univalents and rod bivalents. Meiosis in three hybrid combinations was more irregular than in the parents. Lelley (1974) stated that one of the possible reasons for failure of chromosome pairing in triticale is a genetically controlled decrease of chaisma formation leading to desynapsis. Whereas in wheat, regular bivalent formations regulated by a few specific genes and chromosome pairing in rye is controlled by polygenic systems. This is evident from investigations on the effects of inbreeding and out breeding in rye.

Proportion of rod bivalents (instead of ring bivalent) and univalents were found to be greatly increased in the parents and still more in the F_1 plants. This confirmed previous results of Skutina and Khvostova (1971) and Khvostova and Skutina (1975), who worked with octoploid as well as hexaploid rye material. Weimarck's observations (1976) also supported the conclusion of Skutina and Khvostova (1971) in the way that it is chiefly the rye chromosome that occurs as univalents or rod bivalents at first metaphase.

Rees and Thompson (1956) studied chaisma frequency in F_1 from dialled crosses between various inbred lines with different levels of chaisma frequency. From this work it was said that meiotic behavior in rye is controlled by nonallelic interaction of several loci. Meiotic disturbances in triticale become apparent by the occurrence of univalents at first metaphase. Muntzing (1957) had reasons to say that in octoploid triticale these univalents are predominantly rye chromosomes.

Based on the above description of the meiotic behavior of intergeneric hybrids, one might expect the wheat genome to be responsible for a lowered chaisma frequency and for univalency in triticale, however, in most of the cases it was found to be restricted to the rye genome (Lelley 1975a,b; Pohler *et al*., 1978).

Although bread wheat are superior in quality to rye, some rye varieties possess certain character such as winter hardiness, which would be very valuable if they could be transferred to wheat. A limited success in transferring rye characters to wheat has been achieved, but the manner of transmission is not definitely known. The high degree to sterility characteristic of wide crosses such as that between wheat and rye has not been overcome completely in the new strains possessing rye characters.

Tsuchiya (1975) stated that the cytological instability and development of shriveled seeds in triticale are serious obstacles against commercial use on a large scale. Merker (1974)

emphasizes that one of the goals for triticale improvement is reduction of meiotic disturbances. Several studies on triticale have been reported on relationship between meiotic abnormalities and fertility when plants of the same generation were compared (Merker, 1971; Weimarck, 1973). Among the reproductive abnormalities of triticale the incomplete chromosome pairing observed at first meiotic metaphase has been subject of several studies (Kaltsikes, 1974; Scoles and Kaltsikes, 1974; and Gustafson, 1976. Thomas and Kaltskies (1971) have shown that it is the rye chromosomes that do not pair, and that among the rye chromosomes those with heterochromatin at both telomeres fail to pair more often than those which have heterochromatin at one telomere only. Besides meiosis shows various irregularities, such as, the presence of univalent chromosomes, lagging chromosomes etc.

Triticale (*Triticosecale wittmarck*), a man made species where 'bread' or 'pasta' wheat and rye genomes are the constitutions, shows cytological disorders common in those organisms where evolution is not complete and consequently disturbance during the meiotic process may be responsible for a reduced fertility (De Ferreira, 1983), in reference to the number of seeds produced by a plants spike or spikelet. Because of this there remains a biological way to promote its evolution in order to improve meiotic and fertility performance in triticale. In this complex, one of the most studied meiotic irregularities is the presence of micronuclei in the tetrads (Suarez *et al*., 1987; Falcao *et al*., 1990; Manero De Zumelzu *et al*., 1995) and there several studies have demonstrated the existence of variability which may be useful for plant breeding purpose (Manero De Zumalezu *et al*., 1992, 1995, 1998; Ordonez *et al*., 1997).

In F_1 hybrids (AABBDR) from the crosses of hexaploid triticale (AABBRR) and hexaploid wheat (AABBDD), the expected chromosome association should be 14 bivalents plus 14 univalents, where the bivalents comprise the A-and B- genome chromosomes and the univalents represent seven rye chromosomes and seven D-genome chromosomes, respectively. Some of the univalents are eliminated and the others are transmitted to the following generation, together with the complete A- and B- genomes. Because of the predominant self- pollination of the plants descendants with different chromosome constitutions result, ranging from pure 6*x* triticale to common wheat.

Since the hybrids between wheat and rye are known to produce a certain proportion of unreduced ovules, and since the union of gametes having the same chromosome number generally gives a better result than the union of gametes with different chromosomes numbers, the primary wheat x rye hybrids are exposed to triticale pollen. The union of unreduced ovules with 24 chromosomes and reduced triticale pollen grains with the same chromosome number should give new triticale plants with 56 chromosomes. The hybrid

between *Triticum durum* Desf and *Secale cereale* was reported by Aase in 1930 and the derived allopolyploid by O'Mara in 1948. The hybrids which when doubled constitute the known triticale combinations exist with both tetraploid (*Triticum durum*) and the hexaploid (*Triticum aestivum*) varieties. The combination of *Triticum durum* and *Secale cereale* has been made much less frequently than that involving *Triticum aestivum*, owing to hybridization difficulties.

Since artificial crosses between 6*x* wheat and triticale are standard procedures in triticale breeding and allow to transfer useful genes from wheat into triticale, homologous substitutions can contribute to new variability and to karyotype reconstruction. Positive results with triticales derived from crosses between hexaploid triticale and bread wheat have been obtained by several workers as mentioned earlier.

Obviously a huge number of works have been done in the last 70-80 years on this intergeneric hybrid, and that have been used also in different crossing programmes with various objectives, but success rate is not yet upto the mark. Rather this hybrid has become more interesting because of its cytogenetical and breeding behaviour. And thus the present work is also a consequent upon many many research attributes of this hybrid.

The present investigation deals cytogenetics of triticale in terms of the following objectives.

1. Determination of chromosome combination in triticale derives from the parents *Secale cereale* and *Triticum aestivum* or *T. durum* by studying somatic complements,
2. Studies on their chromosome association and chaisma frequencies, and
3. Studies on chromosomal anomalies in pollen mother cells and pollen grain in triticale lines.

REVIEW OF LITERATURE

As it has been mentioned in the previous chapter of introduction regarding cytogenetical interest of triticales, many scientists such as **O'Mara (1953), Sears (1948), Krishnaswamy (1939), Berg and Oehler (1938), Muntzing (1939), Tsuchiya (1974), Weimarck (1973), Merker (1972), Krolow (1966), Thomas and Kaltsikes (1976), Riley and Chapman (1957), Vakar and Krot (1934), Gustafson and Zillinsky (1973), Heneen (1961), Lee *et al.* (1969), Florell (1931), O'Mara (1940), Bielig and Driscoll (1970) and Bennett and Kaltsikes (1973)** have significant contribution from the decade of thirty of twentieth century. Till-to-day this crop is being used for improving bread wheat quality along with some other important cytogenetical aspects. But due to the problem like chromosome pairing and consequently the occurrence of chromosomal anomalies makes the studies difficult and thus, the works for 30-40 years on triticale have been conducted with no such effective result. However, few important investigations from 1980 to the present decade of 21st century have been made and due to their relevancy to the present study these are reviewed here.

Bozhanova *et al*. (2014) reported about the hexaploid triticale, an amphidiploid, which was obtained from the cross between durum wheat (2n=28) and rye (2n=14). It was characterized with high protein content, resistance to biotic- (powdery mildew and rust) and abiotic (cold and drought) stress factors. The hybridization between durum wheat and triticale is complicated and is rarely applied in durum wheat breeding improvement. They reported the results of long-standing experiments for obtaining of interspecific hybrids between Bulgarian durum wheat genotypes as mother plants and triticale (2n=42) lines from CIMMYT, Mexico. Although the crossability between two species was relatively high, hybrid plants were obtained only by means of embryo rescue method, due to endosperm degeneration. All regenerated F_1 hybrids were with low fertility and produced seeds with reduced endosperm and low viability. The F_1 hybrids were backcrossed to the recurrent durum wheat genotypes. Strict and repeated selection of plants with durum wheat phenotype was performed in the segregation populations. Thirty-three SSRs mapped on the A, B and D genomes and chromosomes of common wheat were used for molecular characterization of one of the obtained advanced backcross lines.

Mirzaghaderi *et al.* (2011) described the short arm of rye (*Secale cereale* L. chromosome 1 (1RS), besides being part of the rye genome, is present in many hundred wheat cultivars as either 1RS. 1BL or 1RS.1DL wheat- rye translocation. The distribution

of the wheat-rye translocation was examined in 33 Iranian winter and spring wheat cultivars, nine of which had a known donor or 1RS.1Bl, translocation and the other 24 were randomly selected cultivars without a known source of 1RS, 1BL in their pedigree. The presence of the translocation was verified in 4 cultivars, using genome insitu hybridization analysis.

Naskidashvili (2009) found 32.1% setting of hybrid seeds in the case of using hexaploid triticale as a female parent and 66.8% in back crosses, but when wheat was used as a female parent under developed, heavily shriveled and practically nonviable grains were obtained. When crossing of triticale was made with wheat the process of grain development proceeds with insignificant deteriorations, hybrid grains were comparatively well filled and their germination capability in the field reached 84%. By productivity plants of the first generation lag behind parental forms and number of grains in the wheat ear varied from 27.5 to 44.0 and from 52.5 to 54.5 in triticale, while in hybrids this index fluctuated between 12.6 and 24.5.

Galindo and Jouve (1989) described meiosis in four primary hexaploid triticale lines in their component (two tetraploid wheat and two rye parents) and the hybrids obtained by crossing within which ploidiy level was studied using Giemsa c-banding. The individual chromosomes were identified and their meiotic behavior at first metaphase was analysed in each line. In each new triticale line, the level of pairing for wheat chromosomes was moderately reduced and for rye chromosomes was very significantly reduced, in comparison with that of the wheat and rye parents used to synthesize it. The pairing intensity observed suggests the presence of a strong negative intergenomic interaction between the rye and wheat genomes in triticale, irrespective of whether the rye is in a homologous or heterozygous genotypic condition. The homologous of heterozygosity in the wheat constituent does not appear to effect the behavior of the rye chromosomes in triticale.

Charmet *et al.* (1986) stated that a very large proportion of plants regenerated from anther culture of triticale F_1 hybrids did not show the euploid number of chromosomes. Of 408 androgenetic plants checked for their chromosome numbers, 228 were aneuploid, of which 39 had one or several telosomes. Two observations suggest that most of the chromosome variations were observed in the F_1 hybrids and would be caused by meiotic irregularities. On the one hand, majority of these phenomena involved R-genome chromosomes, which also give rise to meiotic univalents. On the other hand, the chromosome number and frequency distribution of the micrioscopes from a fairly asyndetic hybrid fits well with that of the androgenetic plants. Thus, aneuploidy and chromosome rearrangement do not implicate the *in vitro* technique itself.

Chaubey and Khanna (1986) described intergeneric hybrids between wheat and rye, which are utilized for the transfer of desirable rye characteristics into wheat and to increase the genetic variability in the amphiploid genus triticale (*Xtriticosecale wittmack*). However, the cross- incompatibility between most agronomically acceptable wheat cultivars and rye presents a serious limitation to their successful hybridization. Immediate success of triticale as a new cultivated cereal in agriculture is rather limited due to its low spike fertility and shrivelled grains. To overcome these problems triticales are crossed to the local wheat varieties or with rye varieties or different lines of triticales are inter crossed.

Bernard and Bernard (1985) examined two F_5 strains of tetraploid triticale (2n=4x=28), obtained from 6x triticale×2 rye progenies. They made crosses involving diploid and tetraploid rye, some durum and bread wheats, and various 8x and 6x triticale lines. Meiosis in the different hybrid combination was studied. The results showed that the haploid complement of these triticales consists of seven chromosomes from rye and seven chromosome from wheat. High frequencies of PMCs showing trivalents were observed in hybrids involving the reference genotype of wheat and triticale. These findings proved that several chromosomes from the wheat component have chromosome segments coming from two parental wheat chromosomes. The origin of these heterogeneous chromosomes probably lies in homoeologous pairing occurring at meiosis in the 6x triticale×2x rye hybrids from which 4x triticale lines were isolated. A comparison among different hybrids combinations indicated that the involvement of D-genome chromosomes in homoeologous pairing is quite limited. In contrast, meiotic patterns in 4x triticale×2x rye hybrids showed a quite high pairing frequency between some R chromosomes and their A and B homoeologous.

Jung *et al.* (1985) analyzed the cytological and morphological expression of interactions between wheat and rye genomes in hexaploid triticale lines that were developed using genetically pure lines of both parental species. The plant material studied by these authors mainly dealt staining technique, but the wheat and rye chromosomes were not individually analyzed.

Jung and Lelley (1985) reported about six primary triticale lines which were produced from two advanced breeding lines of *Triticum durum* and three inbred genotypes of *Secale cereale*. The wheat and rye parents and the triticale derivatives were crossed in all possible combinations within each species group. Chiasma and univalent frequency in parents and hybrids were determined. Primary triticale lines had more univalents and less chiasmata per pollen mother cell than the corresponding wheat and rye parents together.

The starting point in the chain of events concerning fertility is the meiotic division **(Maich*, et al.*, 1999)**. Thus, to improve the meiotic performance of triticale is a sure biological way to promote its evolution. Sixty F_2 – derived families were evaluated during 1997 and 1998, in order to measure the direct and indirect responses to selection for the improved percentage of normal tetrads, or meiotic index, in hexaploid triticale. A significant direct response to selection in respect to the meiotic index was observed, but not indirectly through spikelet fertility. However, they found that there is the possibility for selection at the end of the reproductive process (i.e. per spikelet fertility) without a negative effect on the percentage of normal tetrads.

Lelley (1985) stated that the genetic mechanism which influences the phenotype of triticale is probably the interaction between its wheat and rye components. triticales often suffer meiotic irregularities, sterility, and kernel shriveling, which can seriously affect their economic potential.

Orellana and Lacadena (1983) examined chromosome pairing in wheat- rye addition and substitution lines using the c-banding technique. It was found that both rye and wheat chromosomes affected each other's homologous pairing. The strongest diminution of wheat pairing (measured as bound arms per cell) was produced by chromosome 5R of rye (7.5 and 7.2% in Chinese spring- imperial' and holdfast – King II addition lines, respectively). The weakest diminution of wheat pairing was produced by chromosome 3R in the Chinese spring- Imperial addition line (1.1%). The diminution of rye chromosome pairing produced by wheat chromosomes ranged from 6.9 to 48.4% (Chinese spring – Imperial and holdfast – King II addition lines), respectively. When put into a heat background, the rye chromosomes suffer a worse fate than the wheat chromosomes. For example, chromosome 6R-reduced the wheat complement pairing in the holdfast – King II addition line by 3.8% but its own pairing is reduced by 41.4%. The decrease in pairing of both wheat and rye homologous chromosomes in addition and substitution line is a complex process in which factors such as genes controlling meiotic pairing, constitutive heterochromatin, and cryptic wheat – rye interactions can play important roles.

Gupta and Priyadarshan (1982) studied meiotic irregularities which are characteristic feature of triticale (Muntzing 1979; Scoles and Kaltsikes 1974). The parental lines and F_1 hybrids of triticale show increased meiotic instability. This finding was later confirmed by several authors (Merker 1971; Sopra and Heyhe 1973; Salmon *et al*., 1977; Pohler *et al*. 1978).

May and Appels (1982) reported that triticale (*Xtriticosecale wittmack*) are being employed as a source of rye (*Secale cereale*) chromatin for the introduction of specific

agronomic characters into wheat (*Triticum aestivum*). The rye chromosomes present in plants of the first and second generations of a backcrossing program have been identified using a radioactive in situ probe which hybridizes specific sites of the rye chromosomes. They showed that homologous pairs of rye chromosomes are present by the second generation which should be there by ensuring their eventual substitution. Furthermore, rye telomes and a wheat-rye chromosome translocation involving 5RL were also observed as possibly useful modifications of rye chromosomes in this breeding program.

Larter and Noda (1981) described three hexaploid (2n=6x=42) triticale lines (*Xtriticosecale wittmarck*) in which a specific chromosome of either the A or B genomes was replaced by a homologous chromosome of the D genome of wheat (*Triticum aestivum* L. em Thell.). Two of the substitutions involved the B genome and the third involved the A genome. **Gustafson (1981)** found the progenies of triticale×wheat hybrids, having a mean meiotic behavior 14II+14I, modified rye chromosomes, telosomes and isochromosomes as well as rye- rye and wheat- rye translocations. These variations are clearly the result of meiotic abnormalities in pollen mother cells of hybrids. **Tarnner (1981)** made intergeneric hybridization between six hexaploid wheat (*Triticum aestivum* L.) cultivars and five inbred rye (*Secale cereale*) lines to study the influence of parental genotypes upon chromosome doubling after colchicines treatment. Significant differences were attributed to independent effects of the wheat and rye parents. Self-fertility of the derived amphidiploids was positively correlated with colchicines responsiveness.

Tania *et al.* (1981) described meiotic behavior of four lines of hexaploid triticale (*Triticosecale*) in details. Types of frequency of meiotic abnormalities were comparable to that described in the literature. A high incidence of condensation disturbances was also observed. Two groups of three- way hybrids were produced by crossing F_1 hybrids of petkus prolific rye (2n=14) and prolific×puma rye (2n=14) on to Chinese spring wheat (2n=42). Meiosis was studied in 89 plants from 29 families from the first combination and in 36 plants from 11 families in the second cross. In three families from the first combination (petkus×profilic) five parental amphidiploids with chromosome numbers of 2n=35, 36, 37, 38 and 41 were identified. The mean bivalent frequencies in five hybrids were 6.71,7.73, 8.10, 9.94 and 13.00, suggesting that the number of bivalents was generally equal to the number of chromosomes in excess of expected chromosome number of 2n=28. These five plants were partial or incomplete amphidiploids and their origin was attributed to duplication of a portion of the wheat complement after fertilization.

Lelley and Larter (1980) described the influence of parental genotype on the chromosomal pairing relationships within the wheat and rye genomes of triticale (*Xtriticosecale wittmarck*). Nullisomic 3A/tetrasomic 3B and nullisomic 5B/tetrasomic 5D plants of the cultivar Chines spring wheat were pollinated with inbred strains of rye (*Secale cereale* L.), the latter being selected on the basis of their average chaisma frequency. The level of chaisma number in the F_1 hybrids was found to be influenced by the genotype of both the wheat and rye parents. Homologous pairing was found to be increased in the wheat x rye hybrids produced from inbred rye parents that exhibited high chaisma frequencies. However, this relationship was modified depending upon the genotype of the wheat parent. Increased dosage of chromosome 3B of wheat was found to increase chaisma frequency.

There may be few other research works on triticale in the last decade, but those are not exactly relevant to the present study. Contents of the present study are completely based on the title and thus, many works on triticale do not fit exactly to this study. However, many such irrelevant works on this hybrid plant have not been considered for review specifically.

MATERIALS AND METHODS

3.1 Materials: The experimental materials used in the present study were three lines of triticale (triticale BAT-1, BAT-2, WRF-7), and two species of *Triticum* viz: *Triticum aestivum*, *Triticum durum***.** An account of them are given bellow:

Table-1: An account of three triticale lines and two species of *Triticum*.

Species / lines	Ploidy level	Chromosome number	Source
triticale BAT-1	Octaploid	56	Wheat Research Center, Shaympur, Rajshahi, Bangladesh
triticale BAT-2	Octaploid	56	
triticale WRF-7	Hexaploid	42	
Triticum aestivum	Hexaploid	42	Cytogenetics Laboratory, Department of Botany, Rajshahi University, Bangladesh
Triticum durum	Tetraploid	28	

3.2 Methods: Fresh and dry seeds of the triticale and wheat species were sown in earthen pot during the growing season of 2008-2009 and 2009-2010. The plants were used for different experiments adopting the methods as mentioned below:

3.2.1 Interphase nuclear phenotype and Karyotype study: For this experiment the steps adopted were as follow:

3.2.1.1 Emergence of root tips: Fresh and dry seeds of three lines of triticale and of *Triticum aestivum* and *Triticum durum*, were allowed to germinate in petridishes on moist filter paper at room temperature (22°C-24°C) .

3.2.1.2 Fixation of root tips: Root tips of 1.0-1.5 cm length were collected in glass vials and were treated with saturated solution of paradichlorobenzene (1-4 dichlorobenzene or PDB) for 4-5 hours at 10°C. The saturated solution of PBD was prepared by dissolving 750mg PDB in 50ml distilled water and incubated over night at 60°C. The treated root tips were thoroughly washed with distilled water and fixed in 1:3 acetoalcohol for 48 hours at room temperature. Afterwards they were preserved in 70% ethanol and stored in refrigerator till used.

3.2.1.3 Staining of root tips: The preserved root tips were stained by 0.5% Haematoxylin following the method of Haque *et al*. (1976) with certain modifications.

i) The preserved root tips were washed thoroughly by distilled water for 5 minutes.

ii) After washing with distilled water the root tips were transferred 50% HCl (by dilution) for about 30minutes for dissolving the middle lamella of cells.

iii) The root tips were washed with distilled water for 8-10 minutes.

iv) Then the root tips were transferred in 2% iron alum (ferric ammonium sulphate) solution for 10-15 minutes.

v) Then the root tips were washed again with frequent change of distilled water for 8-10 minutes.

vi) The root tips were then stained with 0.5% haematoxylin for about 10-15 minutes and washed again with distilled water for 5-7minutes.

3.2.1.4 Preparation of slides: The meristematic zone of the stained root tips were squashed in 0.5% acetocarmine on a clean slide and the meristematic cells were covered with a cover glass, then heat-cool and pressures technique was applied until all the chromosome in the cells were scattered in all directions.

3.2.1.5 Photomicrography and observation: Prepared temporary slides for nuclear phenotype and the karyotypic study were examined under a compound microscope with 40×15 magnification. Photomicrographs were taken from the desired preparations for studying nuclear phenotype and chromosomes were measured from photomicrographs based on the times of magnification. The recorded values were then converted in micrometer (µm).

3.2.1.6 Interphase chromosome volume: In order to determine the interphase nuclear volume from root tip cells, nuclear volume was measured by oculometer and the values were converted into micron (µ) with the help of a stage micrometer. The nuclear volume (NV) was calculated using the formula of sphere, $NV=4/3\pi r^3$ (Nayer *et al*. 1971). The mean nuclear volume divided by the somatic chromosome number gave the Interphase chromosome volume (ICV) as mentioned bellow:

$$ICV = \frac{3/4\,\pi r^3}{Somatic\,chromosome\,number}$$

3.2.1.7 Classification of chromosome: The chromosomes were classified primarily based on arm ratios according to Kutarekar and Wanjari (1983). Arm ratios per haploid set were determined according to position of centromere and were calculated adopting the following formula:

$$Arm\ Ratio = \frac{Short\ Arm\ Length\,(SA)}{Long\ Arm\ Length\,(LA)}$$

The chromosomes having the ratios 0.76 and above were termed as metacentric (m), 0.51 to 0.75 as submetacentric (Sb), and less than 0.51 as subterminal (St) chromosome. The length classes of chromosome which were indicated by the symbol 'X' were determined by comparison between longest and shortest chromosome length and all the chromosomes for particular species were classified as:

i) 6.84 and above=large chromosomes (L)

ii) $5.69 \leq \times \leq 6.28$=medium chromosome(M)

iii) $5.13 \leq \times \leq 6.62$=relatively short chromosome (S_1)

iv) 5.14and less= short chromosome(S_2)

3.2.1.8 Analysis of data: Data were analyzed on the basis of following three conceptual events and standard quantitative karyotypes were established:

1. Since the morphology of chromosome were altered by differential construction, the mean length and arm ratio of similar cytologically processed cells provide the best estimate of a 'standard morphology' of chromosome.

2. In a two dimensional scatter diagram of chromosome length and arm ratio of all chromosome per cell, the points representing the same chromosome tened to cluster on a graph.

3. Two homologous chromosomes become individually recognizable by the mean location of chromosomes occurred not less than one standard deviation away from that of the other. When such a difference did not exist, these two chromosomes could not be distinguished individually, unless particular marker feature (such as satellite) existed on one of them. The indistinguishable chromosome could be assigned to different morphological categories on a probabilistic basis. A standard chromosome morphology was developed following three steps:

i) A simple scatter diagram was produced for all chromosomes of each cells by using of which the diploid and the mean values of each chromosome pair were assumed to be haploid complements.

ii) A combined scatter diagram of the haploid complements of all the cells were constructed to establish a standard morphology of chromosome, which could be identified.

iii) These identified chromosomes were characterized individually through the probabilistic inference.

3.2.2 Scatter diagram: Chromosome morphology was studied in each of three cells, selected on the basis of similar degree of contraction of the chromosomes. For the analysis of quantitative karyotype scatter diagram was prepared following the method proposed by Ahmed *et al*. (1983).

Briefly a scatter diagram of the arm ratios and lengths of chromosome in each cell was used to determine homologous pairs of chromosomes and haploid values of the genome.

3.2.2.1 Derivation of the haploid values: Each chromosome and its corresponding point on the diagram was numbered arbitrarily, then the chromosomes were paired by circling the corresponding two points on the basis of their proximity. The different pairs of points were considered as homologous chromosomes. Averages of the length and arm ratios of each pair of chromosomes constituted the haploid complement of the cell. The process was repeated for each of the three cells under study. Chromosome pairs were then numbered from 1-14 for tetraploid, 1-21 for hexaploid and 1-28 for octaploid.

The degree of similarity of distribution of chromosomes among different haploid complements was tested by using contingency table. The non-significant (χ^2) values indicated that observed cells were homologous for the frequency of haploid length and arm ratio classes. But the significant (χ^2) values indicated the heterogeneity of cells and proved that the chromosomes of those cells were morphologically dissimilar in general.

3.2.2.2 Standardization of chromosome: It was necessary to standardize the length of chromosome of different three cells because, the total haploid length obtained in three cells exhibited a small differentiation. The haploid length from each chromosome was standardized using the following formula:

$$x'ij = \varepsilon xij \times \frac{\varepsilon(xi + xii + xiii)}{3}$$

Where, Xij=unstandardized length of the chromosome in its cell.

X’ij= standardized of jth chromosome in its cell.

Xi= haploid total length of ith cell.

i= no of cells (1-3)

j= no of haploid chromosomes for each species as for example (1-7) in diploid, (1-14) in tetraploid and (1-21) in hexaploid species/ genotypes, respectively.

Following this modification, each chromosome complement was found to have equal haploid total length.

3.2.2.3 Identification of chromosome: Next, corresponding chromosomes in different compliment were determined through a technique applied to the combined scatter diagram on graphs for the three haploid values of arm ratio and the standardize haploid values of chromosome length.

Each point in a scatter diagram represented a specific chromosome in a particular haploid compliment. Symbol using in the diagram referred to specific chromosome in particular haploid compliment i.e. three different symbols referred to alphabetically A, B, C for the studied three cells. The three points representing the haploid homologous of each chromosome should cluster closely and such cluster must contain one point from each studied cells (A, B, C). In reality, clear groups were existed for only some sets of three points. Some groups were distinct but somewhat defused and few other groups were overlapped, because of the occurrence of an outlying point. All clear groups fall in the category of individually identifiable ones. It was applicable in case of parent in present study.

For each of the identified groups (chromosomes) of different cells, the mean ($\bar{x}$) standard error (SE) and coefficient of variation in percentage (CV%) were determined for length and arm ratio using the diploid values.

3.2.2.4 Allocation of unidentified chromosome: All chromosomes in three haploid compliments were classified in different morphological categories on the basis of total and length arm ratio within the length classes.

The class interval (1.5) for length was chosen arbitrarily and the ranges for arm ratio as recommended by Kutarekar and Wanjari (1983) were used. This classification was superimposed on the scatter diagram of the haploid compliments as a grid of length and arm ratio classes. Standard length in vertical displacement of the points in the combined scatter diagram. However, the mean of groups of identified chromosomes in scatter diagram did not change a result of standardization. The unidentified chromosomes were distributed to the various morphological classes using probabilistic inferences on:

i) The frequency of chromosomes in a given cells per haploid set,

ii) Occurrence of points in the combined scatter diagram, and

iii) The examination of the original total length and arm ratio of the chromosomes.

The number of unidentified chromosome were allocated to the various morphological classes and counted. All 14, 21 and 28 haploid chromosome compliments were numbered in decreasing order of total length and increasing order of arm ratio within each length classes following the convention of Rhoades (1955). These numbers were used as the identification of each chromosome.

3.2.2.5 Centromeric formula: Centromeric formula was prepared for each species based on its arm ratio.

3.2.2.6 Proposed standard karyotypce: Finally the standard karyotype was derived based on centromeric formula and range of chromatin length of chromosome.

3.2.2.7 Ideogram: Ideogram was prepared from both identified and unidentified chromosomes .Total haploid complements of chromosome of each parental species were considered as diploid complement pointing the long arms upward. In case of hybrid ideogram for identified chromosomes were prepared only.

3.2.3 Crossing programme: For three successive years (2008-09-, 2009-10 and 2010-11) crossing programme was carried out between triticale lines and *Triticum* species. Attempts were made indifferent way to find the intergenic hybrids. All these were made conventionally.

3.2.4 Chromosome association and chaisma frequency: To determine the chromosome association and chiasma frequency the schedules mentioned below were adopted.

3.2.4.1 Collection and preservation of PMC: During the proper growth of the plants (parents and hybrid) young inflorescences were collected between 7.30-9.30 am and immediately fixed in carnoy's fixative (ethanol: 3 chloroform: 1acetic acid). After 48 hours of fixation the inflorescences were transferred to 70% ethyl alcohol and stored in the refrigerator for meiotic study.

3.2.4.2 Preparation of slides: To record the data on meiotic behavior temporary slides were prepared by acetocarmine smear technique as follows:

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

b) The anther wall was then crushed by a curved dissecting needle and the anther wall was removed.

Then the pollen mother cells (PMCs) were covered with a cover glass. Warmed gently over a alcohol flame and a light pressure was exerted by thumb or finger tip to spread out

the chromosomes. Additional heating was applied as needed until the cytoplasm becomes clear.

3.2.4.3 Observation and photomicrography: Temporary slides were examined under compound microscope and the photomicrographs were taken from desired preparations.

3.2.4.4 Recording of data: Data on chromosome association and chiasma frequency was recorded from the temporary prepared slide. Chromosome configurations during diakinesis were used to determine the chromosome pairing and the number of chiasmata. The numbers of bivalent were counted from 30 PMCs of each hybrid and their parents.

3.2.4.5 Technique of analysis: From the recorded data chiasmata per PMC, chiasmata per bivalent and percentage of univalent, bivalent (rod+ring), trivalents and quadrivalent (if present) were calculated by conventional method.

3.2.5 Meiotic irregularity: Data on meiotic irregularity was recorded from the same slides prepared for determining the chromosome association and chiasma frequency. However, the chromosomes irregularities were observed mainly from metaphase-I to anaphase-II stages. Photomicrographs were prepared from the desired slides.

3.2.6 Pollen grain cytology: For studying pollen grain cytology the procedure proposed by Jagathesan and Sreenivasah (1966) was followed with slight modification in the present investigation as follows:

3.2.6.1 Collection of anther: Ripe anthers were collected in a small vial at the time of anthesis and were treated with 0.2% colchicines solution for 1 hour at 26-28^{0}C temperatures. Then the anthers were washed in distilled water for 2-4 minutes and these were treated with a solution of 0.002M hydroxyquinoline for 1 hour at 26-28^{0}C temperature. They washed again with distilled water for 10 minutes.

3.2.6.2 Fixation of anther: Anthers were fixed in Ostergreen and Heneen's (1962) solution (methanol 60ml + chloroform 30ml + distilled water 20ml + picric acid 1gm + mercuric chloride 1gm) for 24 hours. Afterwards, the anthers were stored for a period up to 6-8 weeks in 70% methanol in refrigerator.

3.2.6.3 Staining of anther: The anthers were stained following the schedule as mentioned below:

i) The anther were hydrated in 60%, 50%, 40%, and 35% methanol consecutively and washed by distilled water.

ii) The anthers were hydrolyses in 1N HCL for 20 minutes and then washed thoroughly in distilled water

iii) Then the pollen grains were stained with leuco-basic fuchin (Schiff's reagent) for 30-60 minutes.

iv) After washing, the anthers were placed on a clean slide, a drop of 2% acetocarmine was added, the anther was crashed with dissecting needle and the debris was removed.

v) A cover glass was placed the pollen grains and gently tapped by keeping slide between folds of blotting paper and uniform pressure was applied

vi) Then the slides were placed under a compound microscope for observation.

3.2.6.4 Data recording: Data on normal and abnormal pollen grains in each of studied materials were recorded. Abnormal pollen grains, i.e. mononucleate, binucleate (different nuclear shape), trinucleate and tetranucleate were observed and their frequency were determined.

3.2.7 Study of pollen grain sterility: For studying pollen grain sterility spikelets containing ripe yellow anthers were collected at the time of flowering and they were preserved in 70% ethylalcohol and kept in a refrigerator till used.

3.2.7.1 Staining of pollen grain and preparation of slides: For the study of pollen sterility only mature anthers of selected lines/species were washed in distilled water for 5 minutes. The anthers were smeared in a drop of 2% acetocermine/potassium iodide solution followed by gentle heating and cooling over an alcohol frame. A cover glass was placed over the material after removing the debris and a slight pressure was applied with thumb or fingertip to spread out the pollen grains. Photographs were taken from the desired preparation.

3.2.7.2 Recording of data: Prepared slides were examined under microscope and numbers of fertile and sterile pollen grains were counted and their percentage were determined.

3.2.8 Analysis of meiotic pairing: Pollen mother cells of *Triticum* species, triticale lines and their two hybrids (F_1 of *T. aestivum*×triticale BAT-1, and (F_1 of *T. durum*×triticale WRF) were studied following the preservation and staining methods as described earlier. The recorded data were then compared with the data of *Secale cereale* published by Orellana *et al.* (1984). Mainly the behaviour of univalent were studied.

RESULTS

The results obtained in this investigation are presented under the following heads:

4.1 Interphase nuclear phenotype.

4.2 Intergeneric chromosome combination.

4.3 Analysis of crossing program.

4.4 Chromosome combination studies through chromosome association and chiasma frequencies.

4.5 Chromosomal anomalies in pollen mother cells of triticale lines and *Triticum* species.

4.6 Analysis of meiotic pairing.

4.7 Univalency in hybrid lines of *Triticum* species and triticale.

4.1. Interphase nuclear phenotype

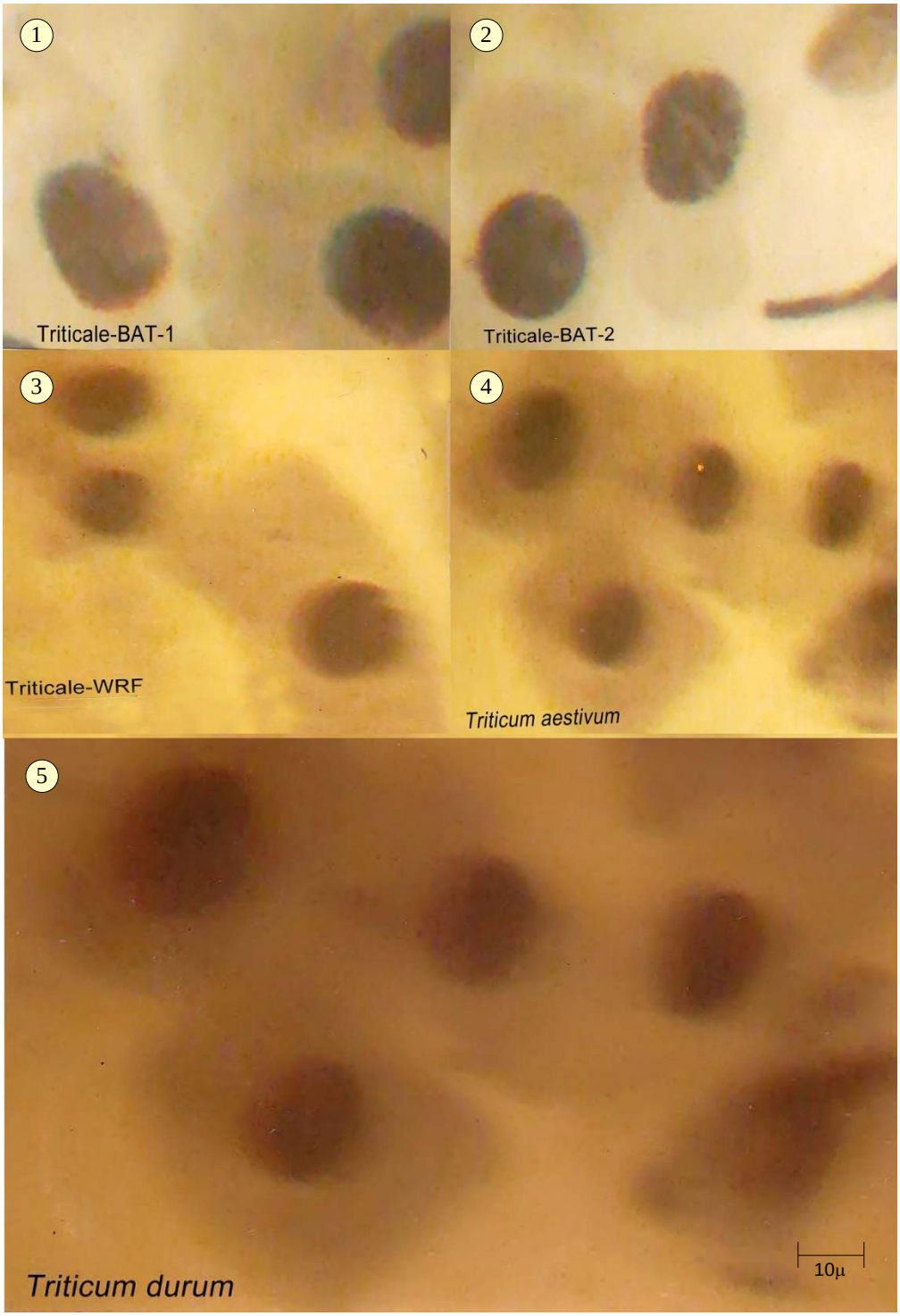
Interphase nuclei in different lines of triticale and *Triticum* species are shown in Figs. 1-5. Nuclear volume (NV) and Interphase nuclear volume (ICV) were determined and the results are given in Table 1.

Table 1. Nuclear volume (NV) and interphase chromosome volume (ICV) in different triticale lines and *Triticum* species.

Name of triticale lines and *Triticum* species	Chromosome number	Nuclear volume NV=4/3πr³ (μ³)		Interphase chromosome volume (ICV) (μ³)	
		$\bar{x}$	SD±SE	$\bar{x}$	SD±SE
triticale BAT-1	56	33.17	10.51±2.102	0.59	0.18±0.036
triticale BAT-2	56	35.99	16.11±3.22	0.64	0.26±0.052
triticale-WRF	42	26.89	11.71±2.34	0.64	0.28±0.06
Triticum aestivum	42	30.74	11.39±2.28	0.73	0.27±0.054
Triticum durum	28	25.12	12.98±2.60	0.90	0.46±0.092

4.1.1. Nuclear volume (NV)

The nuclear volume in three different lines of triticale ranged from 26.89 (triticale WRF) to 35.99 (triticale BAT-2). This was found to be 30.74 in *Triticum aestivum* and 25.12 in *Triticum durum*. It reveals that the values for nuclear volume in triticale BAT-2 is higher than triticale BAT-1 and triticale WRF. On the other hand it was found that all triticale lines were higher for NV than that of *Triticum* species.



Figs. 1-5: Interphase nuclear phenotype of triticale BAT-1, triticale BAT-2, triticale WRF, *Triticum aestivum*, *Triticum durum.*

4.1.2. Interphase chromosome volume (ICV)

The interphase chromosome volume (ICV) in different triticale lines and *Triticum* species varied differentially. The value 0.90µm (*Triticum durum*) was higher than that of all lines of triticale and *Triticum* species. The value 0.59 (triticale BAT-1) was lower than that of other triticale lines and *Triticum* species. The table also reveals that mean values for interphase chromosome volume (ICV) of different triticale lines were lower than both the *Triticum* species.

4.2. Intergeneric chromosome combination

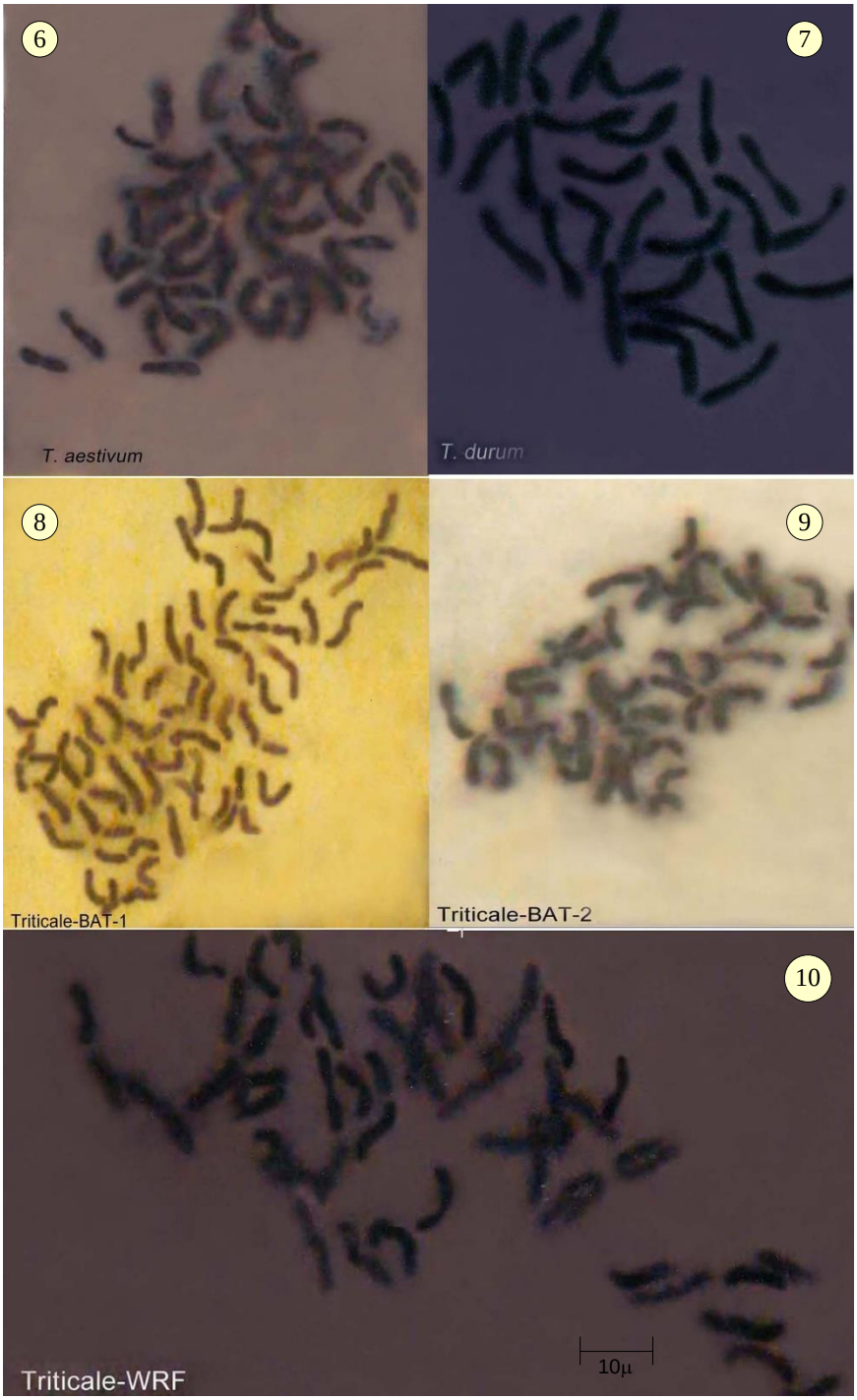
To identify the chromosomes through somatic karyotype, root tips of all the triticale and *Triticum* species were collected. Root tips of 1.5cm length were appropriate for obtaining maximum number of metaphase plates. At least three well spread metaphase plates were considered for this investigation. Somatic chromosomes were measured from photomicrographs. For making quantitative karyotype analysis the method proposed by Ahmed et.al. (1983) was adopted on the basis of scatter diagram of total chromatin lengths (TCL) and arm ratios (AR) of all chromosomes in a number of cells. The cells with well spread metaphase chromosomes having more or less distinct morphology are presented (Fig. 6-10).

The chromosome numbers for octoploid triticale BAT-1 and triticale BAT-2 were found to be 2n=56. The chromosome number of hexaploid triticale WRF was found to be 2n=42. For hexaploid *Triticum aestivum* it was found to be 2n=42, and that for tetraploid *Triticum durum* it was 2n=28. Chromosome morphology was determined quantitatively, and considerable points are described below for this study.

4.2.1. Chromosome morphology

Measurement for the lengths and ratios of representative complement of root tip chromosomes in three lines of triticale and two lines of *Triticum* species are described.

Data were taken at the desirable stage for each of the three cells and were plotted in separate scatter diagrams and combined scattered diagrams. In triticale BAT-1 (Figs. 11-12) pairs of adjacent points were considered to represent homologous chromosome and were circled on the separate scatter diagram and combined scatter diagram.



Figs. 6-10: Metaphase chromosomes in *Triticum aestivum*, *Triticum durum* and three triticale lines.

The average values of total length and arm ratio were calculated constituting the haploid complement of that cell. Then the chromosomes of haploid complements were numbered in decreasing order of total chromatin length and increasing order of arm ratio with in the same length.

The uniformity of the degree of contraction of chromosomes in the studied three cells were determined by comparing haploid total lengths of all chromosome in each cells of this line and standardized haploid length for three cells and chromosomes distribution in this line were determined (Table 2).

The similarity and homogeneity of the distribution of chromosomal morphology in three cells of triticale BAT-1 were tested by the use of contingency table incorporating chromosome length and arm ratio classes (Table 3). Ideogram is also presented (Fig. 13).

Table 2. Standardized haploid length ($\bar{x}$) of observed chromosomes from three cells of triticale BAT-1.

No of chromosome pairs	A cell		AR	B cell		AR	C cell		AR	$\bar{X}=\sum xij/3$
	TCL			TCL			TCL			
	Xij	X'ij		Xij	X'ij		Xij	X'ij		
i	6.84	6.63	0.71	6.68	6.73	0.78	6.56	6.69	0.82	158.54
ii	6.83	6.62	0.78	6.45	6.50	0.72	6.38	6.51	0.87	
iii	6.60	6.40	0.83	6.35	6.40	0.84	6.32	6.45	0.83	
iv	6.58	6.38	0.86	6.28	6.33	0.70	6.21	6.33	0.98	
v	6.53	6.33	0.73	6.23	6.28	0.75	6.16	6.28	0.80	
vi	6.42	6.23	0.86	6.19	6.24	0.90	5.94	6.06	0.93	
vii	6.39	6.20	0.80	6.19	6.24	0.73	5.89	6.01	0.79	
viii	6.31	6.12	0.95	6.00	6.04	0.99	5.86	5.98	0.89	
ix	6.16	5.98	0.78	5.96	6.01	0.82	5.86	5.98	0.85	
x	6.12	5.94	0.85	5.94	5.99	0.85	5.78	5.90	0.81	
xi	6.06	5.83	0.70	5.88	5.93	0.82	5.74	5.85	0.67	
xii	5.92	5.74	1.00	5.75	5.80	0.78	5.62	5.73	0.99	
xiii	5.86	5.68	0.81	5.67	5.72	0.94	5.61	5.72	0.78	
xiv	5.86	5.58	0.73	5.66	5.71	0.89	5.56	5.67	0.74	
xv	5.74	5.57	0.96	5.56	5.60	0.72	5.55	5.66	0.70	
xvi	5.72	5.54	0.91	5.55	5.59	0.69	5.52	5.63	0.85	
xvii	5.71	5.54	0.96	5.40	5.44	0.89	5.44	5.55	0.81	
xviii	5.56	5.39	0.85	5.36	5.40	0.81	5.33	5.44	0.65	
xix	5.56	5.39	0.76	5.34	5.38	1.00	5.29	5.40	0.91	
xx	5.49	5.33	0.67	5.28	5.32	0.93	5.15	5.25	0.78	
xxi	5.48	5.32	0.80	5.18	5.22	0.75	5.14	5.24	0.74	
xxii	5.33	5.17	0.89	5.15	5.19	0.80	5.03	5.13	0.84	
xxiii	5.30	5.14	0.85	5.10	5.14	0.70	5.02	5.12	0.70	
xxiv	5.26	5.10	0.75	5.08	5.12	0.91	5.00	5.10	0.99	
xxv	5.03	4.88	0.72	4.86	4.90	0.83	4.92	5.02	0.65	
xxvi	4.98	4.83	0.84	4.81	4.85	0.72	4.88	4.98	0.78	
xxvii	4.88	4.73	0.78	4.74	4.78	0.98	4.80	4.90	0.74	
xxviii	4.81	4.67	0.85	4.61	4.65	0.80	4.54	4.63	0.84	
∑x=	163.28	158.26		157.25	158.50		155.10	158.21		

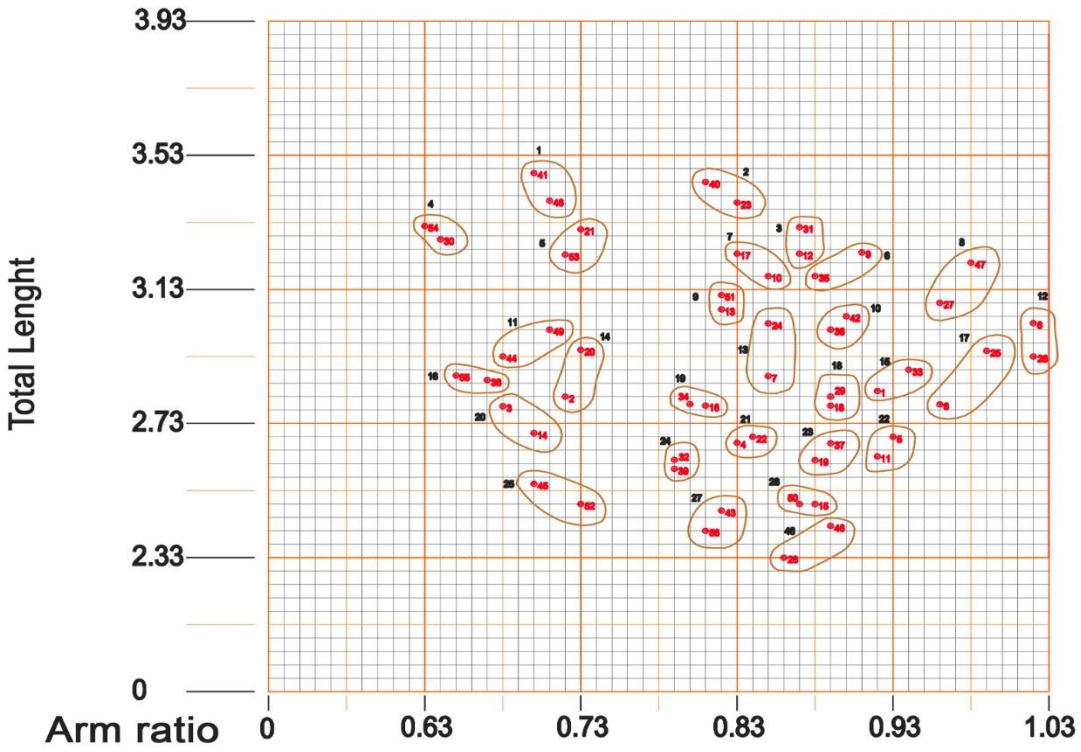


Fig. 11: Scatter diagram of the chromosomes of triticale BAT-1.

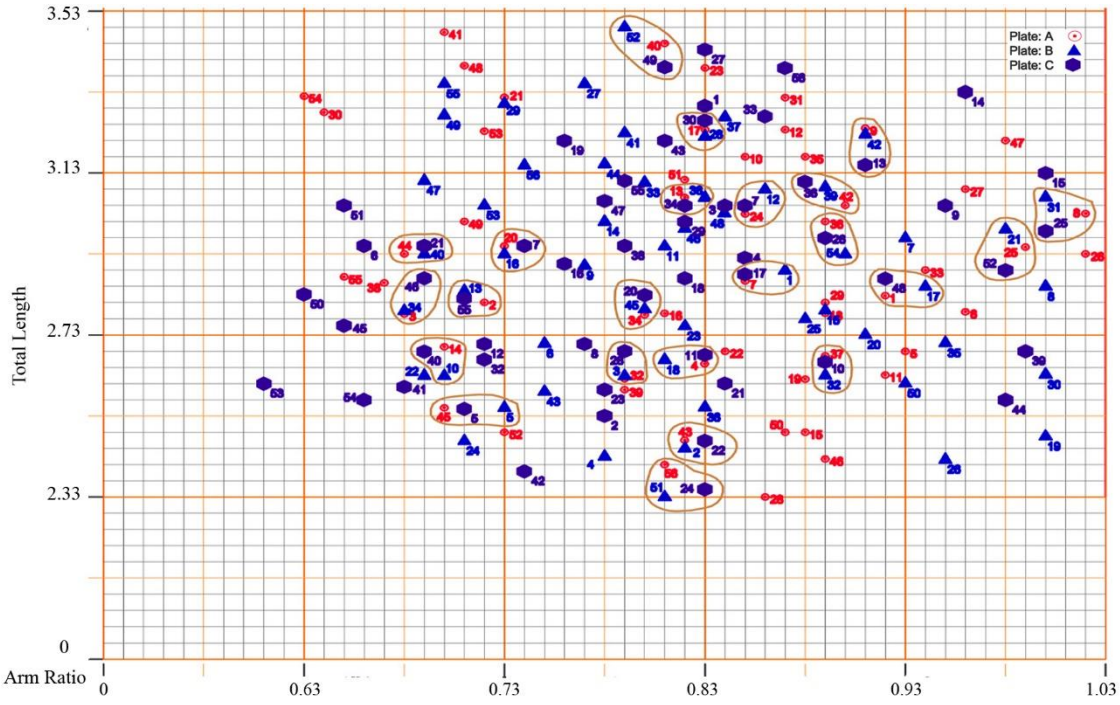


Fig. 12: Combined scatter diagram of the 28 haploid chromosome values, standardized lengths and unstandardized arm ratios from each of the three cells which were recognizably homologous from each of three cells in triticale BAT-1.

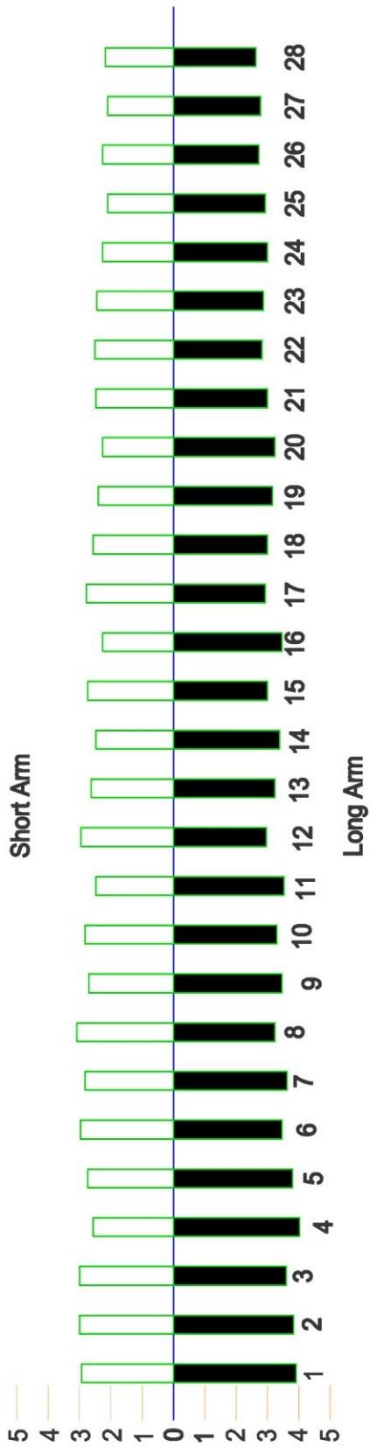


Fig. 13: Ideogram of the chromosomes of triticale BAT-1.

Table 3. Contingency table to test their homogeneity in distribution of chromosomes among different haploid complements in triticale BAT-1.

Length classes X (µm)	Arm ratio Y(µm)	No. of chromosome in the plate			Total
		A	B	C	
6.29-6.84 above	0.76-1.00	6	2	3	11
	0.51-0.75	2	1	-	3
	0.26-0.50	-	-	-	-
Total=		**8**	**3**	**3**	**14**
5.69-6.28	0.76-1.00	7	6	7	20
	0.51-0.75	2	3	1	6
	0.26-0.50	-	-	-	-
Total=		**9**	**9**	**8**	**26**
5.11-6.28	0.76-1.00	5	7	6	18
	0.51-0.75	2	3	4	9
	0.26-0.50	-	-	-	-
Total=		**7**	**10**	**10**	**27**
5.12 less than	0.76-1.00	3	4	4	11
	0.51-0.75	1	2	3	6
	0.26-0.50	-	-	-	-
Total=		**4**	**6**	**7**	**17**
Grand total=		**28**	**28**	**28**	**84**

Similarly in triticale BAT-2, triticale WRF, *Triticum aestivum* and *Triticum durum* (Figs. 14, 15, 17, 18, 20, 21, 23 & 24) pairs of adjacent points were considered to represent homologous chromosomes and circled on the scatter diagrams. Table 4 indicates the standardized haploid complement of three cells of BAT-2. Table 5 shows the contingency table incorporating chromosome length and arm ratio classes. Ideogram for chromosome complement is shown in Fig. 16 for triticale BAT-2.

Figures 17 & 18 also indicated the adjacent points and represent homologous chromosomes and circled on single scatter diagram and combined scatter diagram in triticale WRF. Here Table 6 shows the standardized haploid chromosome complement of triticale WRF and Table 7 reveals contingency test for their homogeneity in distribution of chromosomes among different haploid complement of triticale WRF. Ideogram is also revealed in Fig. 19.

In *Triticum aestivum* Figs. 20 & 21 show separate and combined scatter diagram. Table 8 presents standardized haploid chromosome complement. Table 9 also presents contingency test for their homogeneity in distribution of chromosome among different haploid complement. Fig. 22 shows the Ideogram of *Triticum aestivum*.

Figures 23 & 24 present the separated and combined scatter diagram of the chromosomes of *Triticum durum*. Table 10 shows the standardized haploid chromosome complement and Table 11 presents contingency test. Fig. 25 showed ideogram of *Triticum durum*.

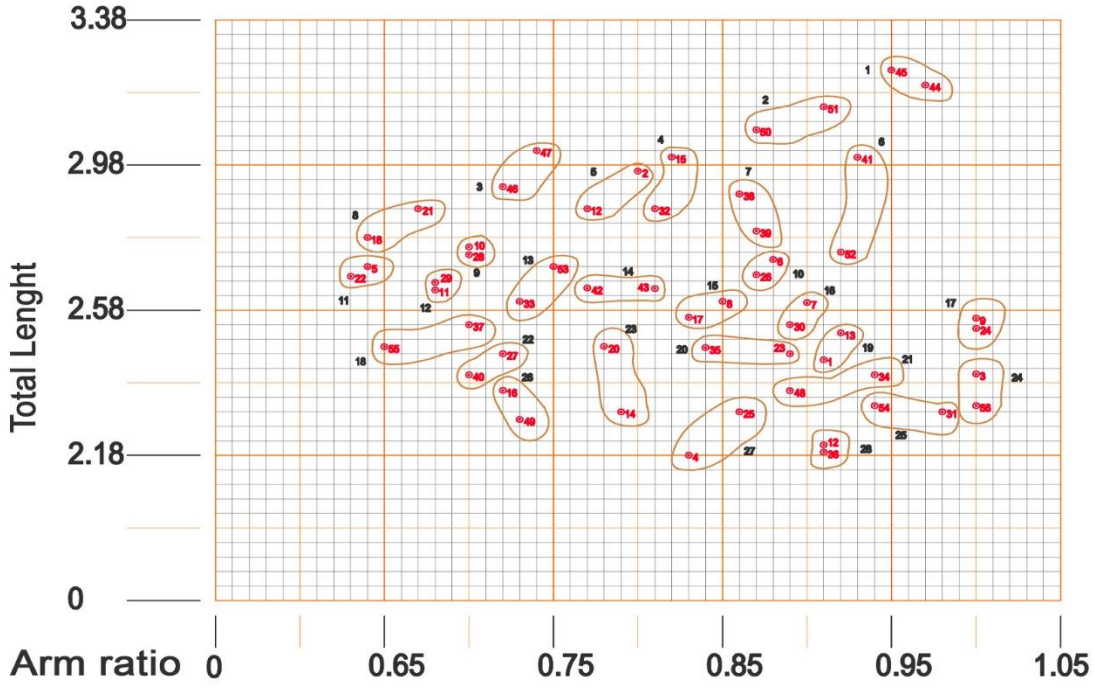


Fig. 14: Scatter diagram of the chromosomes of triticale BAT-2.

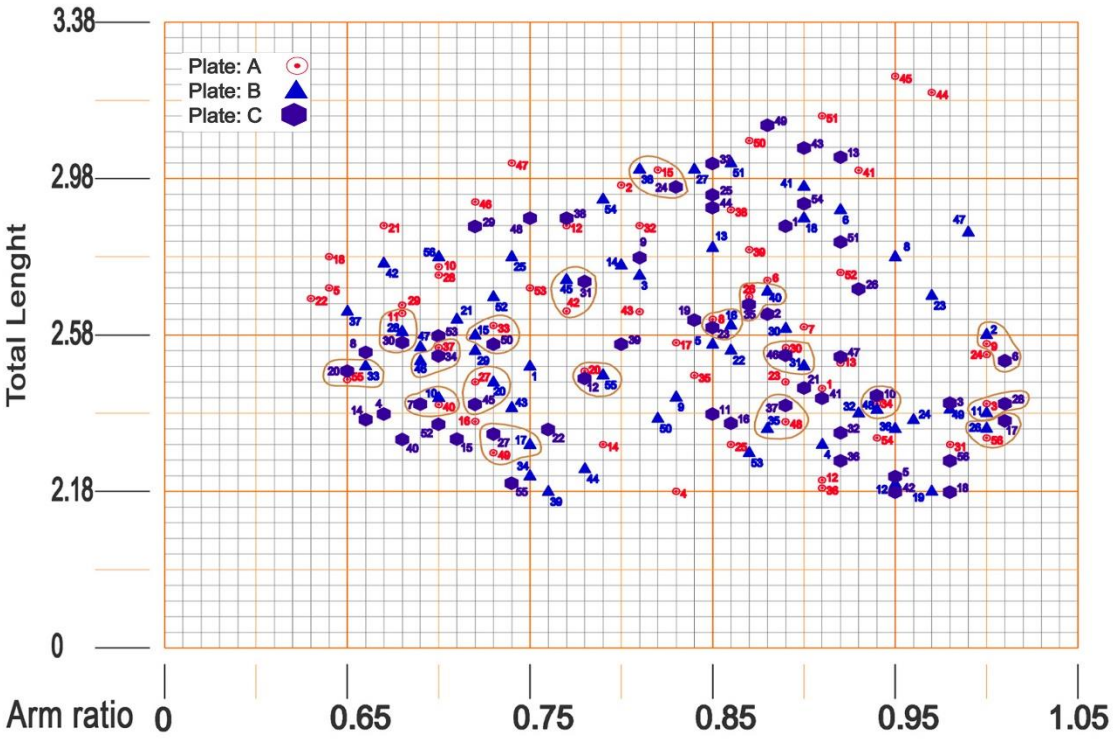


Fig. 15: Combined scatter diagram of the 28 haploid chromosome values, standardized lengths and unstandardized arm ratios from each of the three cells which were recognizably homologous from each of three cells in triticale BAT-2.

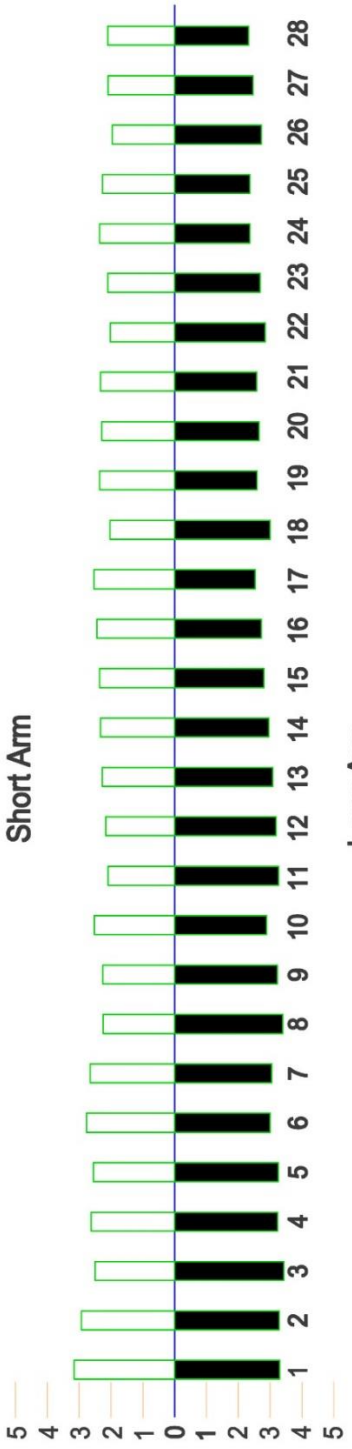


Fig. 16: Ideogram of the chromosomes of Triticale BAT-2.

Table 4. Standardized haploid length ($\bar{x}$) of the observed chromosomes from three cells of triticale BAT-2.

No of chromosome pairs	A cell TCL		AR	B cell TCL		AR	C cell TCL		AR	$\bar{X}=\sum xij/3$
	Xij	X'ij		Xij	X'ij		Xij	X'ij		
i	6.46	6.52	0.96	6.28	6.34	0.85	6.36	6.42	0.86	149.05
ii	6.22	6.28	0.89	6.19	6.25	0.80	6.32	6.38	0.90	
iii	5.93	5.99	0.73	6.06	6.12	0.91	6.08	6.14	0.84	
iv	5.86	5.92	0.82	6.02	6.03	0.88	6.01	6.07	0.89	
v	5.81	5.87	0.79	5.89	5.96	0.97	5.96	6.02	0.81	
vi	5.77	5.83	0.93	5.82	5.88	0.72	5.96	6.02	0.73	
vii	5.71	5.57	0.87	5.75	5.81	0.81	5.81	5.23	0.77	
viii	5.65	5.71	0.66	5.66	5.72	0.64	5.50	5.56	0.87	
ix	5.47	5.52	0.70	5.56	5.62	0.72	5.45	5.50	0.84	
x	5.41	5.46	0.88	5.55	5.61	0.87	5.34	5.39	0.69	
xi	5.36	5.41	0.64	5.46	5.51	0.78	5.27	5.32	0.78	
xii	5.36	5.41	0.74	5.43	5.48	0.99	5.25	5.30	0.65	
xiii	5.34	5.39	0.68	5.42	5/47	0.72	5.24	5.29	0.92	
xiv	5.29	5.34	0.79	5.38	5.43	0.86	5.16	5.21	0.92	
xv	5.16	5.21	0.84	5.35	5.89	0.89	5.15	5.20	0.69	
xvi	5.16	5.21	0.90	5.34	5.39	0.67	5.15	5.20	0.88	
xvii	5.06	5.11	1.00	5.33	5.38	0.69	5.14	5.19	0.99	
xviii	5.03	5.08	0.68	5.15	5.20	0.75	5.07	5.12	0.90	
xix	4.95	4.99	0.92	5.14	5.19	1.72	5.00	5.05	1.00	
xx	4.94	4.99	0.87	5.12	5.17	0.96	4.97	5.02	0.66	
xxi	4.91	4.96	0.92	5.06	5.11	0.83	4.94	4.99	0.85	
xxii	4.87	4.92	0.71	5.01	5.06	0.96	4.93	4.98	0.72	
xxiii	4.78	4.83	0.79	5.00	5.05	1.00	4.91	4.96	0.68	
xxiv	4.72	4.77	1.00	54.90	4.95	0.93	4.86	4.91	0.71	
xxv	4.68	4.73	0.73	4.89	4.94	0.88	4.81	4.86	0.91	
xxvi	4.63	4.68	0.96	4.79	4.84	0.75	4.77	4.82	0.74	
xxvii	4.56	4.61	0.85	4.68	4.73	0.77	4.65	4.97	0.97	
xxviii	4.52	4.46	0.91	4.63	4.68	0.96	4.64	4.69	0.94	
$\sum x=$	147.51	148.77		150.95	152.85		148.70	149.81		

Table 5. Contingency table to test their homogeneity in distribution of chromosomes among different haploid complements in triticale BAT-2.

Type of chromosomes	Length classesX(μ)	Arm ratios (Y)	A	B	C	Total
Large(L)	5.95-6.46 above	0.76-1.00	2	4	5	11
		0.50-0.75	-	-	1	1
		0.26-0.50	-	-	-	-
Total=			**2**	**4**	**6**	**12**
Medium(M)	5.43-5.94	0.76-1.00	4	5	3	12
		0.50-0.75	3	3	-	6
		0.26-0.50	-	-	-	-
Total=			**7**	**8**	**3**	**18**
Relatively short(S_1)	4.92-5.44	0.76-1.00	7	6	8	21
		0.50-0.75	4	5	5	14
		0.26-0.50	-	-	-	-
Total=			11	11	13	35
Short(S)	**4.93 less than**	**0.76-1.00**	**6**	**4**	**3**	**13**

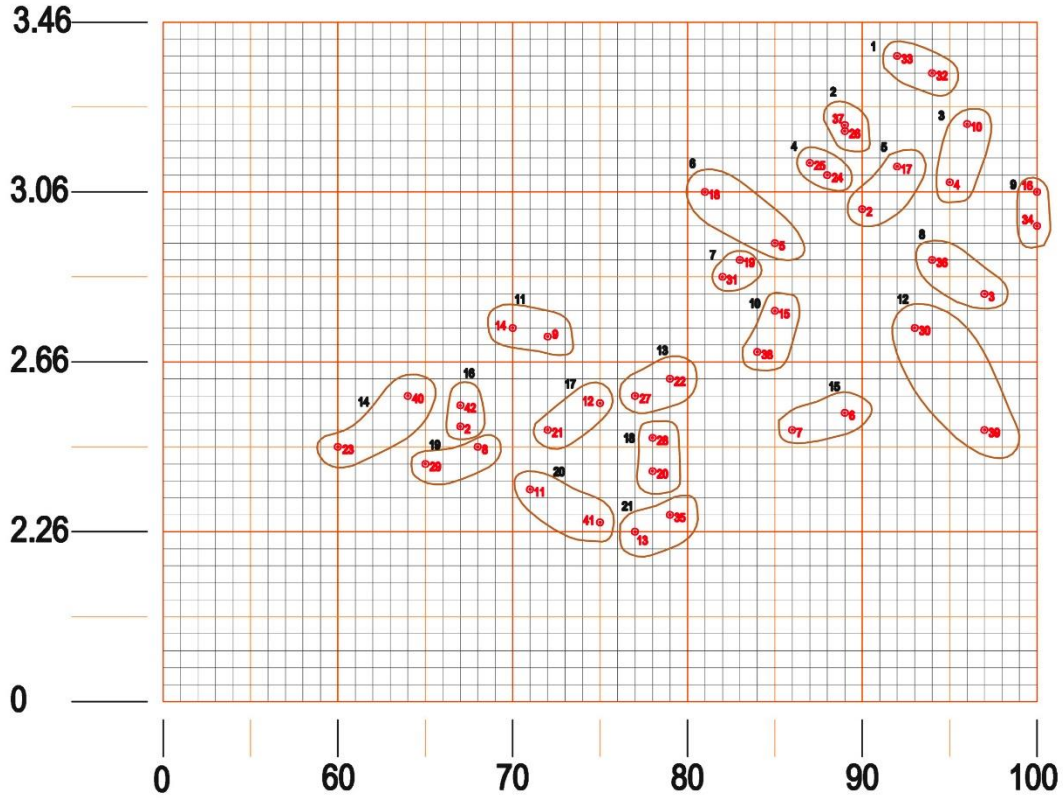


Fig. 17: Scatter diagram of the chromosomes of triticale WRF.

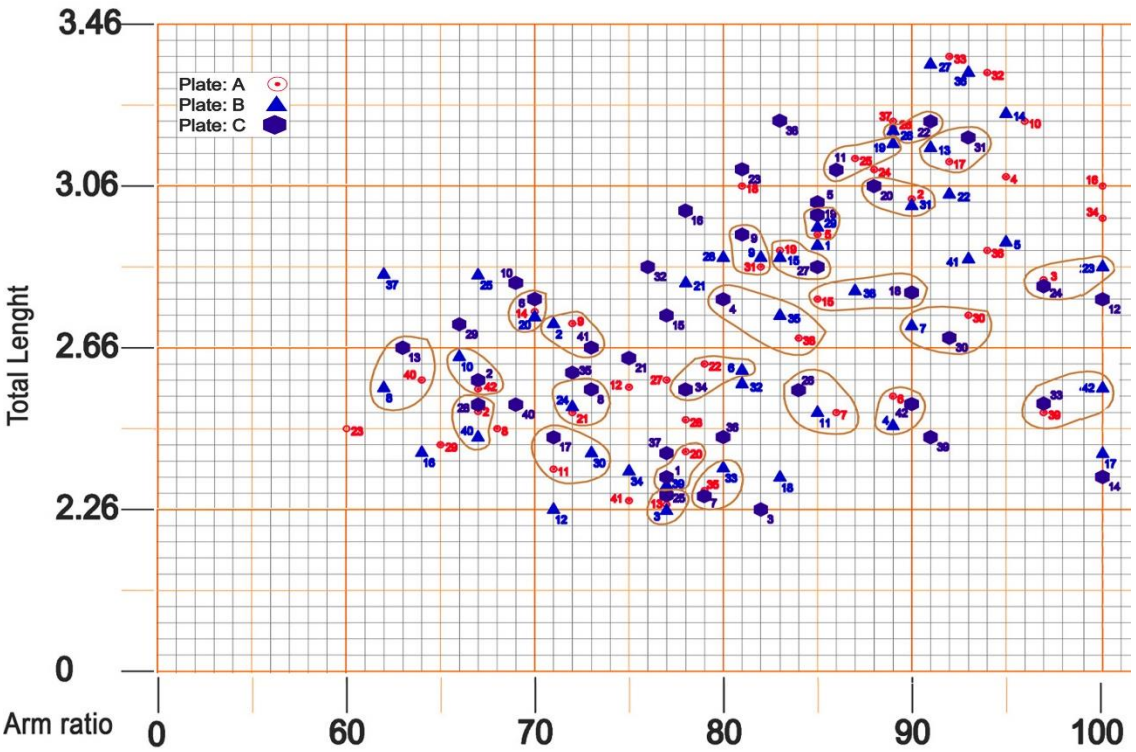


Fig. 18: Combined scatter diagram of the 28 haploid chromosome values, standardized lengths and unstandardized arm ratios from each of the three cells which were recognizably homologous from each of three cells in triticale WRF.

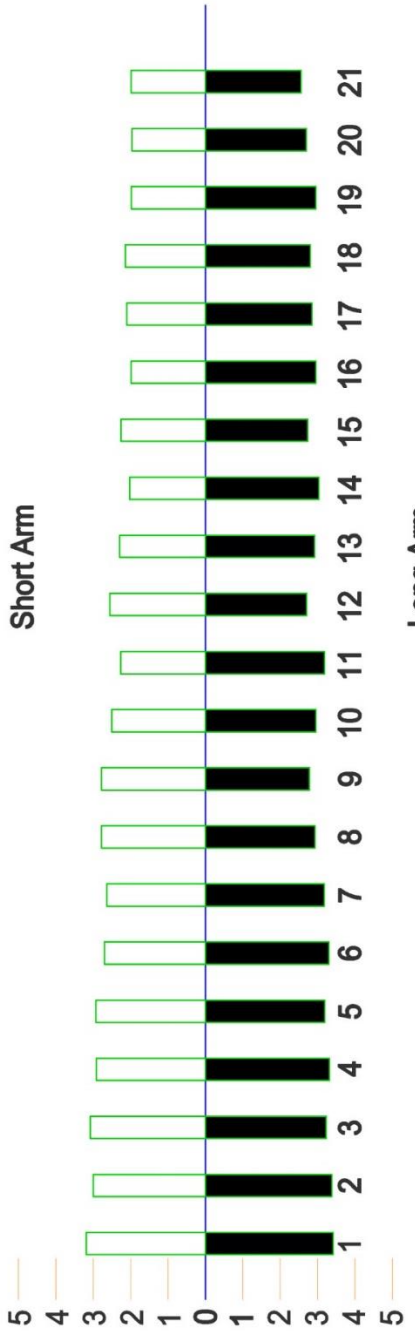


Fig. 19: Ideogram of the chromosomes of triticale WRF.

Table 6. Standardized haploid length ($\bar{x}$) of the observed chromosomes from three cells of triticale WRF.

Chromosome pairs	ACell		AR	B cell		AR	C cell		AR	$\bar{X}=\sum xij/3$
	Xij	X'ij		Xij	X'ij		Xij	X'ij		
i	6.60	6.50	0.93	6.71	6.62	0.92	6.26	6.45	0.93	
ii	6.38	6.28	0.89	6.40	6.31	0.93	6.18	6.36	0.89	
iii	6.31	6.22	0.96	6.38	6.29	0.89	6.02	6.20	0.96	
iv	6.24	6.15	0.88	6.06	5.98	0.91	5.86	6.04	0.88	
v	6.13	6.04	0.92	5.87	5.90	0.85	5.71	5.88	0.92	
vi	5.81	5.91	0.83	5.79	5.71	0.83	5.64	5.81	0.83	
vii	5.76	5.72	0.83	5.79	5.71	0.98	5.45	5.61	0.70	
viii	5.71	5.62	0.95	5.72	5.64	0.79	5.44	5.60	0.99	
ix	5.56	5.48	1.00	5.68	5.60	0.64	5.36	5.52	0.79	
x	5.46	5.38	0.85	5.60	5.52	0.92	5.30	5.46	0.91	
xi	5.45	5.37	0.71	5.55	5.47	0.85	5.21	5.37	0.65	113.44
xii	5.26	5.18	0.95	5.46	5.39	0.71	5.09	5.24	0.73	
xiii	5.21	5.13	0.78	5.19	5.12	0.64	5.03	5.18	0.74	
xiv	5.06	5.21	0.62	5.18	5.11	0.81	4.95	5.10	0.64	
xv	4.99	4.92	0.83	4.97	4.90	0.87	4.84	4.98	0.82	
xvi	4.95	4.88	0.67	4.96	4.89	1.00	4.81	4.95	0.91	
xvii	4.91	4.88	0.74	4.91	4.84	0.73	4.80	4.94	0.78	
xviii	4.88	4.88	0.78	4.84	4.77	0.66	4.78	4.92	0.70	
xix	4.88	4.87	0.67	4.80	4.73	0.73	4.70	4.84	0.99	
xx	4.66	4.59	0.73	4.78	4.71	0.82	4.52	4.66	0.77	
xxi	4.55	4.48	0.78	4.58	4.52	0.77	4.40	4.53	0.81	
$\sum x=$	114.76	113.69		115.22	113.73		110.35	113.64		

Table 7. Contingency table to test their homogeneity in distribution of chromosomes among different haploid complements in triticale WRF.

Length classes X (µm)	Arm ratio(µm)	No of chromosomes in the plats			Total
		A	B	C	
6.15-6.71above	0.76-1.00	4	3	2	9
	0.51-0.75	-	-	-	
	0.26-0.50	-	-	-	
Total=		**4**	**3**	**2**	**9**
5.55-6.14	0.76-1.00	5	7	4	16
	0.51-0.75	-	1	-	1
	0.26-0.50	-	-	-	
Total=		**5**	**8**	**4**	**17**
4.97-5.56	0.76-1.00	4	2	3	9
	0.51-0.75	2	2	4	8
	0.26-0.50	-	-	-	-
Total=		**6**	**4**	**7**	**17**
4.98 less than	0.76-1.00	2	3	6	11
	0.51-0.75	4	3	2	9
	0.26-0.50	-	-	-	-
Total=		**6**	**6**	**8**	**20**
Grand total=		**21**	**21**	**21**	**63**

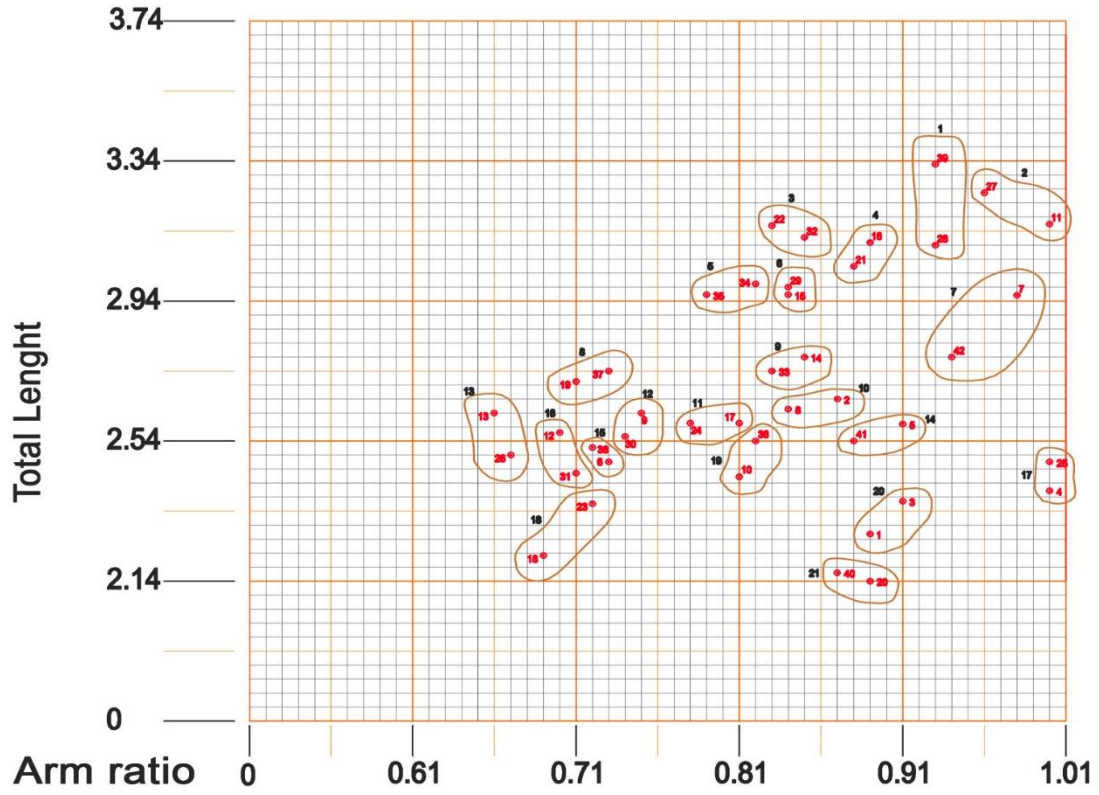


Fig. 20: Scatter diagram of the chromosomes of *Triticum aestivum*.

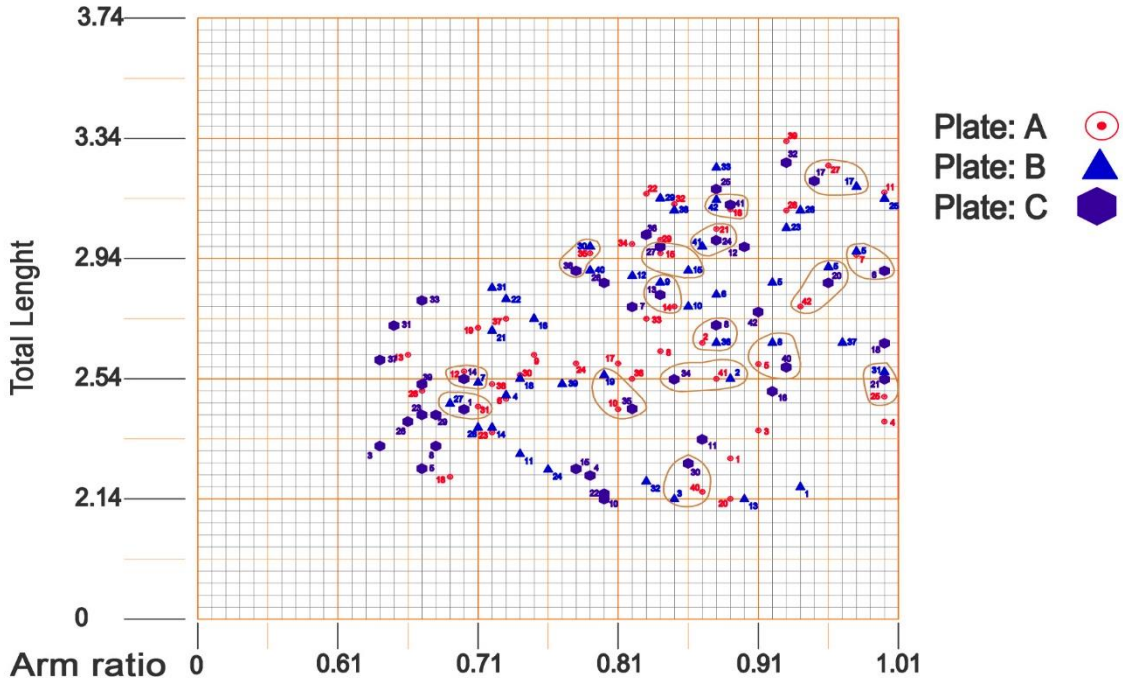


Fig. 21: Combined scatter diagram of the 28 haploid chromosome values, standardized lengths and unstandardized arm ratios from each of the three cells which were recognizably homologous from each of three cells in *Triticum aestivum*.

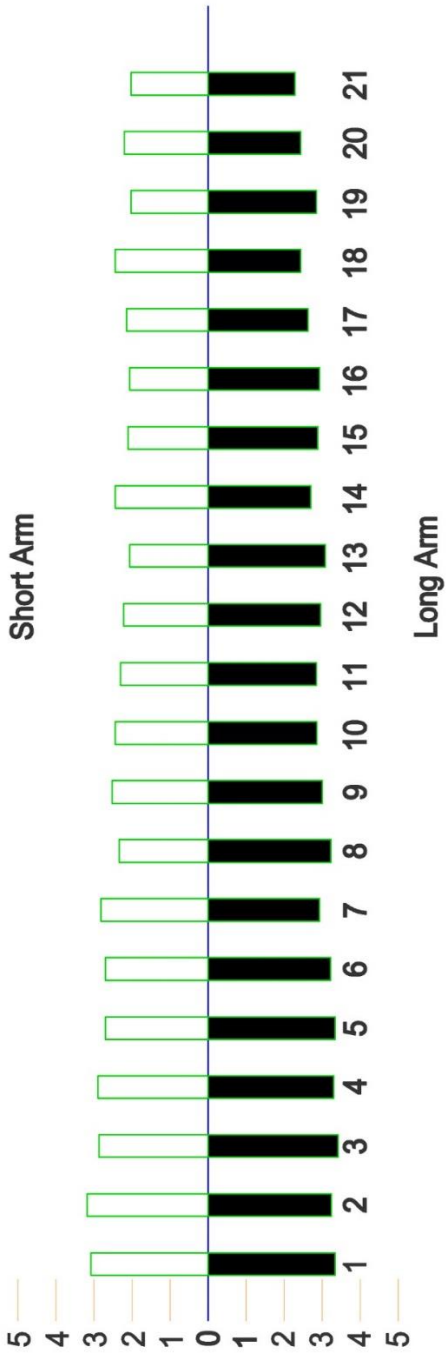


Fig. 22: Ideogram of the chromosomes of *Triticum aestivum*.

Table 8. Standardized haploid length ($\bar{x}$) of the observed chromosomes from three cell of *Triticum aestivum*.

Chromosome pairs	ACell		AR	B cell		AR	C cell		AR	$\bar{X}=\sum xij/3$
	Xij	X'ij		Xij	X'ij		Xij	X'ij		
i	6.42	6.47	0.93	6.37	6.36	0.87	6.60	6.56	0.93	
ii	6.41	6.46	0.98	6.35	6.34	0.99	6.44	6.40	0.88	
iii	6.28	6.33	0.84	6.29	6.28	0.84	6.14	6.10	0.83	
iv	6.19	6.24	0.88	6.16	6.15	0.93	6.12	6.08	0.94	
v	6.04	6.09	0.81	5.93	5.92	0.86	6.12	6.08	0.79	
vi	5.92	5.97	0.84	5.92	5.91	0.78	5.96	5.92	0.78	
vii	5.74	5.79	0.96	5.89	5.88	0.95	5.94	5.90	0.98	
viii	5.56	5.61	0.72	5.81	5.80	0.82	5.75	5.71	0.82	
ix	5.52	5.57	0.84	5.66	5.65	0.73	5.67	5.63	0.71	
x	5.30	5.34	0.86	5.66	5.65	0.89	5.66	5.62	0.89	
xi	5.18	5.22	0.80	5.50	5.49	0.74	5.44	5.40	0.75	114.37
xii	5.15	5.19	0.75	5.45	5.44	0.86	5.32	5.28	1.00	
xiii	5.14	5.18	0.67	5.22	5.21	0.91	5.27	5.23	0.71	
xiv	5.14	5.18	0.90	5.22	5.21	0.99	5.26	5.22	0.92	
xv	4.99	5.03	0.73	5.08	5.07	0.78	5.07	5.04	0.83	
xvi	4.99	5.03	0.71	5.02	5.01	0.74	4.91	4.88	0.70	
xvii	4.88	4.92	1.00	4.98	4.97	070	4.85	4.82	0.66	
xviii	4.87	4.91	0.71	4.74	4.73	0.71	4.81	4.78	0.86	
xix	4.77	4.81	0.82	4.62	4.61	0.74	4.75	4.72	0.73	
xx	4.64	4.68	0.90	4.33	4.32	0.83	4.61	4.58	0.78	
xxi	4.31	4.35	0.88	4.32	4.32	0.91	4.45	4.42	0.79	
$\sum x=$	113.44	114.37		114.52	114.31		115.14	114.37		

Table 9. Contingency table to test their homogeneity in distribution of chromosomes among different haploid complements in *Triticum aestivum*.

Length classes X (µm)	Arm ratios Y (µm)	No of chromosomes in the plates			Total
		A	B	C	
603-6.60 above	0.76-1.00	5	4	5	14
	0.51-0.75	-	-	-	-
	0.26-0.505	-	-	-	-
Total=		**5**	**4**	**5**	**14**
5.46-6.02	0.76-1.00	3	5	4	12
	0.51-0.75	1	2	1	4
	0.26-0.505	-	-	-	-
Total=		**4**	**7**	**5**	**16**
4.87-5.45	0.76-1.00	4	4	3	11
	0.51-0.75	5	2	3	10
	0.26-0.505	-	-	-	-
Total=		**9**	**6**	**6**	**21**
4.88 less than	0.76-1.00	3	2	3	8
	0.51-0.75	-	2	2	4
	0.26-0.505	-	-	-	-
Total=		**3**	**4**	**5**	**12**
Grand total=		**21**	**21**	**21**	**63**

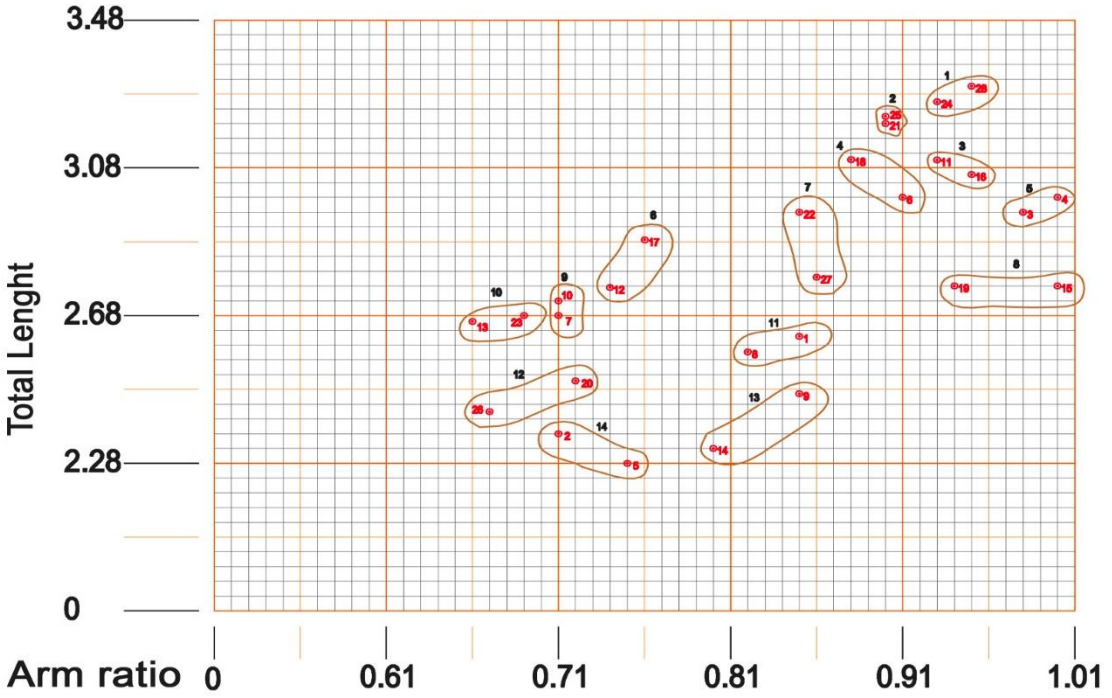


Fig. 23: Scatter diagram of the chromosomes of *Triticum durum.*

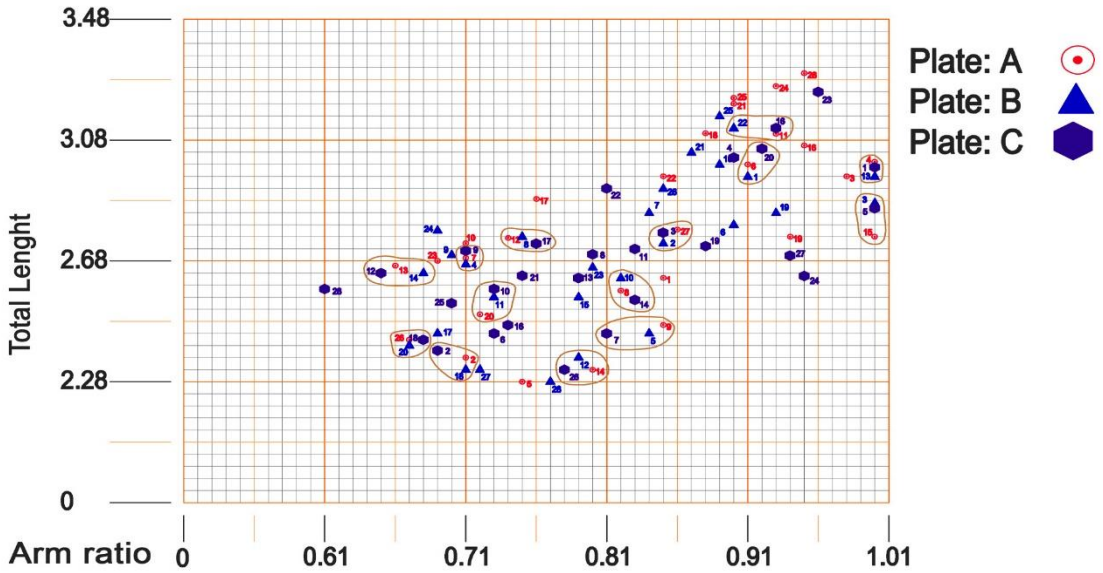


Fig. 24: Combined scatter diagram of the 28 haploid chromosome values, standardized lengths and unstandardized arm ratios from each of the three cells which were recognizably homologous from each of three cells in *Triticum durum.*

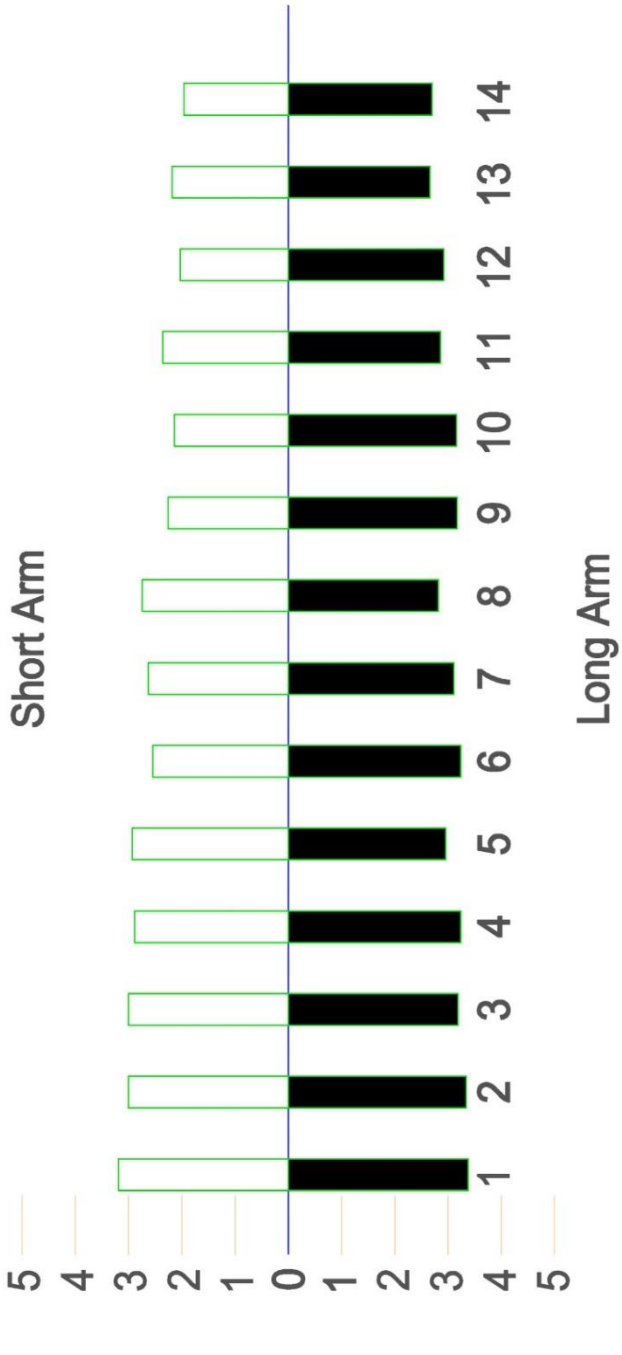


Fig. 25: Ideogram of the chromosomes of *Triticum durum*.

Table 10. Standardized haploid length ($\bar{x}$) of the observed chromosomes from three cells of *Triticum durum*.

Chromosome pairs	A cell		AR	B cell		AR	C cell		AR	$\bar{X}=\sum xij/3$
	TCL			TCL			TCL			
	Xij	X'ij		Xij	X'ij		Xij	X'ij		
i	6.57	6.44	0.94	6.36	6.23	0.90	6.35	6.22	0.95	
ii	6.34	6.21	0.90	6.12	6.00	0.88	6.07	5.95	0.91	
iii	6.19	6.07	0.94	6.01	5.89	0.92	5.86	5.74	1.00	
iv	6.13	6.01	0.90	5.86	5.74	1.00	5.70	5.59	0.79	
v	5.89	5.77	0.99	5.82	5.70	0.84	5.52	5.41	0.86	
vi	5.74	5.62	0.85	5.63	5.52	0.88	5.44	5.33	0.83	
vii	5.64	5.53	0.74	5.46	5.35	0.69	5.33	5.22	0.95	
viii	5.56	5.45	0.97	5.46	5.35	0.71	5.32	5.21	0.80	77.10
ix	5.43	5.32	0.72	5.39	5.28	0.74	5.27	5.16	0.71	
x	5.30	5.19	0.68	5.36	5.25	0.81	5.23	5.13	0.64	
xi	5.22	5.12	0.83	5.10	5.00	0.82	5.22	5.11	0.74	
xii	4.95	4.85	0.70	4.91	4.81	0.68	4.95	4.85	0.74	
xiii	4.84	4.74	0.82	4.74	4.65	0.71	4.83	4.73	0.69	
xiv	4.66	4.57	0.73	4.72	4.63	0.78	4.81	4.71	0.82	
∑=	78.46	76.89		76.94	75.40		75.90	74.36		

Table 11. Contingency table to test their homogeneity in distribution of chromosomes among different haploid complements in *Triticum durum*.

Length classes X (µm)	Arm ratios Y (µm)	No of chromosomes in the plates			Total
		A	B	C	
6.11-6.57 above	0.76-1.00	4	2	1	7
	0.51-0.75	-	-	-	-
	0.26-0.505	-	-	-	-
Total=		**4**	**2**	**2**	**7**
5.46-6.02	0.76-1.00	2	4	3	9
	0.51-0.75	1	-	-	1
	0.26-0.505	-	-	-	-
Total=		**3**	**4**	**3**	**10**
4.87-5.45	0.76-1.00	2	1	4	7
	0.51-0.75	2	3	3	8
	0.26-0.505	-	-	-	-
Total=		**4**	**4**	**7**	**15**
4.88 less than	0.76-1.00	1	2	1	4
	0.51-0.75	2	2	2	6
	0.26-0.505	-	-	-	-
Total=		3	4	3	10
Grand total=		**14**	**14**	**14**	**42**

Table 12 reveals the proportion of the haploid complement and total length occupied by the marked chromosome of triticale and *Triticum* species. Here highest mean value occupied the length percentage was 57.98 in triticale WRF and lowest value was 31.32 in triticale BAT-2.

Table 13 indicates total haploid complement lengths and chromosome distribution of triticale and *Triticum* species. In Table 13 highest CV% was 2.18 in triticale BAT-1 and the lowest value was 0.612 in *Triticum aestivum*. Other CV% values were 0.96 (triticale BAT-2) 1.94 (triticale WRF) and 1.36 (*Triticum durum*). χ^2-values (Table 13) were also highest (10.081) in triticale BAT-1 and lowest (0.044) in triticale BAT-2.

The mean length and arm ratios of the identified chromosomes in different lines of triticale and *Triticum* species are given in Tables 14, 15. 16, 17 & 18.

Table 12. Proportion of the haploid complement and total length occupied by the marked chromosomes of triticale BAT-1, triticale BAT-2, triticale WRF, *Triticum aestivum, Triticum durum*.

Name of triticale lines and *Triticum* species	Cells	Haploid total length	Identified chromosomes		Mean % of occupied length
			Total length	%	
triticale BAT-1	A	163.28	66.24	40.57	40.79
	B	157.25	64.50	41.02	
	C	155.10	63.23	40.77	
triticale BAT-2	A	147.51	45.59	30.91	31.30
	B	150.95	47.82	31.62	
	C	148.70	46.65	31.36	
triticale WRF	A	114.76	67.35	58.69	57.98
	B	115.22	67.13	58.26	
	C	110.35	65.67	57.00	
Triticum aestivum	A	113.44	43.76	38.58	38.81
	B	114.52	44.08	38.49	
	C	115.14	45.31	39.35	
Triticum durum	A	78.46	34.77	44.32	45.36
	B	76.94	35.19	45.74	
	C	75.90	34.94	46.03	

Table 13. Total haploid complement lengths and chromosome distribution of triticale and *Triticum* species.

Name of species/lines	Haploid total length			Mean $\bar{X}$	SD±SE	CV%	χ^2 values
	A	B	C				
triticale BAT-1	163.28	157.25	155.10	158.54	3.46±1.99	2.18	10.081
triticaleBAT-2	147.51	150.95	148.70	149.05	1.43±0.82	0.96	0.044
triticale WRF	114.76	115.22	110.35	113.44	2.20±1.27	1.94	0.13
Triticum aestivum	113.44	114.52	115.14	114.37	0.70±0.41	0.612	16.60
Triticum durum	78.46	76.94	75.90	77.10	1.05±0.61	1.36	0.095

4.2.2 Allocation of unidentified chromosomes: The unidentified chromosome complements were allocated to different morphological categories based on their total length (TCL) and arm ratio (AR) classes. The allocation of unidentified chromosomes of the different lines of triticale and *Triticum* species are given in Tables 19, 20, 21, 22 & 23.

4.2.3 Morphological feature of unidentified chromosome: The unidentified chromosomes were distributed to the various morphological categories using probabilistic inferences, specially on the chromosome frequency in a given class per haploid set (L=Large, M=Medium, S_1=relatively short, and S_2=short). The morphological features of the haploid complement in triticale lines and *Triticum* species are given in Tables 24, 25, 26, 27 & 28.

4.2.4 Proposed standard Karyotype: The standard karyotypes were proposed for triticale lines and *Triticum* species on the basis of centromeric formula, range and average of chromatin length per chromosome. Data on chromosome morphology, i.e., length, arm ratio, relative length, TCL%, TF% and chromosome type are given in Tables 29, 30, 31, 32 & 33.

Morphological features of the proposed standard karyotype (Table 34) are described below:

4.2.4.1 triticale BAT-1 (2n=56): In this case, seventeen pairs (i, ii, iii, iv, v, vi, vii, viii, xi, xii, xv, xvi, xvii, xix, xxi, xxii) were found to be metacentric and submetacentric were six pairs (ix, x, xiii, xiv, xviii, xx). Identified chromosome pairs were i^m, ii^m, iii^m, iv^m, v^m, vi^m, vii^m, $viii^m$, ix^{sm}, x^{sm}, xi^m, xii^m, $xiii^{sm}$, xiv^{sm}, xv^m, xvi^m, $xvii^m$, $xviii^{sm}$, xix^m, xx^{sm}, xxi^m, $xxii^m$, $xxiii^m$ and mean value of these identified chromosomes was 2.79µm. The longest chromosome pairs (counting total twenty eight pairs) was 6.84µm in length and the shortest was 4.81µm in length with a TCL of 158.54. TF% was determined to be 40.42. TCL% was found to be highest as 4.31 and lowest as 2.95 in twenty eight pairs of chromosomes. The proposed karyotype formula (K.F) was as follows: $8L^{6m+2sm}+9M^{7m+2sm}+7S_1{}^{5m+2sm}+4S_2{}^{3m+1sm}$

4.2.4.2 triticale BAT-2 (2n=56): Eleven pairs of chromosomes for this lines were found to be metacentic (i, ii, iii, iv, vii, viii, ix, xiii, xiv, xvi, xvii) and seven pairs submetacentic (v, vi, ix, x, xii, xv, xvii). Eighteen chromosomes were identified (i^m, ii^m, iii^m, iv^m, v^{sm}, vi^{sm}, vii^m, vii^m, ix^{sm}, x^{sm}, xi^m, xii^{sm}, $xiii^m$, xiv^m, xv^{sm}, xvi^m, $xvii^m$, $xvii^{sm}$) and the mean value of eighteen chromosomes was found to be 2.60µm. The longest chromosome pair was 6.46µm in length and shortest chromosome pair was 4.42µm. TF% was highest as 4.38 and lowest as 2.99 among eighteen identified chromosomes. The proposed karyotype formula was found to be $2L^{2m}+7M^{4m+3sm}+11S_1{}^{7m+4sm}+8S_2{}^{6m+2sm}$.

4.2.4.3 Triticale WRF (2n=42): The chromosome complement in this lines was found with thirteen metacentric (i, ii, iii, iv, v, vi, vii, x, xii, xiii, xvi, xvii, and xviii) and five sub metacentric (vii, ix, xi, xiv, xv). Eighteen chromosome (i^{m}, ii^{m}, iii^{m}, iv^{m}, v^{m}, vi^{m},vii^{m}, $viii^{sm}$, ix^{sm}, x^{m}, xi^{sm}, xii^{m}, $xiii^{m}$, xiv^{sm}, xv^{sm}, xvi^{m}, $xvii^{m}$ and $xviii^{m}$).

Eighteen chromosomes were identified and the mean value of eighteen chromosomes was found to be 2.67µm. the longest chromosome 6,71µm in length and shortest was 4.58µm in length with a TCL of 113.44µm. TF% was found to be 45.70 and TCL% was highest as 5.92 and lowest as 4.04 among eighteen identified chromosome. The proposed karyotypic formula was found to be $3L^{3m}+8M^{7m+1sm}+4S_1{}^{2m+2sm}+6S_2{}^{3m+3sm}$.

4.2.4.4 *Triticum aestivum* (2n=42): The chromosome complement in this species was found with fourteen metacentric (i, ii, iii, iv, v, vi, vii, viii, ix, x, xi, xiii, xiv, xvi,) and two sub metacentric (xii, xv). Sixteen identified chromosomes were i^{m}, ii^{m}, iii^{m}, iv^{m}, v^{m}, vi^{m}, vii^{m}, $viii^{m}$, ix^{m}, x^{m}, xi^{m}, xii^{sm}, $xiii^{m}$, xiv^{m}, xv^{sm}, xvi^{m} and mean value of the identified chromosome was 2.77µm. Among twenty one pairs of chromosomes the longest pair was found to be 6.60µm in length and shortest was 4.45µm in length with a TCL of 114.37µm.TF% was found to be 45.62 and TCL% ranged from 5.77-3.89. The proposed karyotypic formula was found to be $5L^{5m}+5M^{4m+1sm}+6S_1{}^{3m+3sm}+5S_2{}^{3m+2sm}$.

4.2.4.5 *Triticum durum*: In this species the chromosome complement were found with eight metacentric (i, ii, iii, iv, v, viii, x, xiii, 0 and five was submetacenrtic (vi, vii, ix, xi, xii). Thirteen identified chromosome were i^{m}, ii^{m}, iii^{m}, iv^{m}, v^{m}, vi^{sm}, vii^{sm}, $viii^{m}$, ix^{sm}, x^{m}, xi^{sm}, xii^{sm}, $xiii^{m}$ and mean value of the identified chromosome was 2.69µm. Among fourteen chromosomes longest chromosome pair was found to be 6.57µm and shortest was 4.66µm with a TCL of 77.10µm. TF% was found to be 46.32 and TCL% was found to be highest as 8.52 and lowest as 6.04. The proposed karyotype formula was found to be $4L^{4m}+3M^{2m+1sm}+4S_1{}^{2m+2sm}+3S_2{}^{1m+2sm}$.

Table 14. Mean length and arm ratios of the identified chromosomes in triticale BAT-1.

No of Identified chromosomes	Chromosomes type	Total length			Arm ratios		
		$\bar{x}$	SD±SE	Cv%	$\bar{x}$	SD±SE	CV%
1	m	3.37	0.08±0.046	2.37	0.79	0.016±9.43	2.03
2	m	3.20	0.052±0.030	1.63	0.82	0.019±0.010	2.32
3	m	3.18	0.20±0.11	6.29	0.98	0.00471±2.72	0.15
4	m	3.13	0.091±0.052	2.91	0.90	0.019±0.011	2.11
5	m	3.03	0.045±0.026	1.49	0.88	0.012±7.20	1.36
6	m	2.99	0.068±0.039	2.27	0.81	0.021±0.012	2.59
7	m	2.98	0.033±0.019	1.12	0.84	0.022±0.013	2.61
8	m	2.95	0.066±0.04	2.24	1	0±0	0
9	m	2.93	0.082±0.047	2.80	0.88	0.026±0.015	2.95
10	sm	2.89	0.09±0.05	3.11	0.74	0.00943±5.44	0.33
11	sm	2.86	0.05±0.03	1.75	0.69	0.00471±2.72	0.16
12	m	2.82	0.033±0.019	1.17	0.84	0.025±0.014	2.98
13	m	2.79	0.045±0.026	1.67	0.92	0.016±9.43	1.74
14	sm	2.77	0.069±0.035	2.49	0.72	0.00471±2.72	0.17
15	sm	2.74	0.03±0.02	1.09	0.69	0.00942±5.44	0.34
16	m	2.61	0.054±0.03	2.07	0.81	0.016±9.43	1.98
17	m	2.59	0.057±0.033	2.20	0.88	0.019±0.010	2.16
18	m	2.58	0.033±0.020	1.28	0.89	0±0	0
19	sm	2.58	0.099±0.058	3.83	0.70	0.0047±2.72	0.182
20	sm	2.47	0.032±0.019	1.30	0.72	0.0047±2.72	0.19
21	m	2.38	0.071±0.041	2.98	0.81	0.022±0.012	2.72
22	m	2.29	0.082±0.047	3.58	0.81	0.029±0.017	3.58
23	m	2.09	0.092±0.053	4.40	0.79	0.019±0.012	2.41
$\bar{x}$=2.79							

Table 15. Mean length and arm ratios of the identified chromosomes in triticale BAT-2.

No of Identified chromosomes	Chromosomes type	Total length			Arm ratios		
		$\bar{x}$	SD±SE	Cv%	$\bar{x}$	SD±SE	CV%
1	m	3.07	0.07±0.04	2.28	0.82	0.0057±0.0033	0.70
2	m	2.77	0.106±0.035	3.82	0.77	0	0
3	m	2.75	0.08±0.046	2.91	0.87	0.01±0.0057	1.14
4	m	2.68	0.061±0.035	2.27	0.85	0.0057±0.0033	0.67
5	sm	2.67	0.032±0.018	1.20	0.68	0.0058±0.0033	0.85
6	sm	2.62	0.06±0.03	3.70	0.69	0.00577±0.0033	0.84
7	m	2.62	0.08±0.05	3.05	1	0	0
8	m	2.61	0.049±0.028	1.88	0.89	0.0057±0.0033	0.64
9	sm	2.57	0.015±0.00.087	0.58	0.72	0.0057±0.0033	0.79
10	sm	2.57	0.078±0.045	3.04	0.65	0.01±0.0058	1.54
11	m	2.57	0.068±0.04	2.64	0.78	0.01±0.0057	1.28
12	sm	2.53	0.060±0.035	2.37	0.72	0.01±0.0058	1.39
13	m	2.51	0.10±0.059	3.98	0.94	0.00577±0.0033	0.61
14	m	2.50	0.031±0.014	1.24	0.88	0.0057±0.0033	0.65
15	sm	2.48	0.076±0.044	3.06	0.69	0.0115±0.0067	1.67
16	m	2.48	0.07±0.04	2.82	1	0	0
17	m	2.43	0.092±0.053	3.79	1	0	0
18	sm	2.38	0.09±0.05	3.78	0.73	0.02±0.0088	2.74
$\bar{x}$=2.60							

Table 16. Mean length and arm ratios of the identified chromosomes of triticale WRF.

No of Identified chromosomes	Chromosomes type	Total length			Arm ratios		
		$\bar{x}$	SD±SE	Cv%	$\bar{x}$	SD±SE	CV%
1	m	3.18	0.019±0.011	0.59	0.90	0.00943±5.44	1.05
2	m	3.11	0.080±0.046	2.57	0.87	0.01±7.20	1.15
3	m	3.02	0	0	0.90	0	0
4	m	2.95	0.20±0.12	6.78	0.88	0.06±0.039	6.82
5	m	2.94	0.00471±2.72	0.16	0.85	0	0
6	m	2.88	0.02±9.81	0.69	0.82	0.00471±2.72	0.57
7	m	2.80	0.074±0.043	2.64	0.87	0.02±0.012	2.29
8	sm	2.73	0.014±8.16	0.51	0.70	0	0
9	sm	2.61	0.14±0.080	0.053	0.71	0.012±7.20	1.69
10	sm	2.56	0.0060±0.035	0.47	0.79	0.012±7.20	1.51
11	m	2.53	0.08±0.045	2.34	0.67	0.00471±2.72	0.70
12	sm	2.51	0.050±0.029	3.16	0.98	0. 014±8.16	1.43
13	m	2.49	0.046±0.027	1.85	0.90	0.00943±5.44	1.05
14	sm	2.46	0.033±0.019	1.34	0.67	0	0
15	sm	2.46	0.046±0.05	1.87	0.73	0.00471±2.72	0.65
16	m	2.33	0.057±0.033	1.45	0.77	0.00471±2.72	0.61
17	m	2.29	0.057±0.032	2.49	0.79	0.00471±2.72	0.60
18	m	2.26	0	0	0.77	0	0
$\bar{x}$=2.67							

Table 17. Mean length and arm ratios of the identified chromosomes in *Triticum aestivum*.

No of Identified chromosomes	Chromosomes type	Total length			Arm ratios		
		$\bar{x}$	SD±SE	Cv%	$\bar{x}$	SD±SE	CV%
1	m	3.24	0.037±0.021	1.14	0.96	0.016±9.43	1.67
2	m	3.15	0.041±0.024	1.30	0.82	0.087±0.050	10.61
3	m	3.05	0.038±0.022	1.24	0.87	0.00471±2.72	0.54
4	m	2.99	0.019±0.010	0.64	0.78	0.00816±4.71	1.05
5	m	2.98	0.056±0.032	1.88	0.84	0.00816±4.71	0.97
6	m	2.97	0.019±0.011	0.64	0.98	0.014±4.71	1.43
7	m	2.86	0.065±0.038	2.27	0.93	0.025±0.014	2.69
8	m	2.85	0.052±0.030	1.82	0.84	0.00943±5.44	1.12
9	m	2.72	0.066±0.038	2.43	0.87	0±0	0
10	m	2.64	0.038±0.022	1.44	0.92	0.00471±2.72	0.51
11	m	2.59	0.08±0.045	3.09	1	0±0	0
12	sm	2.53	0.13±0.073	5.14	0.70	0.00816±4.71	1.17
13	m	2.53	0.065±0.037	2.57	0.80	0.00942±5.44	1.18
14	m	2.53	0.033±0.019	1.30	0.87	0.021±0.012	2.41
15	sm	2.51	0.08±0.048	3.19	0.70	0.00942±5.44	1.35
16	m	2.23	0.102±0.059	4.57	0.85	0.012±7.20	1.41
$\bar{x}$=2.77							

Table 18. Morphological features of the haploid complement of *Triticum durum*.

Chromosome no.	Name of identified chromosome	Total length $\bar{x}$	Arm ratio	Centromeric position	Chromosome Type
1	m_1	6.57	0.94	m	L
2	m_2	6.34	0.90	m	L
3	m_3	6.19	0.94	m	L
4	m_4	6.13	0.90	m	L
5	m_5	5.89	0.99	m	M
6	m_6	5.74	0.85	m	M
7	sm_1	5.64	0.74	sm	M
8	m_7	5.56	0.97	m	S_1
9	sm_2	5.43	0.72	sm	S_1
10	sm_3	5.30	0.68	sm	S_1
11	m_8	5.22	0.83	m	S_1
12	sm_4	4.95	0.70	sm	S_2
13	m_9	4.84	0.82	m	S_2
14	sm_5	4.66	0.73	sm	S_2

Table 19. The allocation of unidentified chromosomes of triticaleBAT-1 to different morphological categories.

Type of chromosomes	Length classes 'X'(µm)	Arm ratio 'Y'(µm)	Total No of chromosome in 3 haploid sets	No. of identified chromosome with names	Proposed unidentified chromosome	Assigned chromosome no.
Large(L)	6.29-6.84 above	0.76-1.00	11	16(m)	-	1, 2, 3, 4, 5, 6, 7, 8, 11, 12, 15, 16, 17, 19, 21, 22
		0.51-0.75	3	6(sm)	-	9, 10, 13, 14, 18, 20
		0.26-0.50				
Medium (M)	5.69-6.28	0.76-1.00	20	1(m)		23
		0.51-0.75	6	-	-	-
		0.26-0.50				
Relatively short (st)	5.11-5.70	0.76-1.00	18	-	-	-
		0.51-0.75	9	-	-	-
		0.26-0.50	-	-	-	-
Short (S)	5.12 less than	0.76-1.00	11	-	-	-
		0.51-0.75	6	-	-	-
		0.26-0.51	-	-	-	-
Total =			**84**	**23**	**-**	

Table 20. Allocation of unidentified chromosomes of triticale BAT-2 to different morphological categories.

Type of chromosomes	Length classes 'X' (µm)	Arm Ratios 'Y' (µm)	Total No of chromosome in 3 haploid sets	No of identified chromosome with names	Proposed unidentified chromosome	Assigned chromosome no.
Large(L)	5.95-6.46	0.76-1.00	11	11(m)	-	1, 2, 3, 4, 7, 8, 11, 13, 14, 16, 17
		0.51-0.75	1	7 (sm)	-	5, 6, 9, 10, 12, 15, 18
		0.26-0.50	-	-	-	-
Medium(M)	5.43-5.94	0.76-1.00	12	-		
		0.51-0.75	6	-	-	-
		0.26-0.50	-			
Relatively short($S_{1)}$	4.92-5.44	0.76-1.00	21	-	-	-
		0.51-0.75	14	-	-	-
		0.26-0.50	-			
Short(S_2)	4.93 less than	0.76-1.00	13	-	-	-
		0.51-0.75	6	-	-	-
		0.26-0.50	-	-	-	-
Total=			**64**	**18**		

Table 21. The allocation of unidentified chromosomes of triticale WRF to different morphological categories.

Type of chromosomes	Length classes 'X' (µm)	Arm ratio 'Y' (µm)	Total No of chromosome in 3 haploid sets	No of identified chromosome with names	Proposed unidentified chromosome	Assigned chromosome no.
Large (L)	6.15-6.72	0.76-1.00		13(m)	-	1, 2, 3, 4, 5, 6, 7, 10, 12, 13, 16, 17, 18
		0.51-0.75	-	5(sm)	-	8, 9, 11, 14, 15
		0.26-0.50	-	-	-	
Medium(M)	5.55-6.14	0.76-1.00	16	-	-	-
		0.51-0.75	1	-	-	-
		0.26-0.50	-	-	-	-
Relatively short(s)	4.97-5.56	0.76-1.00	9	-	-	-
		0.51-0.75	8	-	-	-
		0.26-0.51	-	-	-	-
Short (S_2)	4.98 less than	0.76-1.00	11	-	-	-
		0.51-0.75	9	-	-	-
		0.26-0.51	-	-	-	-
Total=			**63**	**18**	**-**	**-**

Table 22. The allocation of unidentified chromosomes of *Triticum aestivum* to different morphological categories.

Type of chromosomes	Length classes 'X' (µm)	Arm ratio 'Y' (µm)	Total No. of chromosome in 3 haploid sets	No. of identified chromosome with names	Proposed unidentified chromosome	Assigned chromosome no.
Large (L)	6.03-6.60	0.76-1.00	14	14(m)	-	12,15
		0.51-0.75	-	2(sm)	-	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 16
		0.26-0.50	-	-	-	-
Medium(M)	5.46-6.02	0.76-1.00	12	-	-	-
		0.51-0.75	4	-	-	-
		0.26-0.50	-	-	-	-
Relatively short (S_2)	4.87-5.45	0.76-1.00	11	-	-	-
		0.51-0.75	10	-	-	-
		0.26-0.50		-	-	-
Short(S)	4.88 less than	0.76-1.00	8	-	-	-
		0.51-0.75	4	-	-	-
		0.26-0.50	-	-	-	-
Total=			**63**	**16**		

Table 23. Allocation of unidentified chromosomes of *Triticum durum* to different morphological categories.

Type of chromosomes	Length classes 'X' (µm)	Arm ratio 'Y' (µm)	Total No. of chromosome in 3 haploid sets	No. of identified chromosome with names	Proposed unidentified chromosome no.	Assigned chromosome no.
Large (L)	6.11-6.57 above	0.76-1.00	7	8(m)	-	1, 2, 3, 4, 5, 8, 10, 13,
		0.51-0.75	-	5(sm)	-	6, 7, 9, 11, 12
		0.26-0.50	-		-	-
Medium (M)	5.61-6.10	0.76-1.00	9	-	-	-
		0.51-0.75	1	-	-	-
		0.26-0.50	-	-	-	-
Relatively Short(S_2)	5.13-6.62	0.76-1.00	7	-	-	-
		0.51-0.75	8	-	-	-
		0.26-0.50	-	-	-	-
Short (S)	5.14 less than	0.76-1.00	4	-	-	-
		0.51-0.75	6	-	-	-
		0.26-0.50	-	-	-	-
Total=			**42**	**13**	**-**	

Table 24. Morphological features of the haploid complement of triticale BAT-1.

Chromosome no.	Name of identified chromosome	Total length	Arm ratio	Centromeric position	Chromosome type
1	sm_1	6.84	0.71	sm	L
2	m_1	6.83	0.78	m	L
3	m_2	6.60	0.83	m	L
4	m_3	6.58	0.86	m	L
5	sm_2	6.53	0.73	sm	L
6	m_4	6.42	0.86	m	L
7	m_5	6.39	0.80	m	L
8	m_6	6.31	0.95	m	L
9	m_7	6.16	0.78	m	M
10	m_8	6.12	0.85	m	M
11	sm_3	6.01	0.70	sm	M
12	m_9	5.92	1.00	m	M
13	m_{10}	5.86	0.81	m	M
14	sm_4	5.86	0.73	sm	M
15	m_{11}	5.74	0.96	m	M
16	m_{12}	5.72	0.91	m	M
17	m_{13}	5.71	0.96	m	M
18	m_{14}	5.56	0.85	m	S_1
19	m_{15}	5.56	0.76	m	S_1
20	sm_5	5.49	0.67	sm	S_1
21	m_{16}	5.48	0.80	m	S_1
22	m_{17}	5.33	0.89	m	S_1
23	m_{18}	5.30	0.85	m	S_1
24	sm_6	5.26	0.75	sm	S_1
25	sm_7	5.03	0.72	sm	S_2
26	m_{19}	4.98	0.84	m	S_2
27	m_{20}	4.88	0.78	m	S_2
28	m_{21}	4.81	0.85	m	S_2

Table 25. Morphological features of the haploid complement of triticale BAT-2.

Chromosome no.	Name of identified chromosome	Total length	Arm ratio	Centromeric position	Chromosome type
1	m_1	6.46	0.96	m	L
2	m_2	6.22	0.89	m	L
3	sm_1	5.93	0.73	sm	M
4	m_3	5.86	0.82	m	M
5	m_4	5.81	0.79	m	M
6	m_5	5.77	0.93	m	M
7	m_6	5.71	0.87	m	M
8	sm_2	5.65	0.66	sm	M
9	sm_3	5.47	0.70	sm	M
10	m_7	5.41	0.88	m	S_1
11	sm_4	5.36	0.64	sm	S_1
12	sm_5	5.36	0.74	sm	S_1
13	sm_6	5.34	0.68	sm	S_1
14	m_8	5.29	0.79	sm	S_1
15	m_9	5.16	0.84	m	S_1
16	m_{10}	5.16	0.90	m	S_1
17	m_{11}	5.06	1	m	S_1
18	sm_6	5.03	0.68	sm	S_1
19	m_{12}	4.95	0.92	m	S_1
20	m_{13}	4.94	0.87	m	S_1
21	m_{14}	4.91	0.92	m	S_2
22	sm_7	4.87	0.71	sm	S_2
23	m_{15}	4.78	0.79	m	S_2
24	m_{16}	4.72	1	m	S_2
25	sm_7	4.68	0.73	sm	S_2
26	m_{17}	4.63	0.96	m	S_2
27	m_{18}	4.56	0.85	m	S_2
28	m_{19}	4.42	0.91	m	S_2

Table 26. Morphological features of the haploid complement of triticale WRF.

Chromosome no.	Name of identified chromosome	Total length $\bar{x}$	Arm ratio	Centromeric position	Chromosome type
1	m_1	6.71	0.92	m	L
2	m_2	6.40	0.93	m	L
3	m_3	6.38	0.89	m	L
4	m_4	6.06	0.91	m	L
5	m_5	5.87	0.85	m	M
6	m_6	5.79	0.83	m	M
7	m_7	5.79	0.98	m	M
8	m_8	5.72	0.79	m	M
9	sm_1	5.68	0.64	sm	M
10	m_9	5.60	0.92	m	M
11	m_{10}	5.55	0.85	m	M
12	sm_2	5.45	0.71	sm	S_1
13	sm_3	5.19	0.64	sm	S_1
14	m_{11}	5.18	0.81	m	S_1
15	m_{12}	4.97	0.87	m	S_1
16	m_{13}	4.96	1.00	m	S_2
17	sm_4	4.91	0.73	sm	S_2
18	sm_5	4.84	0.66	sm	S_2
19	sm_6	4.80	0.73	sm	S_2
20	m_{14}	4.78	0.82	m	S_2
21	m_{15}	4.58	0.77	m	S_2

Table 27. Morphological features of the haploid complement of *Triticum aestivum*.

Chromosome no.	Name of identified chromosome	Total length $\bar{x}$	Arm ratio	Centromeric position	Chromosome type
1	m_1	6.60	0.93	m	L
2	m_2	6.44	0.88	m	L
3	m_3	6.14	0.83	m	L
4	m_4	6.12	0.94	m	L
5	m_5	6.12	0.97	m	L
6	m_6	5.96	0.78	m	M
7	m_7	5.94	0.98	m	M
8	m_8	5.75	0.82	m	M
9	sm_1	5.67	0.71	sm	M
10	m_9	5.66	0.89	m	M
11	sm_2	5.44	0.75	sm	S_1
12	m_{10}	5.32	1.00	m	S_1
13	sm_3	5.27	0.71	sm	S_1
14	m_{11}	5.26	0.92	m	S_1
15	m_{12}	5.07	0.83	m	S_1
16	sm_4	4.91	0.71	sm	S_1
17	sm_5	4.85	0.66	sm	S_2
18	m_{13}	4.81	0.86	m	S_2
19	sm_6	4.75	0.73	sm	S_2
20	m_{14}	4.61	0.78	m	S_2
21	m_{15}	4.45	0.79	m	S_2

Table 28. Morphological features of the haploid complement of *Triticum durum*.

Chromosome no.	Name of identified chromosome	Total length $\bar{x}$	Arm ratio	Centromeric position	Chromosome Type
1	m_1	6.57	0.94	m	L
2	m_2	6.34	0.90	m	L
3	m_3	6.19	0.94	m	L
4	m_4	6.13	0.90	m	L
5	m_5	5.89	0.99	m	M
6	m_6	5.74	0.85	m	M
7	sm_1	5.64	0.74	sm	M
8	m_7	5.56	0.97	m	S_1
9	sm_2	5.43	0.72	sm	S_1
10	sm_3	5.30	0.68	sm	S_1
11	m_8	5.22	0.83	m	S_1
12	sm_4	4.95	0.70	sm	S_2
13	m_9	4.84	0.82	m	S_2
14	sm_5	4.66	0.73	sm	S_2

Table 29. Analysis of length, arm ratios, relative length, TCL%, centromeric position and chromosome type of metaphase chromosome in triticale BAT-1.

Name of variety		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
triticale BAT-1	Short arm length (µ)	2.93	3.00	3.00	2.56	2.74	2.96	2.82	3.08	2.70	2.82	2.48	2.96	2.63
TF% =40.42	Long arm length (µ)	3.91	3.83	3.60	4.02	3.79	3.46	3.62	3.23	3.46	3.30	3.53	2.96	3.23
TCL = 158.54	Total arm length (µ)	6.84	6.83	6.60	6.58	6.53	6.42	6.39	6.31	6.16	6.12	6.06	5.92	5.86
	Arm ratios	0.71	0.78	0.88	0.86	0.73	0.86	0.80	0.95	0.78	0.85	0.70	1.00	0.81
	Relative length	100	99.85	96.49	96.20	95.47	93.86	93.42	92.25	90.05	89.47	87.87	86.55	85.67
	TCL%	4.31	4.31	4.16	4.15	4.12	4.05	4.03	3.98	3.89	3.86	3.79	3.73	3.70
	Centromeric position	sm	m	m	m	sm	m	m	m	m	m	sm	m	m
	Chromosome type	L	L	L	L	L	L	L	L	M	M	M	M	M

XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII	XXIV	XXV	XXVI	XXVII	XXVIII
2.48	2.74	2.26	2.78	2.56	2.40	2.26	2.48	2.51	2.44	2.26	2.10	2.26	2.10	2.18
3.38	3.00	3.46	2.93	3.00	3.16	3.23	3.00	2.82	2.86	3.00	2.93	2.72	2.78	2.63
5.86	5.74	5.72	5.71	5.56	5.56	5.49	5.48	5.33	5.30	5.26	5.03	4.89	4.88	4.81
0.73	0.96	0.91	0.96	0.85	0.76	0.67	0.80	0.89	0.85	0.75	0.72	0.84	0.78	0.85
85.67	83.92	83.63	83.48	81.29	81.29	80.26	80.12	77.92	77.89	76.90	73.54	70.61	69.15	68.17
3.70	3.62	3.61	3.60	3.51	3.51	3.46	3.46	3.36	3.34	3.32	3.17	3.05	3.02	2.95
sm	m	m	m	m	m	sm	m	m	m	sm	sm	m	m	m
M	M	M	M	S_1	S_1	S_1	S_1	S_1	S_1	S_1	S_2	S_2	S_2	S_2

Table 30. Analysis of length, arm ratios, relative length, TF%, Total chromatin length, TCL%, chromosome type, centromeric position of metaphase chromosome in triticale BAT-2.

Name of variety		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Triticale BAT-2	Short arm length (µ)	3.16	2.93	2.50	2.63	2.55	2.77	2.66	2.25	2.26	2.52	2.09	2.28	2.16
TF% = 44.9	Long arm length (µ)	3.30	3.29	3.43	3.23	3.26	3.00	3.05	3.40	3.22	2.89	3.27	3.08	3.18
TCL = 147.95	Total arm length (µ)	6.46	6.22	5.93	5.86	5.81	5.77	5.71	5.65	5.47	5.41	5.36	5.36	5.34
	Arm ratios	0.96	0.89	0.73	0.82	0.79	0.93	0.87	0.66	0.70	0.88	0.64	0.74	0.68
	Relative length	100	96.28	91.80	90.71	89.93	89.32	88.39	87.46	83.75	83.75	82.97	82.97	82.66
	TCL%	4.38	4.22	4.02	3.97	3.94	3.91	3.87	3.83	3.71	3.67	3.63	3.63	3.62
	Centromeric position	m	m	sm	m	m	m	m	sm	sm	m	sm	sm	sm
	Chromosome type	L	L	M	M	M	M	M	M	M	S_1	S_1	S_1	S_1

XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII	XXIV	XXV	XXVI	XXVII	XXVIII
2.33	2.36	2.44	2.53	2.03	2.36	2.29	2.33	2.02	2.10	2.36	1.96	2.27	2.09	2.10
2.96	2.80	2.72	2.53	3.00	2.59	2.65	2.58	2.85	2.68	2.36	2.72	2.36	2.47	2.32
5.29	5.16	5.16	5.06	5.03	4.95	4.94	4.91	4.87	4.78	4.72	4.68	4.63	4.56	4.42
0.79	0.84	0.90	1.00	0.68	0.92	0.87	0.92	0.71	0.79	1.00	0.73	0.96	0.85	0.91
81.89	79.88	79.88	78.33	77.86	76.63	76.47	76.01	75.39	73.99	73.07	72.45	71.67	70.59	68.42
3.59	3.50	3.50	3.43	3.40	3.36	3.35	3.33	3.30	3.24	3.20	3.17	3.14	3.09	2.99
m	m	m	m	sm	m	m	m	sm	m	m	sm	m	m	m
S_1	S_1	S_1	S_1	S_1	S_1	S_1	S_2	S_2	S_2	S_2	S_2	S_2	S_2	S_2

Table 31. Analysis of length, arm ratios, relative length, TCL%, centromeric position and chromosome type of metaphase chromosome in triticale WRF.

Name of variety		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI
Triticale WRF	Short arm length (µ)	3.22	3.08	3.00	2.89	2.70	2.62	2.86	2.52	2.32	2.67	2.55	2.26	2.03	2.32	2.31	2.48	2.06	1.92	2.02	2.11	1.99
TF% = 45.70	Long arm length (µ)	3.49	3.32	3.38	3.17	3.17	3.17	2.93	3.20	3.45	2.93	3.00	3.20	3.16	2.86	2.66	2.48	2.85	2.92	2.78	2.67	2.59
TCL = 113.44	Total arm length (µ)	6.71	6.40	6.38	6.06	5.87	5.79	5.79	5.72	5.68	5.60	5.55	5.46	5.19	5.18	4.97	4.96	4.91	4.84	4.80	4.78	4.58
	Arm ratios	0.92	0.93	0.89	0.91	0.85	0.83	0.98	0.79	0.64	0.92	0.85	0.71	0.64	0.81	0.87	1.00	0.73	0.66	0.73	0.82	0.77
	Relative length	100.00	95.38	95.08	87.48	87.48	86.14	86.14	85.25	84.65	83.46	82.71	81.37	77.34	77.20	74.07	73.92	73.17	72.13	71.54	71.23	68.26
	TCL%	5.92	5.64	5.62	5.34	5.17	5.10	5.10	5.04	5.01	4.94	4.89	4.81	4.58	4.57	4.38	4.37	4.33	4.27	4.23	4.21	4.04
	Centromeric position	m	m	m	m	m	m	m	m	sm	m	m	sm	sm	m	m	m	sm	sm	sm	m	m
	Chromosome type	L	L	L	M	M	M	M	M	M	M	M	S_1	S_1	S_1	S_1	S_2	S_2	S_2	S_2	S_2	S_2

Table 32. Analysis of length, arm ratios, relative length, TCL%, centromeric position and chromosome type of metaphase chromosome in *Triticum aestivum.*

Name of variety		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI
Triticum aestivum	Short arm length (µ)	3.18	3.00	2.78	2.86	2.96	2.62	2.94	2.59	2.36	2.66	2.33	2.66	2.18	2.52	2.29	2.06	1.99	2.22	1.99	2.02	1.97
TF% = 45.62	Long arm length (µ)	3.42	3.44	3.36	3.26	3.16	3.34	3.00	3.16	3.31	3.00	3.11	2.66	30.90	2.74	2.78	2.85	2.86	2.59	2.76	2.59	2.48
TCL = 114.37	Total arm length (µ)	6.60	6.44	6.14	6.12	6.12	5.96	5.94	5.57	5.67	5.66	5.44	5.32	5.27	5.26	5.07	4.91	4.85	4.81	4.75	4.61	4.45
	Arm ratios	0.93	0.88	0.83	0.94	0.79	0.78	0.98	0.82	0.71	0.89	0.75	1.00	0.71	0.92	0.83	0.70	0.66	0.86	0.73	0.78	0.79
	Relative length	100.00	97.58	93.03	92.73	92.73	90.30	90.00	84.39	85.91	85.76	82.42	80.61	79.85	79.70	76.82	74.39	73.48	80.17	71.97	69.85	67.42
	TCL%	5.77	5.63	5.37	5.35	5.35	5.21	5.19	4.87	4.96	4.94	4.76	5.65	4.61	4.60	4.43	4.29	4.24	4.21	4.15	4.03	3.89
	Centromeric position	m	m	m	m	m	m	m	m	sm	m	sm	m	sm	m	m	sm	sm	m	sm	m	m
	Chromosome type	L	L	L	L	L	M	M	M	M	M	S_1	S_1	S_1	S_1	S_1	S_1	S_2	S_2	S_2	S_2	S_2

Table 33. Analysis of length, arm ratios, relative length, TCL%, centromeric position and chromosome type of metaphase chromosome in *Triticum durum*.

Name of variety		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Triticum durum	Short arm length (µ)	3.19	3.00	3.00	2.89	2.93	2.63	2.40	2.74	2.26	2.14	2.36	2.03	2.18	1.96
TF% = 46.32	Long arm length (µ)	3.38	3.34	3.19	3.24	2.96	3.11	3.24	2.82	3.17	3.16	2.86	2.92	2.66	2.70
TCL = 77.10	Total arm length (µ)	6.57	6.34	6.19	6.13	5.89	5.74	5.64	5.56	5.43	5.30	5.22	4.95	4.84	4.66
	Arm ratios	0.94	0.90	0.94	0.90	0.99	0.85	0.74	0.97	0.72	0.68	0.83	0.70	0.82	0.73
	Relative length	100.00	96.50	94.22	93.30	89.65	87.37	85.84	84.63	82.65	80.67	79.45	75.34	73.67	70.93
	TCL%	8.52	8.22	8.03	7.95	7.64	7.44	7.32	7.21	7.04	6.87	6.77	6.42	6.28	6.04
	Centromeric position	m	m	m	m	m	m	sm	m	sm	sm	m	sm	m	sm
	Chromosome type	L	L	L	L	M	M	M	S_1	S_1	S_1	S_1	S_2	S_2	S_2

Table 34. Morphological features of the proposed standard karyotype of triticale BAT-1, *Triticum aestivum*, triticale WRF, and *Triticum durum*.

Name of triticale lines and *Triticum* species	Chromosome number	Total no of identified chromosome	Total no of unidentified chromosome	Chromosome type				Proposed chromosome formula	Karyotype formula
				L	M	S_1	S_2		
triticale BAT -1	n=28	23	5	8	9	7	4	21m+7sm	$8L^{6m+2sm}+ 9M^{7m+2sm}+ 7S_1^{5m+2sm}+ 4S_2^{3m+1sm}$
triticale BAT-2	n=28	18	10	2	7	11	8	19m+9sm	$2L^{2m}+ 7M^{4m}+ 3^{sm}+ 11S_1^{7m+4sm}+ 8S_2^{6m+2sm}$
triticale WRF	n=21	18	3	3	8	4	6	15m+6sm	$3L^{3m}+ 8M^{7m+1sm}+ 4S_1^{2m+2sm}+ 6S_2^{3m+3sm}$
Triticum aestivum	n=21	16	5	5	5	6	5	15m+6sm	$5L^{5m}+ 5M^{4m+1sm}+ 6S_1^{3m+3sm}+ 5S_2^{3m+2sm}$
Triticum durum	n=14	13	1	4	3	4	3	9m+5sm	$4L^{4m}+ 3M^{2m+1sm}+ 4S_1^{2m+2sm}+ 3S_2^{1m+2sm}$

4.2.4.6 Chromosome identification of *Triticum aestivum*, *Triticum durum* and *Secale cereal* based on karyotypic formula through graphical representation

The sets of values of the commonly identified chromosomes of three triticale lines and two *Triticum* species were plotted on a dimensional scatter diagram (Fig. 26). Those points (TCL and arm ratios) which were close to each other and belonged to different symbols on the diagram were considered. Here triticale BAT-1 (*Triticum aestivum* × *Secale cereal)* is a hybrid line. Triticale WRF (*Triticum durum* × *Secale cereal*) is another hybrid line. In this graph the circle indicated the relationship between triticale BAT-1, *Triticum aestivum* and *Secale cereale* and also between triticale WRF, *Triticum durum* and *Secale cereale*, analyzed with the help of the data taken from chromosome morphology in inbred rye by Waheeb K. Heneen (1961).

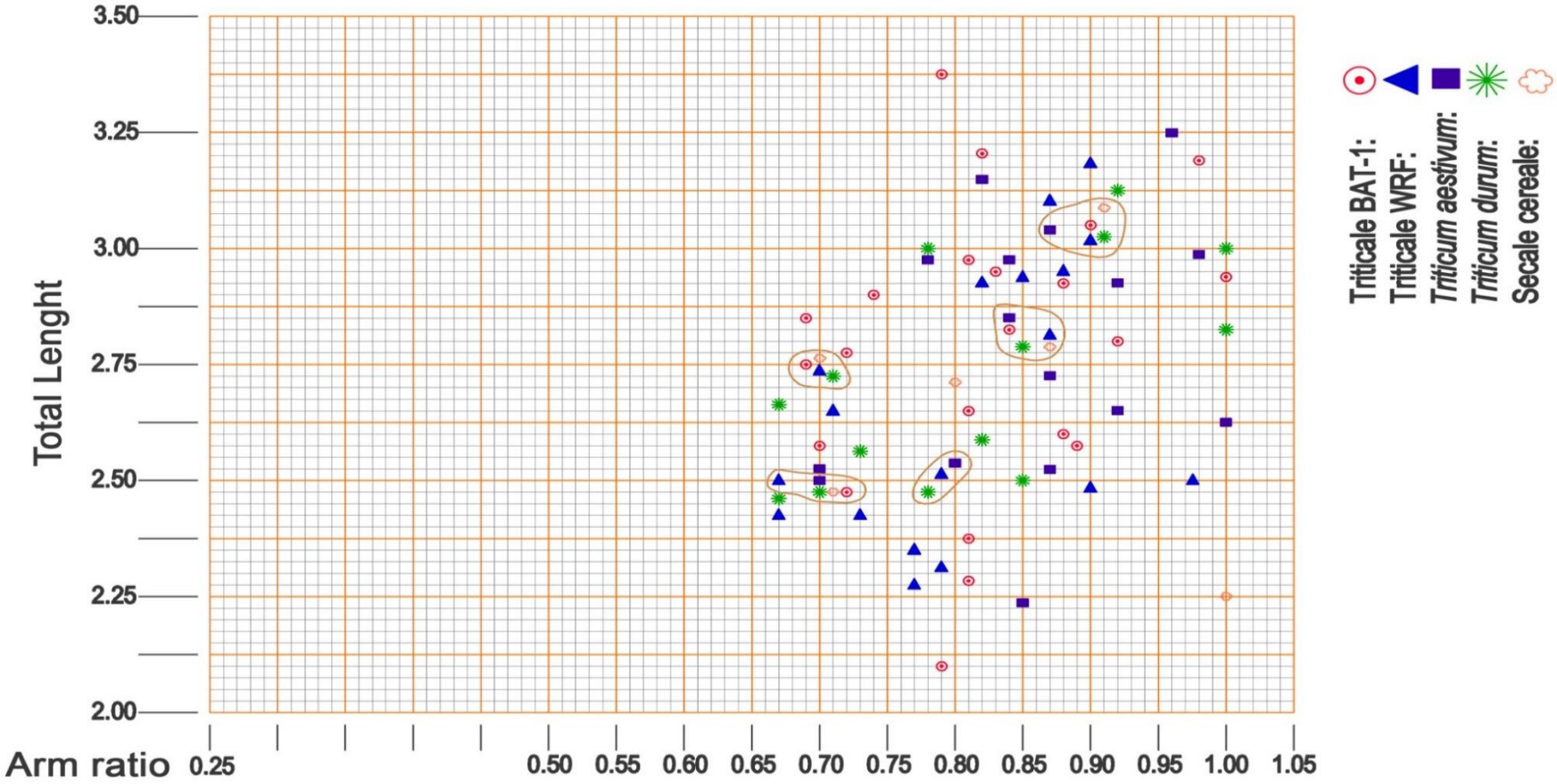


Fig. 26: Diagram showing relationship in case of identified chromosomes of triticale BAT-1, triticale WRF, *Triticum aestivum* and *Triticum durum* and *Secale cereale* (the data of *Secale cereale* was used from the findings of Heneen, W.K., 1961).

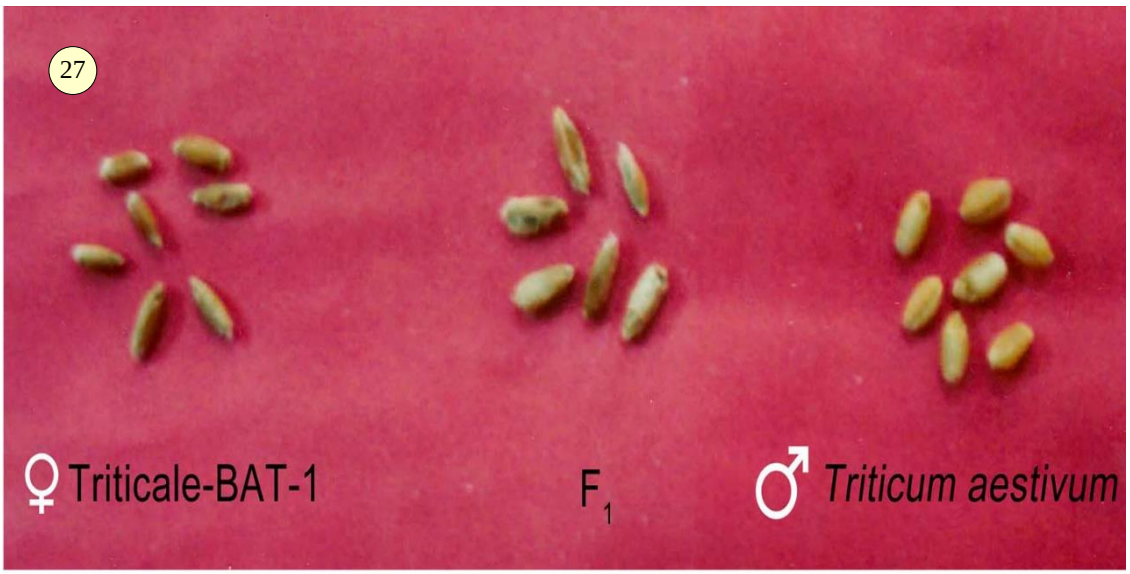
4.3 Analysis of crossing program: Crossing program among triticale lines BAT-1 and WRF and two *Triticum* species were made reciprocally during three growing seasons (2008-09, 2009-10 and 2010-11). To obtain the intergeneric hybrid attempts were made in several ways. But results were very poor (Table 35).

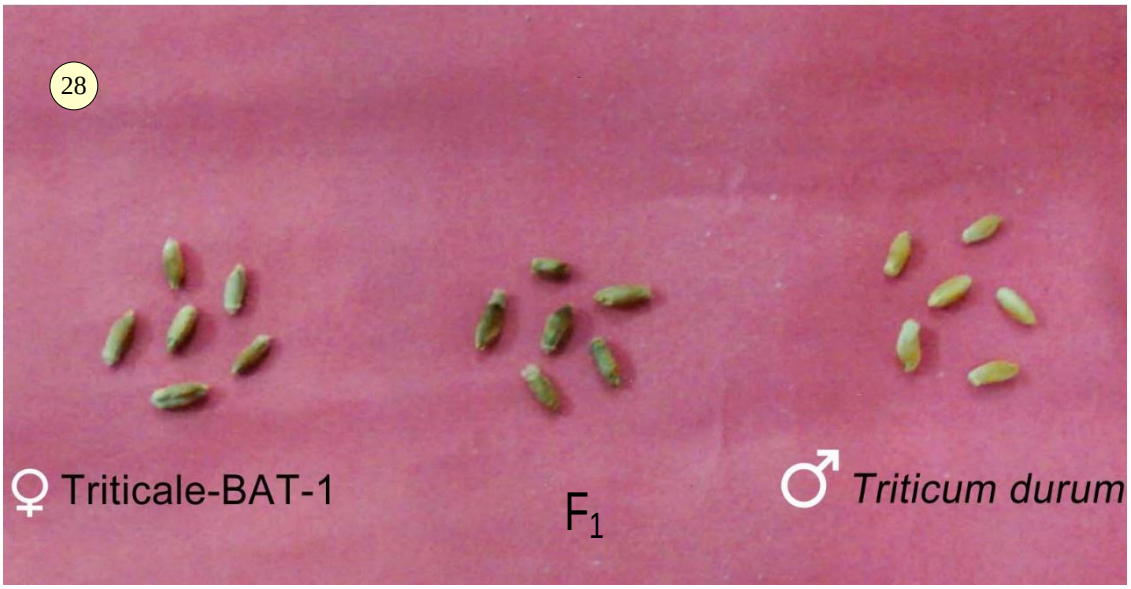
Table 35. Seed set, seed germination, seedling viabilities and seed set in F_1 plants of eight cross combinations.

Cross combination	Pollinated florets during 2008-09, 2009-10 &2010-11	Total seed set	% of seed set	Seed germi-nation	% of seed germi-nation	Seedling viabilities	% of Seedling viabilities	F_1 plants obtained	Seed set in F_1 Plants
triticale BAT-1× *Triticum aestivum*	315	15	4.76	4	26.67	2	50	1	Nil
Triticum aestivum ×triticale BAT-1	300	12	4.00	3	25.00	1	33.33	1	Nil
triticale BAT-1× *Triticum durum*	310	12	3.87	4	33.33	0	0	0	Nil
Triticum durum× triticale BAT-1	320	16	5.00	3	18.75	1	33.33	0	Nil
triticale WRF× *Triticum aestivum*	250	10	4.00	2	20.00	1	50.00	1	Nil
Triticum aestivum ×triticale WRF	280	14	5.00	2	14.29	1	50.00	1	Nil
triticale WRF× *Triticum durum*	320	15	4.69	3	20.00	1	33.33	1	Nil
Triticum durum× triticale WRF	270	11	4.07 $\bar{x}$=4.42	2	18.18 $\bar{x}$=21.77	1	0 $\bar{x}$=31.25	1	Nil

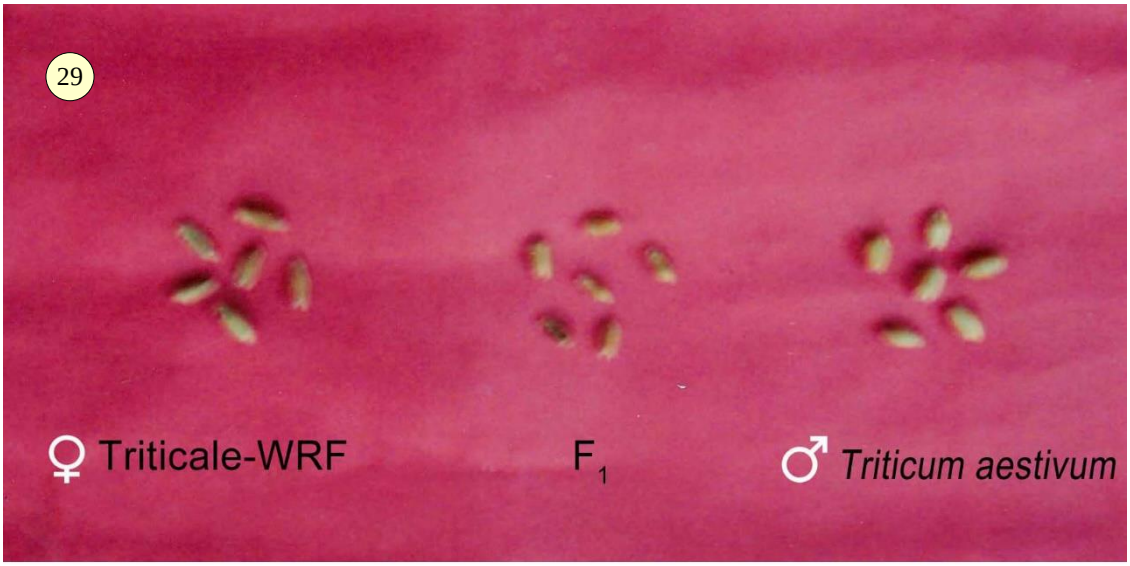
4.3.1 Morphology of hybrid seed: Hybrid seeds of triticale BAT-1×*Triticum aestivum* triticale BAT-1×*Triticum durum*, triticale WRF×*Triticum aestivum*, triticale WRF× *Triticum durum* (Figs. 27-30) were found to be very weak, shriveled and smaller in size. Rest of the crosses showed hybrid seeds to be intermediate in size and shape compared to that of the parents.

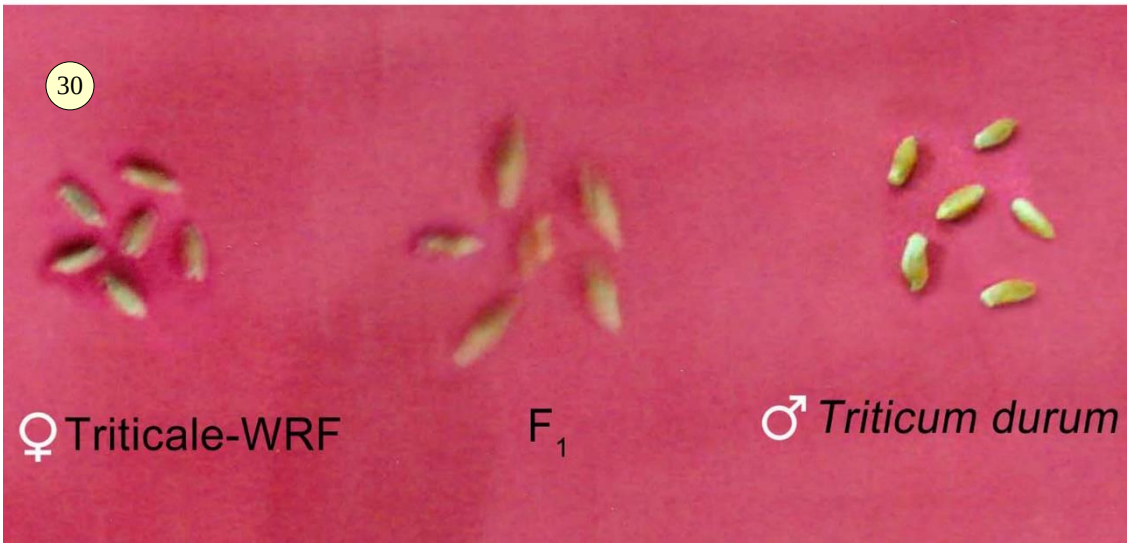
4.3.2 Seedling morphology: At the age of 30-40 days, morphologically F_1 plants were very weak. Their height was found to be intermediate mostly. The hybrid plants were unhealthy. A few hybrid seedlings were grown in MS medium (Figs. 31-33). The germinating seeds were transferred in plastic pots (Fig. 34) containing moist soil, but they were not found to survive. F_1 plants did not set seed but F_1 of *T. aestivum*×triticale BAT-1 and F_1 of *T. durum*×triticale WRF gave very weak inflorescence, which have been described later.



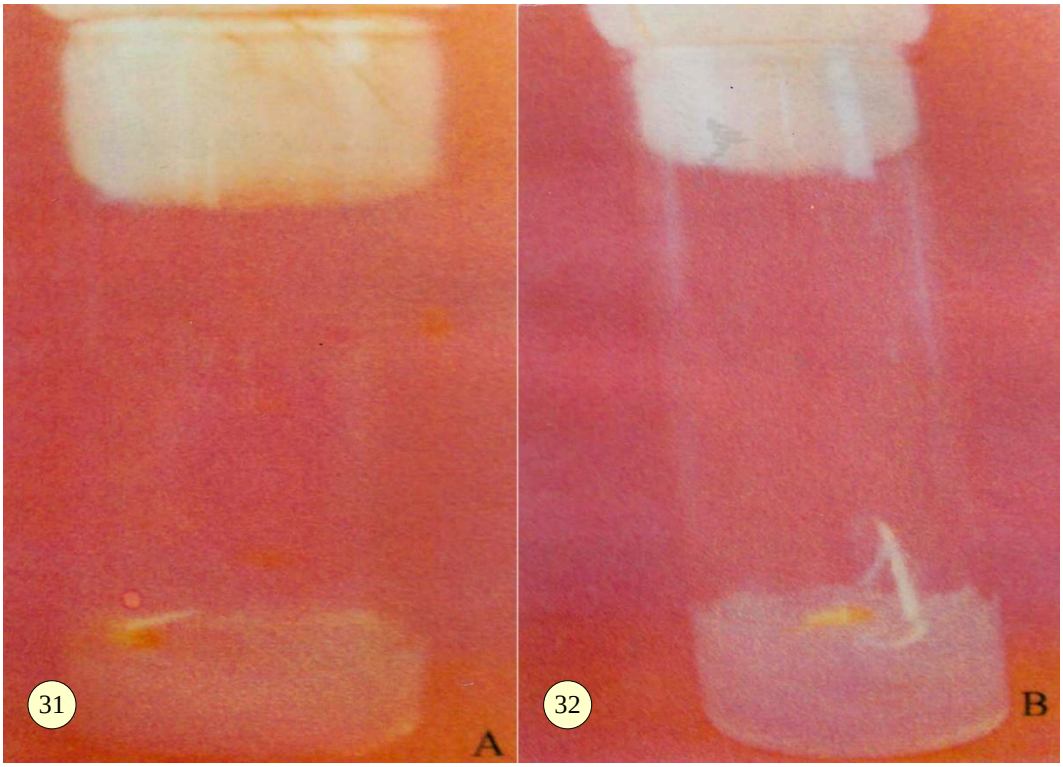


Figs. 27-28: Seed morphology of the F_1s and their respective parents.

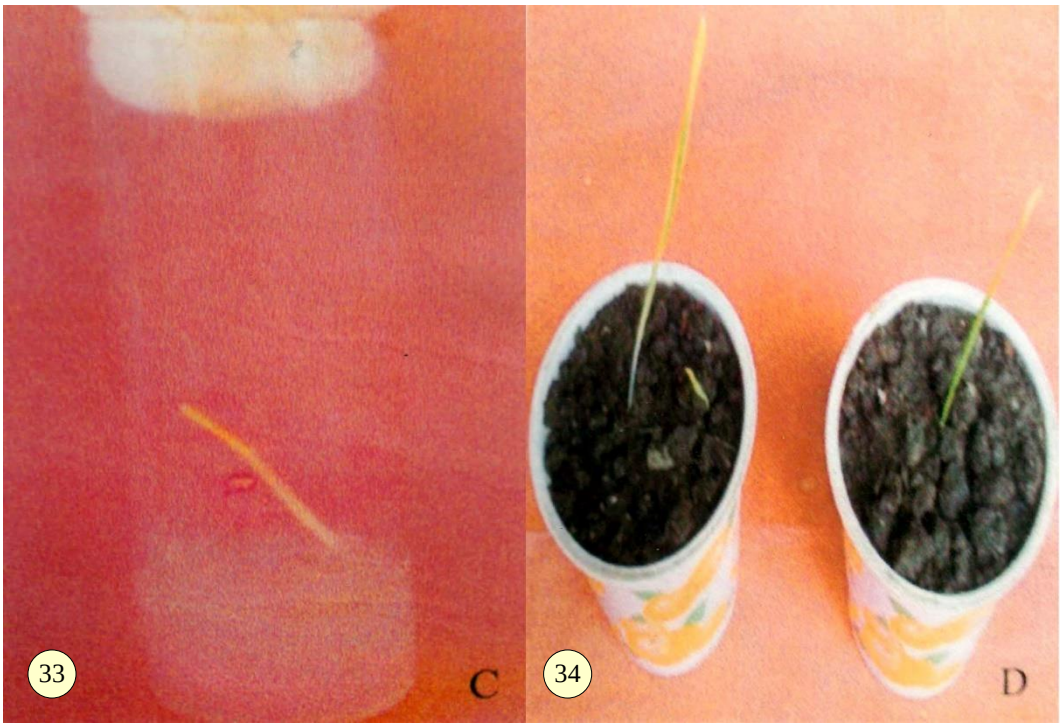




Figs. 29-30: Seed morphology of the F_1s and their respective parents.



Figs. 31-32: Emergence of root and shoot of the triticale×*Triticum aestivum* crossed seeds (shriveled) on MS medium.

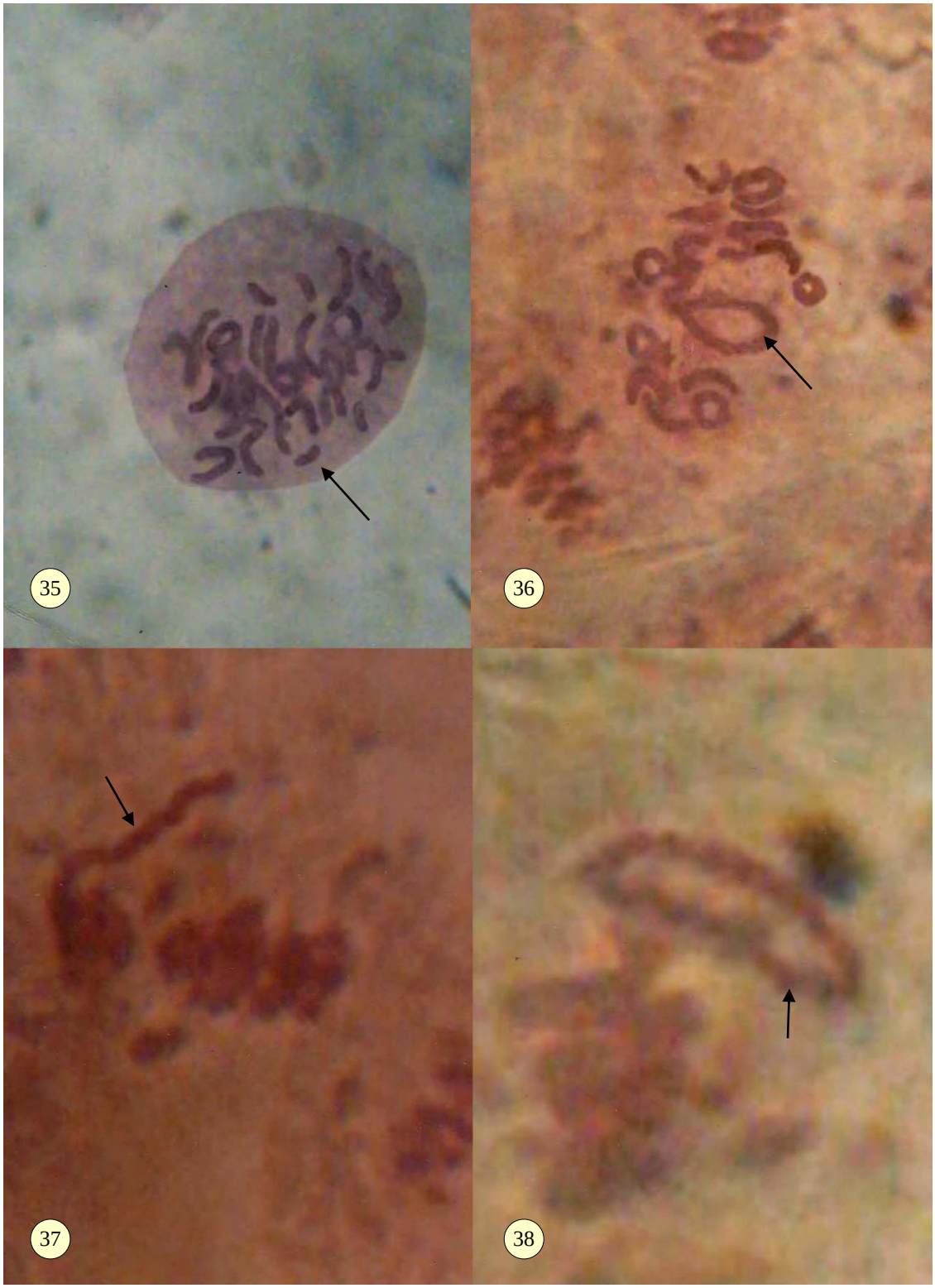


Figs. 33-34: Emergence of root and shoot of the *Triticum* species and triticale crossed seeds (shriveled) on MS medium; (34) Transplanted plantlets in plastic pots.

4.4 Chromosome combination studies through chromosome association and chiasma frequencies

The chromosome association and chiasma frequency in three different lines of triticale and *Triticum* species were studied. The data was recorded from diakinesis/metaphase-1 stages of meiosis Figs. 35-38.

Pattern of chromosome association and chiasma frequency in different genotypes in the present investigation were found to vary. Frequency of normal bivalent was very low and that of univalent was very high in all triticale lines. The values obtained for chromosome association and chiasma frequency are given in Table 36 & 37. The Table 36 reveals that the presence of univalent was highest in triticale WRF (1.41%) and the lowest value in triticale BAT-2 (0.99%). Trivalent was found in all triticale lines. The highest value 0.29% was found in case of triticale BAT-2 and the lowest value 0.13% was found in triticale BAT-1. Another configuration like quadrivalent was also found. The highest value for quadrivalent (0.36%) was found in triticale WRF and the lowest value (0.23%) was found in triticale BAT-1. The highest value for chiasmata per bivalent was found in triticale BAT-1 and in triticale WRF. On the other hand chiasma frequency for *Triticum* species (Table 37) were recorded and compared with that of triticale. The value for univalent was 0 in *Triticum aestivum* and the value of univalent was 2 in case of *Triticum durum*. Similarly trivalent and quadrivalent were not present in *Triticum aestivum* but in *Triticum durum* trivalent and quadrivalent were found and these values were very low. The chiasmata per bivalent was found to be 1.85 in *Triticum aestivum* (Table 37) which is highest than all triticale line and also than that of *Triticum durum*. Chiasmata per cell in *Triticum aestivum* was higher (38.95) than all triticale lines and also from that of *Triticum durum*.



Figs. 35-38: Chromosome association in triticale lines and *Triticum* species (arrow headed are univalent, trivalent and quadrivalent).

Table 36. Chromosome association and chiasma frequency in different lines of triticale.

Name of varieties	Number of chromo-some	Total cells	year	% of association					% of chiasmata		Chiasmata	
				Ring	Rod	Univ-alent	Triv-alent	Quari-valent	Term-inal	Inter-stitial	Per cell	Per bivalent
triticale	56	140	I	35.35	64.65	1.11	0.23	0.23	34.87	0.11	19.59	0.71
BAT-1	56	115	II	38.52	61.48	1.13	0.13	0.38	37.95	0.09	21.30	0.77
Total=				73.87	126.13	2.24	0.36	0.61	72.82	0.20	40.89	1.48
triticale	56	140	I	32.74	65.49	1.19	0.29	0.24	33.90	0.10	19.09	0.69
BAT-2	56	121	II	36.98	63.02	0.99	0.15	0.24	36.54	0.13	20.54	0.74
Total=				69.72	128.51	2.18	0.44	0.48	70.44	0.23	39.63	1.43
triticale	42	137	I	34.51	67.26	1.09	0.21	0.31	43.06	0.10	18.13	0.66
WRF	42	148	II	38.61	61.39	1.41	0.23	0.36	37.93	0.11	15.98	0.77
Total=				74.12	128.65	2.50	0.44	0.67	80.09	0.21	34.11	1.43
				$\bar{x}$=36.12	**$\bar{x}$=63.88**							

Table 37. Chromosome association and chiasma frequency of varieties of *Triticum aestivum* and *Triticum durum.*

Name of varieties	Number of chromosome	Total cells	year	% of association					% of Chiasmata		Chiasmata	
				Ring	Rod	Univ-alent	Triv-alent	Quari-valent	Term-inal	Inter-stitial	Per cell	Per bivalent
Triticum aestivum	42	133	I	2588	205	0	0	0	5175	5	38.75	1.85
Triticum durum	28	121	ii	1495	195	2	2	0	2990	4	24.74	1.77

Table 38. Percentages of meiotic irregularities in different lines of triticale.

Name of line/ varieties	No of cell studies	Year	Percentages of laggards	Percentages of bridges	percentages of fragment	Total percentages of irregularities
triticale	185	I	3.24	3.78	1.62	8.56
BAT-1	195	II	3.58	4.10	2.56	10.26
Total	**380**		**$\bar{x}$=3.41**	**$\bar{x}$=3.94**	**$\bar{x}$=2.09**	**$\bar{x}$=9.46**
triticale	200	I	4.00	5.00	2.00	11.00
BAT-2	210	II	4.76	5.71	1.90	11.90
Total	**410**		**$\bar{x}$=4.34**	**$\bar{x}$=5.36**	**$\bar{x}$=1.95**	**$\bar{x}$=11.45**
triticale	190	I	3.12	4.21	1.58	8.95
WRF	220	II	4.55	4.55	1.36	10.45
Total	**410**		**$\bar{x}$=3.84**	**$\bar{x}$=4.38**	**$\bar{x}$=1.47**	**$\bar{x}$=9.70**

Table 39. Percentages of meiotic irregularities and pollen sterility in *Triticum aestivum* and *Triticum durum*.

Name of line / varieties	No. of cell studies	year	Percentages of laggards	Percentages of bridges	Percentages of fragment	Total percentages of irregularities	Percentages of sterility
Triticum aestivum	150	I	1.33	1.33	0.66	3.33	5.33
	180	II	1.67	1.11	1.11	3.44	5.00
Total	**330**		**$\bar{x}$=1.50**	**$\bar{x}$=1.22**	**$\bar{x}$=0.89**	**$\bar{x}$=3.39**	**$\bar{x}$=5.12**
Triticum durum	165	I	1.82	1.21	0.60	3.64	4.24
	175	II	1.71	1.14	0.57	3.43	2.86
Total	**340**		**$\bar{x}$=1.77**	**$\bar{x}$=1.18**	**$\bar{x}$=0.58**	**$\bar{x}$=3.54**	**$\bar{x}$=3.55**

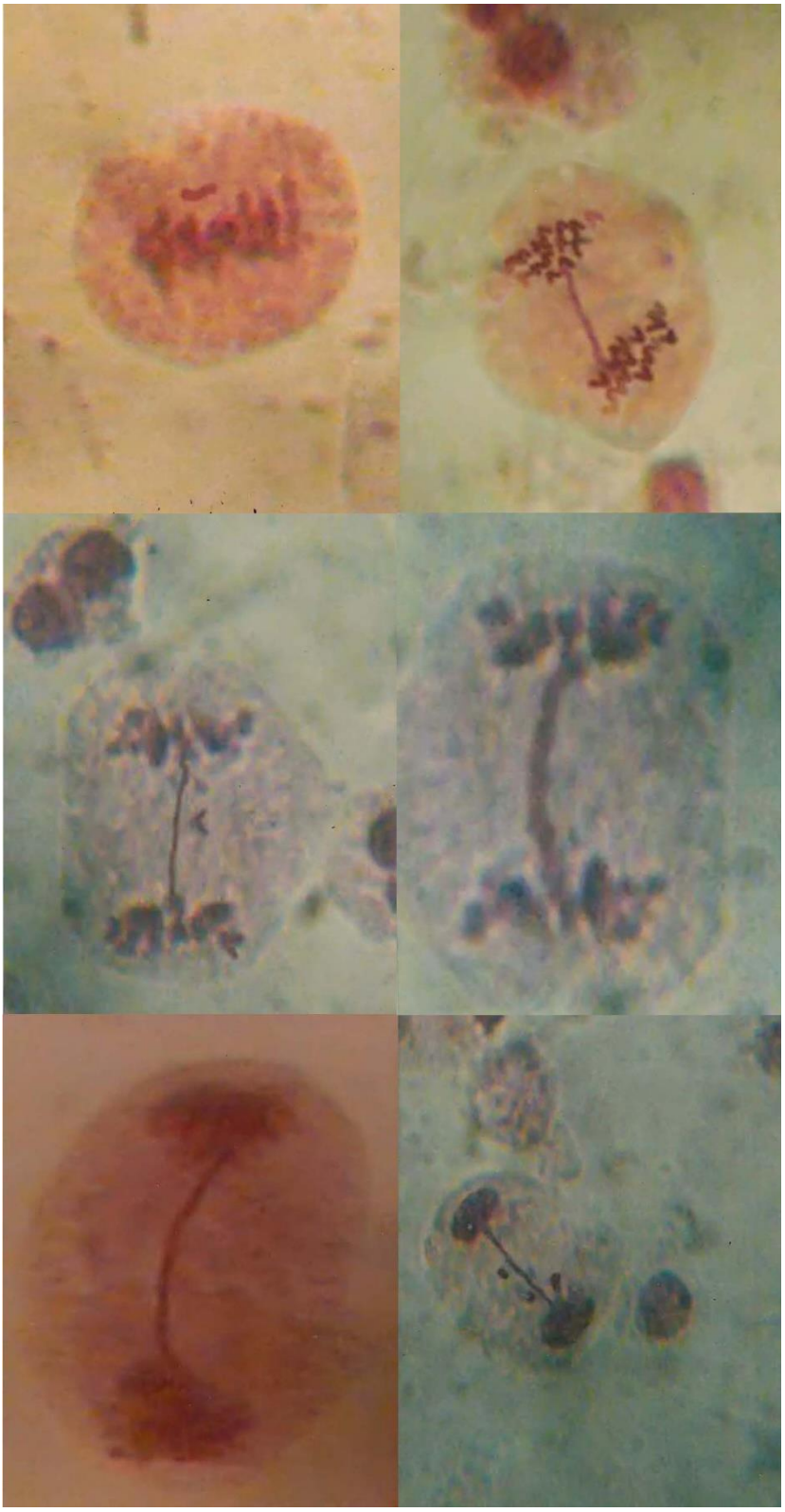
4.5 Chromosomal anomalies in pollen mother cells of triticale lines and *Triticum* species

4.5.1 Meiotic irregularities: Pollen mother cells collected from triticale lines and *Triticum* species were examined for asserting the meiotic irregularities. It was made at different stages of meiosis and the frequency of the irregularities was recorded.

The different type of irregularities such as laggards, bridges, fragments, unequal chromosome distributions etc. were observed frequently and these are shown in Figs. 39-44. The values recorded for meiotic irregularities are presented in Tables 38-39.

The highest percentage (4.38) for laggard chromosome was found triticale BAT-2 and lowest was found to be 3.41 in triticale BAT-1. The highest percentage of chromosome/ chromatid bridges was found in triticale BAT-2 (5.36) and lowest in triticale BAT-1 (3.94). The highest percentage of fragment was found to be 2.09 in triticale BAT-1 and the lowest was 1.47 in triticale WRF. The percentage of irregularities varied from 11.45 (triticale BAT-2) to 9.46 (triticale BAT-1). In *Triticum aestivum* and *Triticum durum* the percentage value for laggard was found to be 1.77, 1.50, respective. The highest percentage of bridges was found to be 1.22 in *Triticum aestivum* and lowest 1.18 in *Triticum durum*. The highest percentage of fragment was found to be 0.89 (*Triticum aestivum*) and the lowest value found to be 0.58 (*Triticum durum*).

4.5.2 Study of pollen grain abnormality and pollen sterility: The values for pollen sterility in *Triticum* species and that for pollen grain abnormality in triticale lines are presented in Tables 39 and 40, respectively. The percentage of pollen sterility was found to be 5.12 (*Triticum aestivum*) and the value was found to be 3.55 (*Triticum durum*) (Fig. 53). In triticale line pollen sterility were found to be 48.90% in triticale BAT-1, 55.58% in triticale BAT-2 and 54.86% in triticale WRF. The highest percentage 37.80 was found in triticale BAT-2 and the lowest percentage value 30.25 was found in triticale BAT-1. This result exposed that percentages of pollen sterility in triticale lines were higher



Figs. 39-44: Chromosome irregularities in triticale lines and *Triticum* species.

than the *Triticum* species (Fig. 53). The pollen grain abnormalities as like as binuclei, trinuclei, tetranuclie etc. were frequently found. The highest percentage value found to be 43.14 in triticale BAT-1. The lowest value found to be 34.55 in triticale BAT-2. Figs. 45-52 showing different types of pollen grain abnormality and pollen sterility.

Table 40. Different types of pollen grain abnormalities and percentages of pollen sterility in different lines of triticales.

Name of line / varieties	No. of cell studies	No. of pollen grain nucleus				Total	% of pollen grain abnormality	% of pollen sterility
		Mono-nucleate	Binucleate	Trinucleate	Tetra-nucleate			
triticale BAT-1	51	9	7	4	2	22	43.14	48.90
triticale BAT-2	55	8	5	3	3	19	34.55	55.58
triticale WRF	60	10	7	4	2	23	38.33	54.86
Total=	**166**	**$\bar{x}$=9.00**	**$\bar{x}$=6.33**	**$\bar{x}$=3.67**	**$\bar{x}$=2.33**	**$\bar{x}$=21.33**	**$\bar{x}$=38.67**	**$\bar{x}$=53.11**

4.6 Analysis of meiotic pairing: To identify the chromosomes of wheat (*Triticum aestivum*) and Rye (*Secale cereal*) in triticale lines this analysis was made and are presented in Table 41.

Here hexaploid wheat (*Triticum aestivum*) and Rye (*Secale cereal*) chromosomes made the meiotic pairing in all triticale lines. Table 41 reveals that there were 1560 ring and 930 open bivalent chromosome of wheat, and 290 ring and 136 (OL-102 & OS-34) open bivalent chromosome of Rye (*Secale cereal*) use present in triticale BAT-1. There the association of wheat chromosome and 8I+2III+2IV rye chromosome were found to be present in triticale BAT-1.

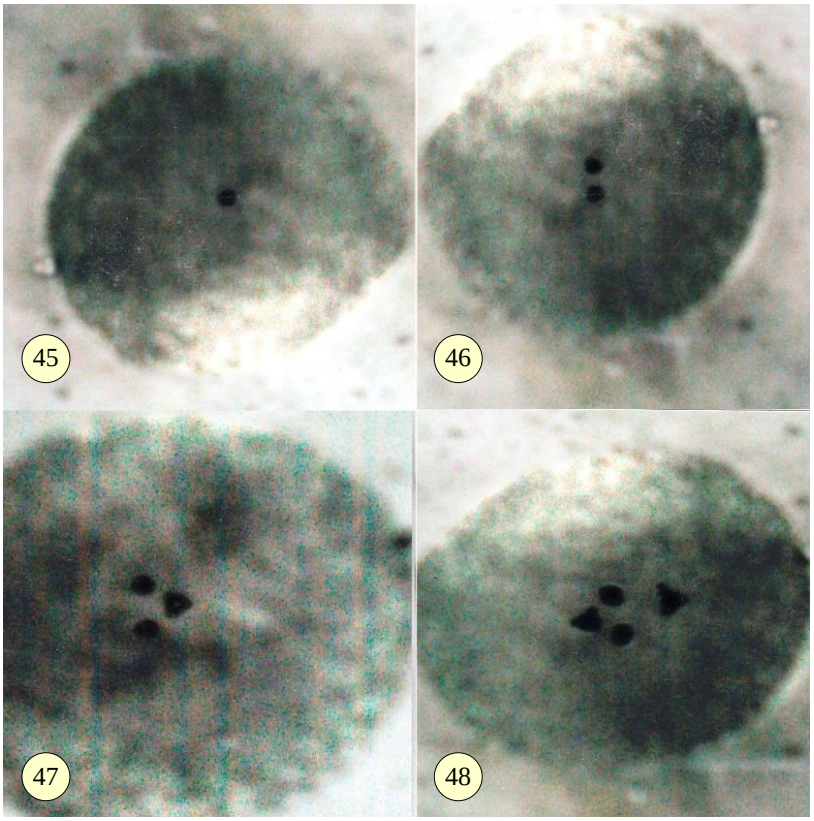
In triticale BAT-2 there were 1414 (Ring), 928 open bivalent of wheat and 305 ring and 130 (OL-105, OS-25) open bivalent chromosome of rye. The association of wheat chromosome (8I+2III+3IV) and rye (7I+1III+2IV). In triticale WRF there were 1565 (ring), 860 open bivalent of wheat and 295 (ring) and 142 (110-OL & OS-32) open bivalent of rye. The association of wheat 10I+2III+2IV and 6I+2III+2IV rye chromosome found in triticale WRF.

Table 41: Meiotic pairing in different type of triticale lines.

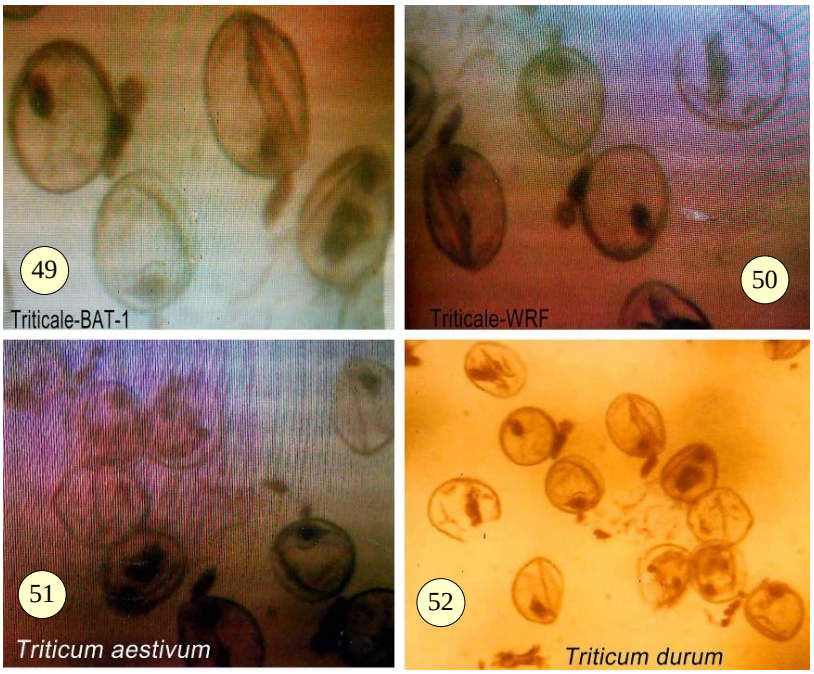
Name of lines	Total cell	Chromo-some number	Wheat (*Triticum aestivum and Triticum durum*) chromosome						Rye (*Secale cereal*) chromosome*					
			Ring	Open bivalent		Univ-alent	Triv-alent	Quadri-valent	Ring	Open bivalent		Univ-alent	Triv-alent	Quadri-valent
				OL	OS					OL	OS			
triticale BAT-1 (*T. aestivum* × *Secale cereale*)	105	2n=56	1560	710	220	10	2	3	290	102	34	8	2	2
triticale BAT-2 (*T. aestivum* × *Secale cereale*)	100	2n=56	1414	618	310	8	2	3	305	105	25	7	1	2
Triticale WRF (*T. durum* × *Secale cereal*)	102	2n=42	1565	575	285	10	2	2	295	110	32	6	2	2

Note: OL= Long arm bound, OS= Short arm bound.

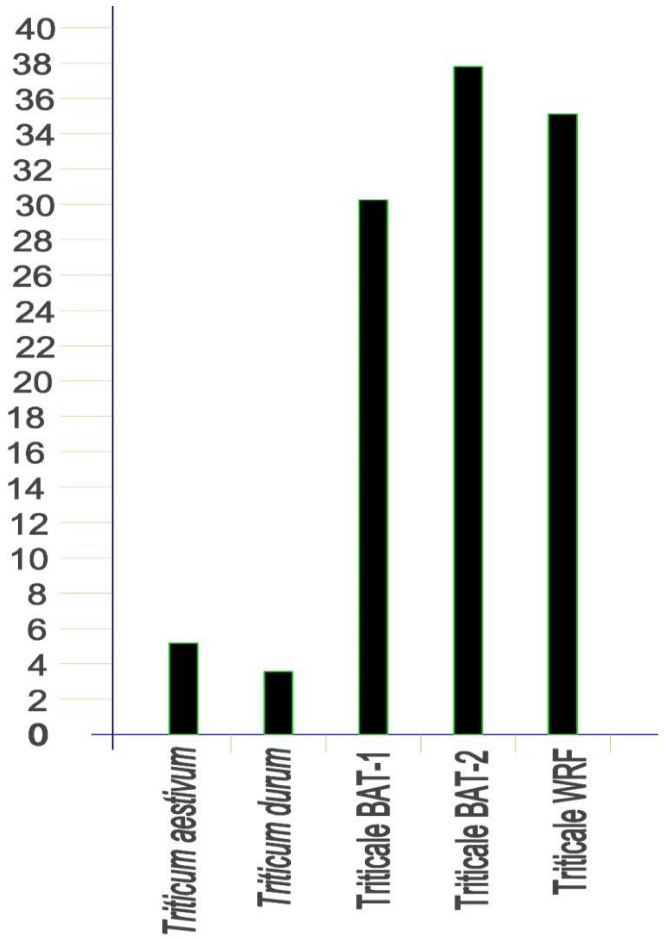
* Data obtained from Orellena J. *et al.* (1984)



Figs. 45-48: Pollen grain abnormalities in triticale lines.



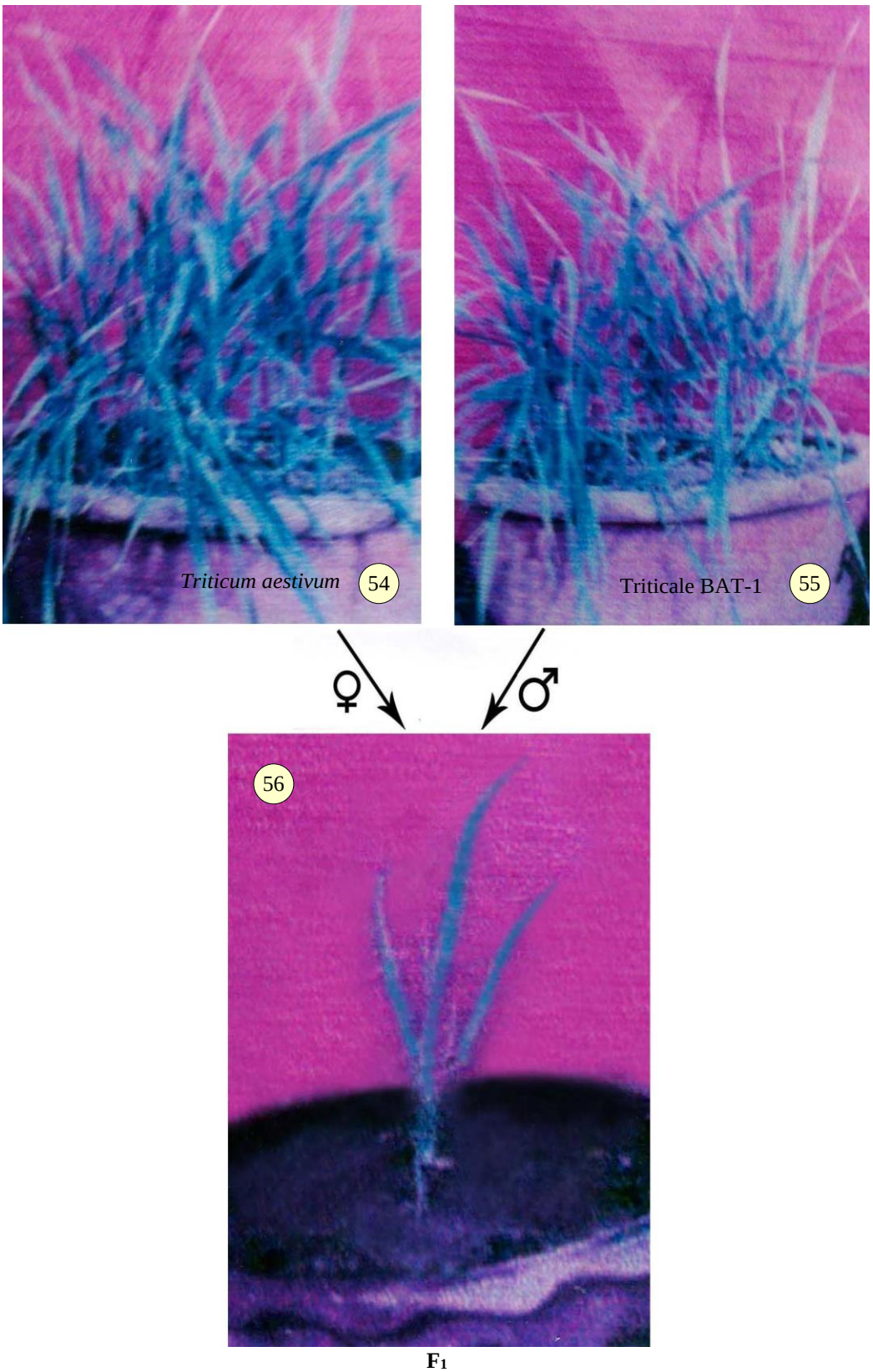
Figs. 49-52: Pollen sterility in triticale lines and *Triticum* species.



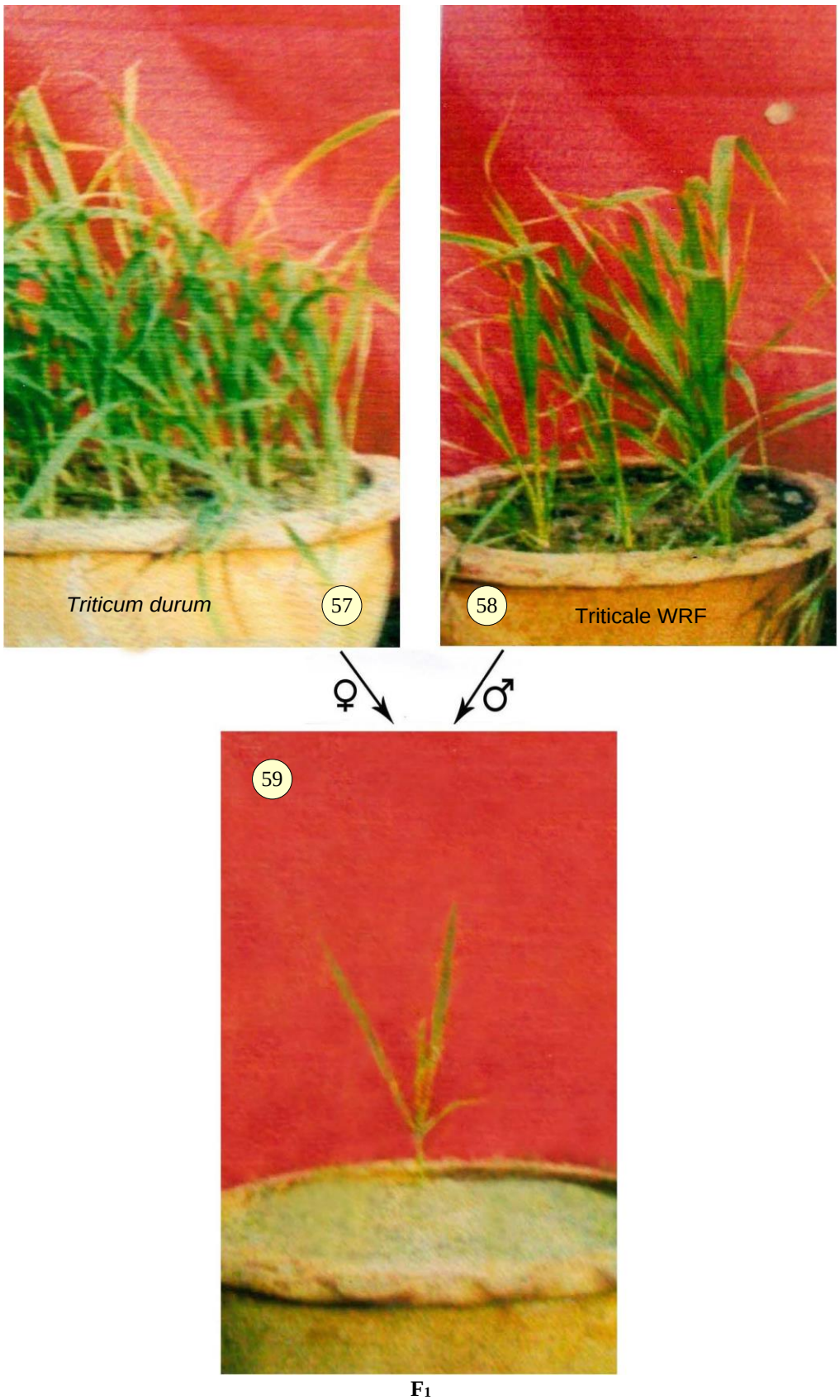
Figs. 53: Histogram showing percentage of pollen sterility in *Triticum aestivum*, *Triticum durum* and in triticale BAT-1, BAT-2 and triticale WRF.

4.7 Univalency in hybrid lines of *Triticum* species and triticale: Two F_1 plants viz F_1 of *Triticum aestivum* and triticale BAT-1, and F_1 of *Triticum durum* and triticale WRF were found to survive somehow in nature (Fig. 54-59). They failed to set the seed, but they had been able to emerge the inflorescence, although they were weak.

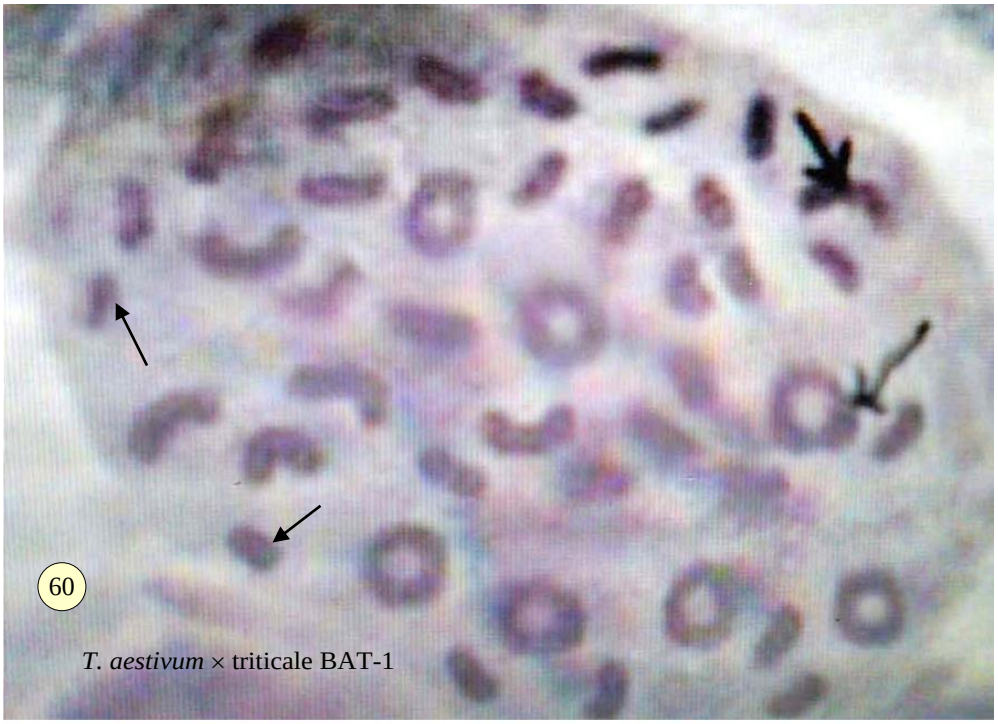
However, their inflorescence were collected and preserved for studying their meiotic behaviour. In both the lines preponderance of univalent was observed (Figs. 60-61).

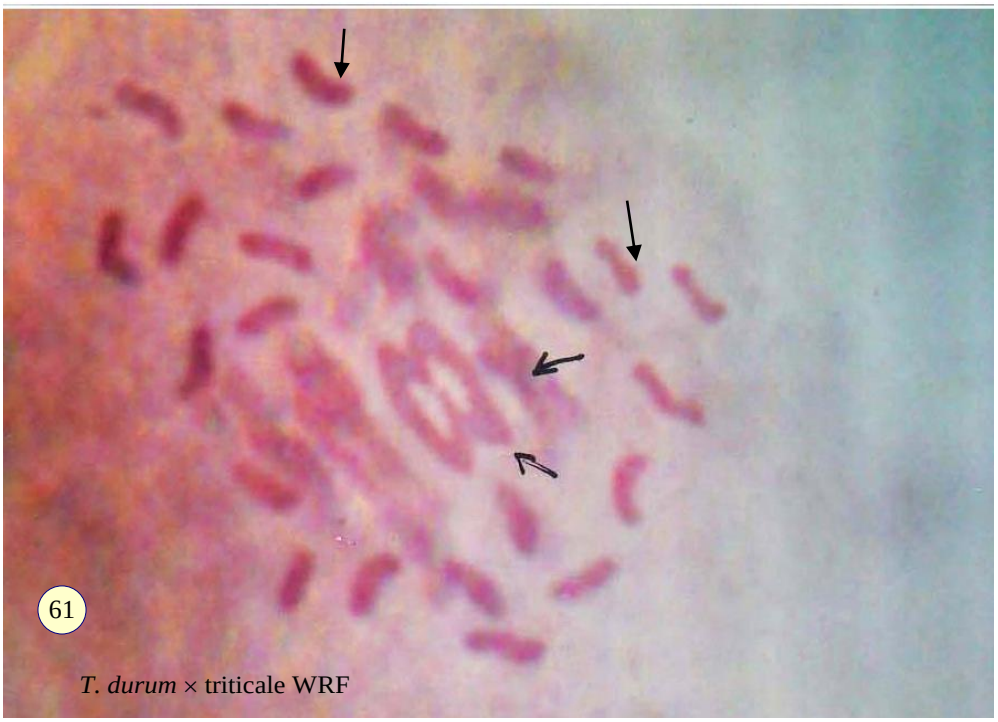


Figs. 54-56: Morphology of the hybrid seedling along with their parents.



Figs. 57-59: Morphology of the hybrid seedling along with their parents.





Figs. 60-61: Diakinesis showing chromosomal association of hybrid lines. (60) Preponderence of univalent (arrow headed) in F_1 (*Triticum aestivum* × triticale BAT-1; (61) Preponderence of univalent in F_1 (*Triticum durum* × triticale WRF). Ring and rod bivalent (arrow headed) in PMC of both the hybrid.

Table 42. Univalency in hybrid of *Triticum* species and triticale.

Name of hybrid varieties	Chromosome number	No of univalent	Percentages of univalent	No of bivalent		Percentages of bivalent	
				Ring	Rod	Ring	Rod
Triticum aestivum × triticale BAT-1	49-50	26	59.18	7	5	14.29	10.20
Triticum durum × triticale WRF	35-36	18	65.71	3	6	8.57	17.14

In addition with univalent percentage that of ring and rod bivalent were also estimated and the values are given in Table 42. In both the hybrids frequency of ring and rod bivalents were almost same. Percentage of univalent in both the cases were many times higher compared to that of bivalent. The findings indicated the dominancy of univalent which is enough for making any pollen mother cell sterile.

DISCUSSION

Triticale is the first man made cereal by intergeneric breeding of wheat (*Triticum aestivum*, a hexaploid) and Rye (*Secale cereale*, a euploid) grass species. Triticale is not completely fertile because they have one set chromosome from each parent and these cannot combine during meiosis and thus normal gametes are not formed. In meiosis duplication and deficiency are reported to have the role for this behaviour in triticale. In **1875 Wilson** reported in his work that crosses between wheat as a female and rye as a male, F_1 triticale was found infertile because of the reason mentioned above i.e. failure of parental chromosome combination. Present Investigation on meiosis revealed a frequent occurrence of irregularities caused by the presence of univalent chromosome. These were observed in all the cases studied in present investigation. It was therefore regarded to be characteristic of wheat –rye amphidiploids in general and from that point of view different parameters were taken under consideration for finding the reasons behind this behaviour and those are justified by significant discussion as follows:

5.1 Interphase nuclear phenotype: Lafontaine (1974) stated that the structural organization in plant cell nuclei are two types, chromocentric and reticulate. In the present study interphase nuclei of meristametic cells of triticale and *Triticum* species were found to be reticulate. Interphase nuclear phenotype in terms of nuclear volume (NV) and interphase chromosome volume (ICV) in three lines of triticale and two species of *Triticum* in the present investigation were found to vary among them. Mean values for nuclear volume (35.99) was highest in triticale BAT-1 and lowest (26.89) in triticale WRF. Mean value for NV and ICV in *Triticum durum* were lowest. Nuclear volume and interphase nuclear volume were found to be dependent proportionally on the number and size of chromosomes. The number and size of triticale chromosomes except WRF were always highest and more than that of *Triticum* species. But ICV was found with decreased value in all triticales lines compared to that of *Triticum* species. It might be due to hybrid character where chromosome complement from two different sources were of different nature in many aspects. **Linde-Laursen and Von Bothmer (1984)** reported in case of *Hordeum vulgare* × *Psathyrostaclys fragillis* hybrids that two genomes in the hybrid were differentiated by lengths. In *P. fragillis* chromosomes by 31% longer than the *Hordeum vulgare* chromosome. The *P. fragillis* chromosomes were characterized by diminished centric constrictions, suppression of nuclear constrictions and nucleolus activity. Almost similar phenomenon of chromosomes of hybrid have exhibited at the stage of interphase where chromosomes are stranded by the synthesis of nucleic acid.

5.2 Intergeneric chromosome combination:- First of all in order to identify the somatic chromosomes of triticale and *Triticum* species, their root tip cells were treated with saturated solution of paradichlorobenzene (PDB) for 4 to 5 hours at 10°C and this treatment gave better results for spreading the metaphase chromosomes. The somatic chromosome numbers were found to be 2n=56 in triticale BAT-1 and triticale BAT-2, 2n=42 in triticale WRF, 2n=42 *in Triticum aestivum* and 2n=28 in *Triticum durum*.

Ahmed *et al.* (1983) stated that a quantitative method of karyotypic analysis following conceptual basis and standard morphology, establish the standard karyotype and this give the knowledge of the morphological properties of the chromosomes in details. Present findings followed this concept and on that basis, two assumptions were involved. First, in a scatter diagram of the total length and arm ratios of all chromosomes in a number of cells, the points produced by the same chromosome would tend to cluster in a specific region. The present result revealed secondly, two nonhomologous chromosomes would be identifiable individually on a morphological basis if in a scatter diagram of several cells, the mean location of one chromosome occurred no less than one standard deviation away from that of the other, with respect to either total length or arm ratio. The chromosomes which were not distinguishable individually could be assigned to different morphological categories on a probability basis. The chromosomes were paired by circling the corresponding points on the scatter diagram on the basis of proximity of two points. In cases where more than two points occurred close together, the chromosomes were re-examined under the microscope to establish how each pair should be the established. The 28 pairs of points were considered to represent homologous chromosomes in triticale BAT-1 and triticale BAT-2, on the other hand 21 pairs of points are considered in triticale WRF and *Triticum aestivum,* and 14 pairs of points were considered in *Triticum durum*.

In present investigation the chromosome pairs were numbered from 1 to 28 in triticale BAT-1 and triticale BAT-2, 1 to 21 in triticale WRF and in *Triticum aestivum*, and 1 to 14 in *Triticum durum*. The unidentified chromosomes were allocated to the various morphological classes. The haploid chromosome complement were numbered from decreasing order of length following **Rhoades (1955).** The number was used as identification of mark and thus each chromosome was allocated to a serial identification number. In case of aneuploid species (**Hadley and Hymowitz, 1976; Palmer, 1974, 1976a&b; Palmer and Heer, 1976**), the specific chromosomes involved could not be identified. It is considered that this method of analysis will allow such identification, where aneuploidy involves a chromosome which was individually identifiable, and the precise nature of this aberration may be specific with respect to the proposed karyotype. Conversely, if the aneuploidy involves a chromosome which was not individually

identifiable, it will be possible to specify the morphological category where that chromosome belongs. Quantitative method helped to develop a standard karyotype based on what the chromosomes belonging to different lines of triticales and two *Triticum* species were identified. The chromosomes may not always have the same total length (**Sindhu *et al*., 1982**) because of variation from cell to cell and differences in fixation. The chromosome morphology varies from cell to cell and thus, major changes are associated with the cell division process also. The chromosome length of all triticale lines and *Triticum* species varied from cell to cell. The class interval 0.49µm for chromosome length was chosen arbitrarily and the range for arm ratio as recommended by **Kutarekar and Wanjari (1983)** were followed.

In present investigation the range of mean values of the identified chromosome were 3.37-2.09µm in triticale BAT-1, 3.18-2.26µm in triticale WRF and 3.07-2.38µm in triticale BAT-2. Similarly the range of mean value were 3.24-2.23 in *Triticum aestivum* and 3.13-2.36µm in *Triticum durum*. Present results reveals significant differences in terms of chromatin length (158.54µm) and TF% (40.42) in triticale BAT-1, TCL (147.95µm) and TF% (44.97) in triticale BAT-2, total chromatin length 113.44µm and TF% (45.70) in triticale WRF. On the other hand the total chromatin length 114.37µm and TF% (45.62) in *Triticum aestivum* and TCL (77.10µm) and TF% (46.32) in *Triticum durum*. Detailed description of the karyotype was made by **Bhattacharya and Jenkis (1960)** on Dakold rye and by **Evans and Jenkins (1960)** on wheat- rye addition products, containing individual pairs or single chromosome types of rye. In their system chromosomes I-III are combined into a separate type, considering devoid of secondary constrictions, while the other entire chromosome were individually differentiated. The chromosome pairs ii and iv with submedian and subterminal centromeres corresponded to chromosomes 3 and 5, respectively. In present investigation the identified chromosome of triticale BAT-1 were 17 metacentric and 6 submetacentric but here no satellite chromosome pair was found. In triticale BAT-2, 11 metacenric and 7 submetacentric chromosomes were found with no sat chromosome. Similarly triticale WRF showed that 13 chromosomes were metacentric of which a pair showed satellites and 5 were submetacentric. This result was almost similar to that reported by **Shigenag and Larter (1971)**. Their report revealed 5 satellited, 5 median, 9 submedian, and 2 subterminal pairs of chromosome in the hexaploid triticale. In present study of *Triticum aestivum*, 14 metacentric and 2 submetacentric, and also in *Triticum durum* 8 metacentric and 5 submetacentric chromosomes could be identified and no satellite chromosome was found in both the *Triticum* species. All this results supported the

results reported by **Ahsan *et al.* (1998).** Thus, the chromosome formula there were found to be similar from morphological point of view among triticale lines as well as between two *Triticum* species. From karyotypic features of triticale, when *Triticum* species is one of the parents of the polygenetic relationship among them has not been confirmed yet cytologically and genetically. The Fig. 26 indicated the possible pathway for the identification the specific chromosome from triticale lines which received the chromosomes from *Triticum aestivum, Triticum durum* and *Secale cereale*. The indication is consistent with postulations of several authors particularly of **Sears (1948 & 1969) and Morrises and Sear (1967)**. In Fig 26, the data of *Secale cereale* was incorporated from the report given by **Hennen (1961)** were the result reveals that, there is a close relationship between *Triticum* species and *Secale cereale*. Triticale BAT-1 derived from the hybrid *Triticum aestivum*×*Secale cereale* and triticale WRF derived from the hybrid between *Triticum durum*×*Secale cereale*. It is stated that *Secale cereale* character is formed in both of octoploid and hexaploid triticale, but *Triticum* species does not show it always. In octoploid triticale (triticale BAT-1), there has a close relationship between *Triticum aestivum* (Hexaploid) and *Secale cereale*. On the other hand, in hexaploid triticale (triticale WRF), there has a close relationship between *Triticum durum* and *Secale cereale.* Rye (*Secale cereale*) is euploid. The basic number of rye is 7. When *Secale cereale* is hybridized with *Triticum aestivum* then one set chromosome of rye come and make gamete but not normal. In F_1 plant most of the rye chromosomes remain as univalent. Fig. 26 indicates that euploid and aneuploid line are hybridized with each other, which make amphidiploid line. **Chaubey and Khanna (1986)** reported that intergeneric hybrids between wheat and rye are utilized for transferring the desirable rye characteristics into wheat and to increase the genetic variability in amphidiploid genus triticale.

5.3 Data analysis of crossing program: *Triticum aestivum* and *Triticum durum* were hybridized with triticale lines (triticale BAT-1, triticale BAT-2 and triticale WRF) for studying all lines which have basic number of 7. Hybrids between wheat and rye were among the first intergeneric combinations obtained. Besides natural hybrid, wheat-rye hybrids have been made by breeders artificially. Triticale is a hybrid line already. Crosses of wheat with *Agropyron* species in attempts to transfer certain desirable characters to wheat sometimes are called *Agrotritium* hybrids. The F_1s between common wheat and *Agropyron trichophorum* are generally intermediate, but with many characters dominantly of *Agropyron* **(McFadden and Sears, 1947; Riley *et al.*, 1958)**. Such hybrids appear to be stable for certain morphological characters, but they carry genes for meiotic instability **(Marshall and Schmidt, 1954)**. Hybridization was also attempted between triticale cv. Welsh and *A. caninum* (4x) but no hybrid seeds were obtained

(Gupta and Fedak, 1986). In present study intergeneric crosses between two *Triticum* species (*Triticum aestivum, Triticum durum*) and three triticale lines (triticale BAT-1, triticale BAT-2 and triticale WRF) were made reciprocally for producing hybrids in the successive three growing seasons. The crosses were made in ambient condition. After hybridization between triticale BAT-1×Hexaploid wheat, triticale WRF×hexaploid wheat, triticale BAT-1×Tetraploid wheat and triticale WRF ×Tetraploid wheat (*Triticum durum*) seeds were found but their viability was very poor. Some of these seeds normally germinated on moist filter in petridishes. When they were transferred in field from petridishes they did not survive. A few of them were found to survive temporarily in MS medium in tissue culture laboratory. The percentages of germination of triticale BAT-1×*Triticum aestivum* was similar to that of another hybrid lines of triticale×*Triticum* species. Seed germination was found to range from 26.67 (triticale BAT-1×*Triticum aestivum*) to 14.29 (*Triticum aestivum*×triticale WRF). However, **Abdulaeve and Kurkiev (1991)** found 65.50% and 45.25% karyotypes to set seed and their 45.20% and 33.30% germinability when crosses were made between *Triticum boeticum* (2n=4x=28) with rye (2n=14) and triticale (2n=28), respectively. Seed set percentages in present study were found to range from 5.00-3.87 with mean value of 4.42%. **Martin and Chapman (1977)** reported seed set of *Hordeum chilense* and *Triticum aestivum* with very low percentage. **Fedak (1980)** stated that the wheat (*Triticum aestivum* cv. Chinese spring) and barley (*Hordeum vulgare*. Beetez) hybrids resulted at a frequency of 0.80% of pollinated florets.

Viability means the seeds capability of germinating and producing a normal seedling. Thus, it matters synonymously with germination capability. In this sense, a seed is either viable or nonviable, depends on its ability to germinate and produce a normal seedling. In the present observation the shriveled seeds were cultured in growth media for finding the plantlet and the young seedlings were transplanted in to potted soil maintaining the environmental condition. Several workers **(Mujeeb *et al.*, 1978; Fedak, 1982; Mujeeb and Rodrigauez, 1982, 1984)** raised the hybrid plants successfully after cutting the embryo. In present study some hybrid seed culture in media in laboratory and growing 1-2 plants were produced here. In that case the inflorescence of the hybrid could not be collected to study their meiosis. It may be mentioned here that the hybrid plants were quite similar to their respective parents from morphological points of view but did not survive and thus failed to set seed. **Fedak (1985)** propagated intergeneric hybrids of triticale through callus culture of immature inflorescence. **Fedak and Armstrong (1986)** found a total 16 seeds from 250 pollinating florets of *Secale cereale*×*Thinopyrum intermedium*. Only 5 of them contained embryo and striking only two germinated to produce seedlings. Similarly **Abdulaeva and Kurkiev (1991)** found less seedling

viability compared to the total seed setting of intergeneric hybrid in the cross of *Triticum boeticum*×rye and in the cross of *T. boeticum*×triticale. Almost similar results were found in the present study. Seed in all F_1 plants are nil. All the hybrid plants were sterile. However, **Dewey (1971)** found that the hybrid plants were particularly self-sterile. The F_1 plants of *Triricum aestivum*×*A. junceum* were completely male sterile because of non-dehiscent anthers **(Charpenties and Feldman, 1986a).** Percentages of seedling viabilities is higher in *Triticum aestivum*×triticale BAT-1 and percentages of seedling viabilities is in triticale BAT-1×*Triticum durum* and *Triticum durum*×triticale WRF in the present investigation.

In order to identify the somatic chromosomes of triticale lines and *Triticum* species and their intergeneric hybrids root tips of 40-45 days old hybrid seedlings were used for study as suggested by **Mujeeb *et al.* (1978).** The root tip cells were treated with saturated solution of paradichlorobenzene (PDB) for 4 to 5 hours at 10°C and this treatment gave better results for spreading the metaphase chromosomes. The somatic chromosome numbers were found to be 2n=49 in triticale BAT-1×*Triticum aestivum*, 2n=42 in triticale BAT-1×*Triticum durum*, 2n=42 in triticale WRF×*Triticum aestivum* and 2n=35 in triticale WRF×*Triticum durum*. The present results support the results of **Muntzing (1979)** who made crosses between triticale and bread wheat. triticale A and C were used as female parents. As expected, most of the F_1 plants in his experiment had 49 chromosomes but a few of them were aneuploid, a total of 194 F_2 plants were obtained almost all with chromosome numbers ranging from 42 to 49. Only 10 plants had chromosome numbers between 51 to 56.

Gupta and Fedak (1986) studied partial amphidiploids from wheat x rye crosses of 89 plants from 29 families and they reported five partial amphidiploids with chromosome numbers of 2n=35, 36, 38 and 41. In the hybrid between *Triticum aestivum*×*E. giganteus, Hordeum vulgare*×*E. giganteus* and *Hordeum vulgare*×*Triticum aestivum* chromosome composition was observed to be 2n=5x=35, 2n=4x=28, and 2n=2x=21, respectively by **Mujeeb and Rodriguez (1980 & 1984).** The morphology of chromosomes was found to vary from species to species and hybrids to hybrids. The karyotype analysis of triticale×Wheat F_1 hybrids was reported by **May and Apples (1982).** In the plants of F_1 generation, the total chromosome number varied from 2n=49, (n=24.5) where ½ denotes a telosome to 50 of which from one eight were rye chromosomes. In present investigation somatic chromosome number of hybrid triticale BAT-1×*Triticum aestivum* is 2n=49. Here chromosome pairs are 24 but a single one was unpaired. Another hybrid line triticale

WRF×*Triticum durum*, showed chromosome number to be 35 and here chromosome pairs were 17 and the single chromosome was like previous one.

In case of triticale BAT-1×*Triticum aestivum* hybrid among the identified chromosomes 13 were found to be metacentric and 7 submetacentric in pairs and which is single one that was also metacentric. This observation is in agreement with the study of **Santos *et al.* (1984)** for the wheat barley addition lines where they found two sattalied chromosomes. But in present investigation no sattalied chromosome was found. **Naskidashvili *et al.* (2009)** compared the seed viability and germinability of hybrid of triticale and hexaploid wheat reciprocally. When triticale as female parent and wheat varieties as pollinators were countered then comparatively viable seeds were obtained. The other way when hexaploid was used for female parent and triticale was used as pollinators then hybrid seed were not germinable. Present results support this result. Present investigation also indicated that when triticale BAT-1 was used as female plants and hexaploid wheat was used as pollinator the seeds obtained were comparatively viable for germination. Another way when hexaploid wheat (*T. aestivum*) was used as female parent and triticale BAT-1 was used as pollinator the hybrid seeds did not germinate.

In hybrid of triticale BAT-1×*Triticum durum* seeds were found very week for germinability. When triticale BAT-1 was used as female and *T. durum* used as pollinator then seed germinability was low. Again when *T. durum* was used as female and triticale BAT-1 was used as pollinator then seeds were viable or germinability was comparatively high. Here, the chromosome number of the hybrid line (triticale BAT-1 ×*T. durum*) was 42. The first hybrid between *Triticum durum* and rye was described in **1925 by Schegalow**. The first amphidiploids between tetraploid wheat and rye and their reciprocal crosses were reported by **Derzhavin (1938).** More interest from the breeding point of view was the amphidiploids between *Triticum durum* and cultivated rye (*Secale cereale*) produced by **O'Mara (1948)** and the combination of *Triticum durum* and rye made by **Nakajima (1950).** The first material used for the work in Canada was the tetraploid *durum*×rye amphiploid produced by **O'Mara (1948).** Several additional hexaploid triticales were developed from new crosses. In the present investigation seeds were obtained from the cross between octoploid triticale and tetraploid wheat. However, their viability was very poor. They revealed chromosome number as 42. It was also a hexaploid, where chromosome pairs were 21. **Dardey and Gustafson (1975)** reported the same result in triticale BAT-1×*Triticum durum*.

In **1973 Gustafson and Zillinsky** reported for the first time that hexaploid triticale× hexaploid wheat produced seeds with low germiability. All of the plants were sterile.

Lukaszewski (1986) reported about the hybrid between hexaploid triticale×hexaploid wheat where the seed viability was also poor. The present results were found to be supported by this results. Here, when triticale WRF was used as female and *Triticum aestivum* as pollinator then seed germination percentages was 20.00 and when *Triticum aestivum* was used as female and triticale WRF as pollinator then seed germination percentage was 14.29. The seed set in F_1 plant was nil in both of the hybrids.

The hybrid between *Triticum durum* Desf. and *Secale cereale* L. was reported by Aase in 1930 and the derived allopolyploid by O'Mara in 1948. The first hybrid between *Triticum durum* and rye was described in **1924 by Schegalow, Krolow (1964) and Sanchez-Monge (1956),** intercrossing tetraploid wheat and rye. **Kiss (1966a,b, 1971, 1974a,b)** produced his first hexaploid triticale in 1950 after hybridization between *Triticum turgidum* and *Secale cereale*. In present investigation triticale WRF is hexaploid wheat and *Triticum durum* is tetraploid. The chromosome number of their hybrid line was 35, where 17 were paired chromosomes and 1 chromosome was unpaired. Hsam *et al.* (1974) reported that seed development in triticale with tetraploid wheat cytoplasm is less good than in those with hexaploid wheat cytoplasm.

5.4. Chromosome combination studies though chromosome association and chaisma frequencies: From many studies of meiosis in triticale, it has become clear that chromosome pairing is not complete at metaphase-1. The genomic origin of the resultant univalent has become a subject of many investigations. It appears that the unpaired chromosomes are either proportional or non-proportional rye or wheat genome representatives. These univalents are considered to be the source of subsequent anaphase misdistributions. These univalents can also result either from lack of pachytene pairing (asynapsis) or from a precocious separation of associated chromosomes (desynapsis). The cytological details of meiosis in triticale are of interest because of fertility of the allpolyploid combination which depends on a normal meiotic division. Since triticales are not fully fertile, the nature of the anomalies is important. The chromosome number in triticale should be 56, which is twice the total genetic sum of the parents. If the meiosis were wholly normal, the cell at meiosis would have 28 pairs and each gamete would contain 28 different chromosomes, of which 21 would be wheat and seven would be rye. This allpolyploid, however, is not perfect and univalents do occur at meiosis. The number is different for different triticales, but all have the same. The result, therefore, is that numbers other than 56 are frequently found **(Berg and Oehler, 1938)**. The present result reflects the above statement. 56 chromosomes were frequently found from triticale (triticale BAT-1 and triticale BAT-2). The cytological analysis of triticale made by **Lewitsky and Benetzkja (1929, 1930 & 1931)** showed that the mode of meiosis in the

pollen mother cells as well as on the female side, revealed a frequent occurrence of irregularities caused by the presence of univalent chromosomes. **Lebedeff (1934),** the first one who reported the occurrence of aneuploidy in such material. A few plants had less than 56- chromosomes. Lebedeff discussed the reduced fertility of the wheat-rye amphidiploids and pointed out that poor fertility and other disturbances are characteristic of inbred lines of rye. Pattern of chromosome association and chaisma frequency in the different lines of triticale and *Triticum* species obtained in the present investigation were found to vary.

Weimarck (1975) studied meiosis of 9 F_1 in comparison with that of their parental lines and found chromosome combination to vary. In present study chromosome number in triticale BAT-1 and triticale BAT-2 were found to be 56 and that of triticale WRF was 42, which is reported to be of *Triticum durum*×*Secale cereale*. Due to lack of *Secale cereale* seeds in Bangladesh triticale lines were used to find out the race of gene part with *Triticum aestivum* and *Triticum durum*. This was confirmed by previous results of **Skutina and Kvostova (1971),** who worked with octoploid as well as hexaploid material. **Weimarck's (1975)** observations also supports the present findings. However, it is mainly the rye chromosomes that occur as univalents at metaphase-1. **Schlegel (1982)** reported meiotic chromosome pairing behavior of two monosomic rye-wheat additions. He found ring bivalent 4.44% (from 30 PMC) and rod bivalent 0.57%. In present investigation highest ring bivalent percentages (38.61) was recorded in hexaploid triticale WRF and lowest percentage (32.74) in triticale BAT-2. On the other hand, highest rod bivalent (65.49%) was found in triticale BAT-2 and lowest (61.39%) in triticale WRF. **Miller and Riley (1972)** observed meiotically, where the mean bivalent chromosome pairing was triticale (*Triticum durum*×*Secale cereale*) 20.57. Present result revealed that the mean values of ring bivalent was 36.12 and that of rod was 63.88 in the triticale lines. **Millar and Riley (1972)** also observed bivalent of their parental lines *Triticum aestivum* and *Triticum durum* and *Secale cereale*. The mean value of *Triticum aestivum* was 21. In *Triticum durum* it was 20. The present result showed that ring bivalent in *Triticum aestivum* was 25.88%, rod was 20.5% and in *Triticum durum* ring bivalent was 14.96% and rod was 19.5%. Here *Triticum aestivum* and *Triticum durum* exposed normal bivalent almost.

Triticale is a hybrid so not only univalent but also quadrivalent and trivalent are found among with meiotic irregularities. Triticale, is allopolyploid, however, is not perfect and thus the number is different for different triticales. The gametes from such hybrid are not normal, rather remain with anomalous chromosome numbers. Most of the male gametes that function are euploid or nearly so, owing to the strong selection in favour of euploid

pollen, but the female gametes can function with much anomalies. Euploid gametes are more functional in competition in the cereals and the aneuploid plants are less fertile than the euploid ones. However the failure to secure precise euploid numbers for the gametes is not the only source of cytological anomaly in this allopolyploid. It has been shown in various species, that univalent chromosomes can be a source of anomaly other than that which is simply numerical. Present investigation reveals that the highest univalent was found to be total of 2.5% in triticale WRF which is hexaploid, and the lowest value was found to be total of 2.18% in triticale BAT-2 which is octoploid. In two consecutive years univalent found to vary from 1.11%- 1.13% in octoploid triticale BAT-1, 1.19%-0.99% in Octoploid triticale BAT-2 and 1.09%-1.41% in hexaploid triticale WRF. Meiotic observations have earlier been reported by **Sanchez-Monge (1958), Pieritz (1966) and Tuschiya (1968a,b).** The line investigated by Pieritz (1966) is the most stable one so far reported for 94.24% normal cells and 0.11 univalents per cell at first metaphase. **Tsuchiya (1968a,b)** investigated meiosis in four lines and they varied from 30.0% to 48.0% normal cells and from 2.29 to 1.42 univalents per cells. **Sanchez-Monge (1958)** investigated first metaphase in three lines of primary hexaploids. They had 21.0%, 31.3% and 31.5% normal cells and mean of 2.3, 2.2, and 2.0 univalents per cell. In present investigation out of three triticale lines two were octoploid (triticale BAT-1 and triticale BAT-2) and one was hexaploid (triticale WRF). However, *Triticum* parental lines *Triticum aestivum* and *Triticum durum* showed no univalent. Another parental line *Secale cereale* was not examined and it has been known that *Secale cereale*'s chromosome is present both in octoploid triticale and hexaploid triticale. So, univalent or irregularities are frequently found. Most of the univalent chromosome come from rye and some from wheat.

O' Mara (1954) reported the failure of the rye chromosomes to pair as regularly as the wheat chromosomes. It may represent some inability of rye to operate normally in a cell which is almost wholly wheat the tendency to asynapsis may also be due to the rye chromosome and the consequent uncovering of recessive factors which have deleterious effects on regularity of meiosis. However, all such factors now known to affect meiosis as a whole and not merely the single chromosome which bears them. **Merker (1971)** reported that univalent is frequently found in rye. Present result reveals that *Secale cereale* chromosome is response for univalent in triticale. It has been also observed that hexaploid and tetraploid wheat also response for univalent in triticale line with low frequency.

Vakar and Krot (1934) reported the presence of tetravalent in a few cases, and their "end-to-end" way of conjugation, make it triticale (wheat-rye) hybrids there takes place

an interchange of chromosome parts. The formation of tetravalent may, of course, be due to the accumulations of homologous, or to be exact, wheat chromosomes as a result of repeated pollination of F_1 of wheat-rye hybrids triticale with wheat-pollen and subsequent distribution of chromosomes in the reduction division. It is evident that a trivalent association occurs in frequently in haploid *Triticum aestivum* and these associations are the result of chromosome pairing and chromatid exchange as indicated by the occurrence in the progeny of haploids of reciprocal translocation which are most easily accounted for crossing over between nonhomologous chromosomes **(Sears 1939).** The present result supports this result. Trivalent were frequently found in triticale lines. The highest percentages of trivalent was found to be 0.44 in triticale WRF and lowest percentages was found to be 0.36 in triticale BAT-1. Quadrivalent and trivalent were frequently found in all the three triticale lines. In hexaploid wheat (*Triticum aestivum*), quadrivalent and trivalent were not found but in tetraploid wheat (*Triticum durum*), quadrivalent and trivalent were found. In *Triticum aestivum*, qudrivalent was not found because they exhibit normally bivalent. In meiosis, triticale contains many types of translocation (quadrivalent, trivalent etc.) and that might be due to rye chromosome. Small number of chromosomes were found to be involved in translocation. Similar result was found **(Zeller, 1973; Schlegel and Korzum, 1999)** in veery line. The veery line was derived from crosses between a Mexican spring semi dwarf and the Siberian winter bread wheat variety Kavkaz, which carried an IRS. IBL translocation chromosome with the IRS arm coming from Pethus rye.

Miller and Riley (1972) found value of trivalent in triticale (*T. aestivum*×*Secale cereale*) to be 0.23% and that of quadrivalent in this line was 0.30%. They reported also the value of trivalent (0.21%) and the quadrivalent (0.35%) in hexaploid triticale (*T. durum*×*Secale cereale*). Present investigation revealed the similar result, where the quadrivalent percentage was found to be 0.61 in triticale BAT-1 and 0.48 in triticale BAT-2. Similarly in hexaploid triticale, quadrivalent percentage was found to be 0.67 in triticale WRF. The chaisma frequency per cell was highest (21.30) in triticale BAT-1 and lowest (18.13) in triticale WRF. **Mujeeb *et al.* (1978)** observed that chaisma frequency was very low in hybrid varieties. He observed it in *Hordeum*×*Triticum* hybrids.

Sears (1941) stated that *Secale cereale* is normally self-sterile and therefore very heterozygous. **Miller and Riley (1972)** found univalent, trivalent and quadrivalent in *Secale cereale*. Trivalent and quadrivalent are very low in number but univalent is found frequently. All the rye chromosomes found as univalents are distributed in the

random fashion. The univalent can always be identified as rye chromosomes and not wheat by their larger size and consistent form. On the other hand hexaploid wheat (*Triticum aestivum*) normally conjugates with it complete homologous at meiosis and only bivalents are formed. It is functionally diploid so normally 21 bivalents are found frequently. The chaisma per cell in hexaploid wheat is higher than that of triticale lines. The value of chaisma frequency in *Triticum aestivum* is 38.95 per cell and in triticale lines chaisma frequency in two consecutive are 19.59, 21.30 per cell in octoploid triticale BAT-1 and 19.09 and 20.54 per cell in triticale BAT-2 and 18.1 and 15.98 per cell in hexaploid triticale WRF.

Triticum durum is cytologically tetraploid and here the percentages of univalent and trivalent is very low than that of triticale lines. The chiasmata per cell in this line is 24.74 which is higher than all triticale lines. All the hybrids exhibited very low chaisma frequencies which provide enough opportunity for misdivision of the univalents. **Martin and Sanchez-Monge Laguna (1982)** reported the level of pairing in the hybrids of *H. chilense*×*Triticum aestivum* which supports the present result. **Fedak (1983)** observed that the chaisma frequency was very low in the hybrids of *Triticum*×*Secale cereale*. **Gupta and Fedak (1986)** studied the inheritance of genetic variation in rye (*Secale cereale*) which affected homologous chromosome pairing in hybrids with bread wheat (*Triticum aestivum*).

Rye promotes homologous pairing in wheat×rye hybrids through a polygenic system **(Feldman, 1966; Lelley, 1976; Dovrak, 1977).** Chaisma frequency is very useful for comparing species and in some cases it is considered as the precise parameter than the karyotype itself. The chaisma formation reflects similarities both in genetic content and in the arrangement of genes (Roy and Singh, 1968). From this point of view it may be stated here that the chaisma frequency in different hybrids and their parents reflected mostly the dissimilarities between the genomes of wheat and rye species. On the other hand, the disruption of the normal outbreeding habit of *Secale cereale* has been reported to have a negative effect on the chaisma frequency **(Lamm 1936; Riley and Law, 1965)**. Similarly in this work the inbred lines of rye showed significantly lower chaisma numbers and more univalents in their F_1 hybrids.

In both wheat and rye, dominance and non-allelic gene effects have been suggested to be involved in the control of chromosome pairing **(Rees and Thompson, 1956; Watanabe, 1962).** Triticale F_1s often had lower chaisma frequencies than their parents **(Muntzing, 1939; Merkar, 1971; Pohler *et al.*, 1978).** But even after several generations of inbreeding and selection, **Tsuchiya and Larter (1969)** reported an average of 11.6% aneuploids in bulk seeds. Based on the above description of the meiotic behaviours of

hybrids of parental species, one might expect the wheat genome to be responsible for a lowered chaisma frequency and for univalency in triticale F_1s. Present results reflected the same concept. Hexaploid and tetraploid wheat genome were found to be responsible for lowering the chiasma frequency was triticale and Rye genome, and also *Triticum* genome also responsible for univalency in triticale F_1s. Pairing failure in triticale, however, was in most cases found to be restricted to the rye genome **(Lelley 1975a,b and Pohler *et al.*, 1978)**. In triticale genotypes which were heterozygous only for the rye genome also had significantly lower chaisma frequencies than the parental lines with both genomes of wheat and rye homozygous **(Lelley, 1981).**

5.5 Chromosomal anomalies in pollen mother cells of triticale lines and *Triticum* species: Meiotic irregularities like laggards, chromosome elimination, non-disjunction, presence of one or more micronuclei, etc. are caused due to different reason. Meiocytes are influenced to give anomalous relation of chromosome due to presence of more univalents and also due to lack of chromosome pairing. So meiotic irregularity is common occurrence for the intergeneric hybrids.

Muntzing (1939) described the cytological instability in triticale and this might be due to physiological disturbances, which increase the degree of meiotic irregularities and this causes the reduced fertility. The two lines investigated by **Pieritz (1966)** represented the same two categories on the octoploid level. If these results can be regarded as representative for the two ploidy levels there seems to be a fundamental differences between the meiotic disturbances of the two levels. Since aneuploidy is a result of pairing failure, lagging and meiotic elimination of chromosomes in micronuclei. The present investigation reveals many irregularities in triticale lines, but in hexaploid wheat and tetraploid *durum* there were no such irregularities. Bridge, laggard, unequal distribution and micronuclei were found in all triticale lines. In triticale meiotic irregularities sometimes make the plants sterile. **Merker (1971)** reported the frequency of aneuploid plants determined in progenies of euploids. All meiotic observations were made on euploid plants. The frequency of univalent was investigated at first metaphase; frequency of lagging chromosomes and of bridges at first anaphase and frequency of micronuclei in tetrads. He also discussed the disturbances of first metaphase that have been observed in low frequencies. Fragmentation of bivalents and univalents in all lines and bivalents oriented outside the equatorial plate in all the lines and also multivalent in all lines were observed. Present investigation revealed, lagging chromosomes at first anaphase. In large majority of the laggards there is a separation of chromatids in this division. Only a small number of laggards go to one pole with both chromatids. The variation in frequency of laggards is about the same as that of univalents at first metaphase. In **1971 Merker** reported the line 110 as the most disturbed one with 15.52% normal cells and a mean of

3.08 laggards per cell. Rosner and Line 141 were the least disturbed with 58.00% and 58.30% normal cells and 0.98% and 0.82% laggards per cell, respectively. The heterogeneity with in lines for this disturbance was much larger than for univalent in first metaphase. Homogeneity was predominantly observed in the lines with lowest frequency of laggards. The present result was found to be supported mostly in this regard. The highest mean value of laggard was 4.34, found in triticale BAT-2 and the lowest value 3.41 was found in triticale BAT-1. **Merker (1971)** described bridges at first anaphase elaborately. These bridges are caused by stickiness of the bivalents which supported the present findings. Fragments have not been observed in association with bridges and hence, inversion heterozygosity can be ruled out as a reason for bridge formation. Present result revealed that the highest mean value (5.36) of bridges was found in triticale BAT-2 and lowest value (3.94) in triticale BAT-1. Fragmentation was also found in these triticale lines. Highest mean value of fragmentation 2.09 was found in triticale BAT-1 and lowest value 1.47 was found in triticale WRF. The highest mean percentages of irregularities were 11.45, found in triticale BAT-2 and lowest 9.46 was in triticale BAT-1. It has been known that maximum irregularities were found in octoploid triticale. On the other hand, *Triticum* species were also examined cytologically for irregularities, but there were very small percentages of irregularities in *Triticum* species (*Triticum aestivum* and *Triticum durum*) which are very negligible and not effectible. **Bjurman (1957)** briefly reported the material studied which consists of two strain of rye-wheat (2n=56), triticale A, and C, their F_1 hybrids and a uniform recombination product, derived from a cross between these strains. The frequency of univalent at first metaphase of meiosis in triticale A and C was studied by **Muntzing (1939),** who found meiosis in triticale A to be much more irregular than in triticale C. Present investigation indicates that meiosis of triticale BAT-2 was much more irregular than in triticale BAT-1 and triticale WRF.

Roupakias and Kaltsikes (1977a,b,c,d) reported micronuclei along with many other cytological events in triticale genotype. Highest mean value of micronuclei was 4.61 in A_3A_4 and lowest mean value was 1.24 in A_5A_6 genotype to triticale. **Marker (1971)** also described the tetrad micronuclei. Line 110 showed highest frequency of micronuclei with 14.07% normal tetrads and a mean of 3.07 micronuclei per tetrad. Line 128 had the lowest value with 60.03% normal tetrads and 1.08 micronuclei per tetrad. Line 6 had the widest distribution with up to 16 micronuclei. The present result showed that highest percentages of micronuclei (p.g. nuceli) was (43.14%) and normal tetrads (55.86%) in triticale BAT-1 and lowest percentages of micronuclei is 34.55 and normal tetrads were 65.45 in triticale BAT-2. Meiotic observation have earlier been reported by **Sanchez and Monge (1985); Pieritz (1966) and Tsuchiya (1969, 1970)**. The line investigated by **Pieritz (1966),** was most stable one with 97.87% normal tetrads. **Tsuchiya (1968a,b)** investigated meiosis in four lines and found them to vary from 64.40% to 79.40% normal

tetrads. **Alam and Kabir (1983)** however, reported this sort pollen grain abnormality in barley due to mutagenic effect of insecticides.

Merker (1974) emphasized that one of the goals for triticale improvement is reduction of meiotic disturbances. Furthermore, he pointed out that such disturbances can lead to formation of aneuploid individuals in populations which, by having reduced vigor and low fertility, can lower the yield potential of the lines.

The present investigation reveals that the fertility of triticale lines were low. The highest pollen sterility percentage is 55.58 in hexaploid triticale BAT-2, and lowest percentage is 48.90 in triticale BAT-1 and 54.86 in triticale WRF. It has been known that almost all the hybrid lines /varieties are self-sterile.so based on present findings it can be said that triticale BAT-1, triticale-BAT-2 and triticale WRF are semi sterile. *Secale cereale* is said to be semisterile but the other parental common species of octoploid and hexaploid triticale are very much fertile. The mean percentage value of sterility is 5.17 in *Triticum aestivum* and 3.55 in *Triticum durum*. These values are low than that of even triticale lines. The mean percentage value of irregularities is 3.39 in *Triticum aestivum* and 3.54 in *Triticum durum*. Thus, it may be said that *Secale cereale* is the origin of chromosomal anomalies in triticale. It has been reported that meiotic irregularities and abnormalities are frequently found in rye (*Secale cereale*). **Kihara (1919) and Sax (1922)** have shown that the cereals furnish an excellent example of polyploidy. The basic chromosome number is 7. Among wheat species diploid, tetraploid, and hexaploid are known and the bread wheat having 21 haploid chromosomes is familiar. In rye the haploid number is always 7, so the hybrids between 14- and 21- chromosome species of wheat show at the heterotypic division 14 bivalent and 7 univalent chromosome. Those between 7 and 14-chromosome species show 7 bivalents and 7 univalents. The chromosome of rye have been examined individually for their ability to correct the male sterility, where two full components of rye chromosomes (14) were represented in it. In spite of this, meiosis shows various irregularities such as the presence of 6 univalent chromosomes with lagging behaviours.

The triticale hybrid lines are intergeneric line and it has intergeneric chromosome combination. **Fedak (1985)** studied the intergeneric hybrids of *Hordeum vulgare* with *Agropyron canium* and *A. desystachym*, and reported abnormal chromosomes in hybrid meiocytes, which was found to be attributed to abnormal spindle fiber function. Chromosomal abnormalities or irregularities categorized as anaphase bridges, and aberrant chromatid separation gave rise to chromosome instability in subsequent cell division **(Mujeeb *et al.*, 1978).** Anaphase separation were quite irregular, and lagging and micronuclei were also observed **(Gupta and Fedak, 1986)** in the intergeneric

hybrids. **Narkhede and Meshram (1981)** studied intergeneric hybrids and observed meiotic irregularities like irregular separation of chromosome, laggards and chromosome fragment. **Veronica *et al.* (1984)** studied meiotic behaviours of hybrids and reported that lagging, univalents and irregular distribution of chromosome to the gametes were the major contributing factors for causing sterility among the hybrids. The parents *Triticum aestivum* and *Triticum durum* were found with pollen sterility but with a very low percentages. On the contrary different hybrids were found with a very high percentages of pollen sterility. Several workers **(Vasil, 1967; Edwardson, 1970; and Laser and Larson, 1972)** stated that abnormalities might have adverse effects on meiosis and pollen development. In the present study frequency of meiotic irregularities and pollen sterility in different hybrids and their respective parents indicated a positive relationship between them.

The simplest examples of anomaly are the duplicate and deficiency of different chromosomes so that the total number is unaltered. Such changes have their parallel in the nullisomic-tetrasomic combinations in *Triticum aestivum* **(Sears, 1944).** If such alteration is possible in a pure species which should be sensitive to deficiency and duplication even though polyploidy, it should be less noticeable in its effects than such alteration in a new allpolyploid which consists of two independent and unintegrated genomes. That such duplication and deficiency may occur is shown by the occurrence of quadrivalents and trivalents in triticale A and C the Rimpau and Wiebe strains **(Muntzing, 1939).** Another kind of duplication and deficiency which occurs as a result of meiosis with univalent chromosomes is caused by irregular divisions of the univalents so that is chromosomes are formed **(Upcott, 1937; Darlington, 1940; Sears 1944; O'Mara, 1952).** The consequence of such misdivision is that the kind of deficiency and duplication which more difficult to detect because the characteristic quadrivalents of whole chromosome duplication and deficiency do not occur.

One fact, however, is clear but difficult to explain. As mentioned above hybrids between primary lines of octoploid as well as hexaploid triticale are generally more sterile and have a more irregular meiosis than the parents, whereas F_1 hybrids between inbred lines of rye have a much more regular meiosis than their parents. Now a days, increased sterility and meiotic irregularity in F_1 combinations of hexaploid triticale could be caused by substitutional differences between the parents and also by homozygosity for different kind of translocations involving the A and B genomes **(Larsen 1973; Linde-Laursen and Larsen, 1974).** In octoploid triticale only the translocations could be a cause of F_1 sterility, whereas no substitutional differences can be expected. Possibly there are also other causes of the F_1 sterility and increased meiotic irregularity in triticale which are not fully understood. Sterility is related to laggard at anaphase, chromosome elimination,

non-disjunction and presence of one or more micronuclei. Meiocytes are similarly influenced to give irregular number of bivalents and univalents. The present result showed the different types of nucleus in pollen grain as like as mononuclei with highest mean value (9.00), bi-nuclei (6.00), trinuclei (3.67) and tetra nuclei (2.33). These are caused due to chromosomal anomalies, and caused the plants directly sterile.

5.6 Analysis of meiotic pairing: The mutual influence between wheat and rye genomes on meiotic pairing has been reported in many cases of wheat-rye combinations **(Naranjo *et al.*, 1979; Naranjo and Palla, 1982).** Wheat chromosomes seem to produce a decrease of homologous pairing with rye chromosomes **(Lacadena, 1967; Lelley, 1976),** whereas the homologous pairing between wheat chromosomes increases when the dosage of rye genomes increases **(Miller and Riley, 1972; Naranjo *et al*., 1979)**.

In present observation it was found that wheat and rye chromosomes paired together in all triticale lines. Rye chromosome influences the meiotic pairing of wheat homologous chromosomes and due to that hexaploid wheat and rye have made triticale BAT-1 and triticale BAT-2 by hybridization and also tetraploid wheat and rye have made triticale WRF by hybridization. In Table 41 wheat and rye chromosomes have revealed these meiotic pairing. Here, In triticale BAT-1 hexaploid wheat (*Triticum aestivum*) have ring bivalent 1560, open bivalent: (Long arms 710, short arms 220), univalent 2 and quadrivalent 3 and rye (*Secale cereale*) have ring bivalent 290, open bivalent: (long arms 102, short arms 34), univalent 8, Trivalent 2 and quadrivalent also 2. In triticale BAT-2 hexaploid wheat have ring bivalent 1414, open bivalent: (long arms 618, short arms 310) univalent 8 trivalent 2 quadrivalent 3, and rye have ring bivalent 305, open bivalent: (long arms 105, short arms 250), univalent 7, Trivalent 2 quadrivalent 2. In triticale WRF tetraploid wheat (*Triticum durum*) have ring bivalent 1565 open bivalent: (long arms 575, short arms 285), univalent 10 trivalent 2 quadrivalent 2 and rye have ring bivalent 295, open bivalent: (long arm 110 short arms 32), univalent 6, Trivalent 2 quadrivalent 2. In each triticale line, the level of pairing for wheat chromosomes was moderately reduced and for rye chromosomes it was very significantly reduced, in comparison with that of the wheat and rye parents used to synthesize it. The pairing intensity observed suggests the presence of a strong negative intergenomic interaction between the rye and wheat genomes in triticale, irrespective of whether the rye is in a homozygous or heterozygous genotypic condition. **Chapman and Miller (1973)** suggested that the rye promotive effect is mediated by chromosome 5R and more specifically by its short arm, the suppressor activity detected in long arm being lower in effectiveness.

Orellana *et al*. (1984) analyzed meiotic configuration of wheat homologous pairs (ring, open bivalent, and univalent pairs) and their frequency distributions in the addition lines

as well as from the comparison with the respective parental wheat. They also observed that rye chromosomes influenced the meiotic pairing of wheat homologous chromosomes. For analysis it had been shown that wheat chromosome paired as: ring bivalents found 3744, (out of 200 cells) open bivalent found 439, and univalent found 17 in wheat-rye (triticale) hybrid line. Again, rye chromosomes paired as: ring bivalent found 155, long arm bound found 27 short arm found 14, and univalent found 4. Present findings supports this result.

Riley and Miller (1970) reported the effect of the rye genome on homologous pairing of wheat chromosomes. It has been shown that in, wheat x rye hybrids, the rye (*Secale cereale*) genome interferes with the diploizing system and thus, promotes homologous chromosome pairing **(Nakajima and Zennyozi, 1966; Miller and Riley, 1972; Mettin *et al.*, 1976; Lelley, 1976; Dvorak 1977).** Rye has been also shown to induce homologous pairing in intergeneric *Hordeum*×*Secale cereale* hybrids **(Gupta and Fedak, 1985a, 1985b).** Different species of *Secale* and different cultivar of *Secale cereale* exhibit variation in their effect on induced homologous chromosome pairing in the various hybrids as mentioned above.

5.7 Univalency in hybrid lines of *Triticum* species and triticale:

The genomic formula of hexaploid triticale is AABBRR, octoploid triticale is AABBDDRR and that of bread wheat is AABBDD. Thus, it is evident that both of triticale genome have RR which have come from euploid rye (*Secale cereale*). Genome of tetraploid wheat (*Triticum durum*) is AABB. Hybrids between hexaploid triticale and hexaploid wheat have constitutions AABBRD. Hybrid between octoploid triticale and tetraploid wheat have constitutions also AABBRD. In **1973 Gustafson** and Zillinsky reported for the first time in strains of x 'Armadilo' triticale rye chromosome 2R replaced by chromosome pair 2D of hexaploid wheat. The lines resulted from spontaneous crosses with Mexican bread wheat having a 'Norin 10' dwarfing gene. They stood out for high fertility. Several workers **(Larter *et al.*, 1968; Jenkins, 1969; 1975; Popov and Tsvetkov, 1975; Kolev, 1975)** reported artificial crosses between hexaploid triticale and bread wheat and found in meiosis the typical chromosome pairing with 14 bivalents+ 14 univalents, where the bivalents comprised of all the A and B chromosomes and the univalents represent R chromosomes and 7D chromosomes. During meiosis some of the univalents were eliminated and the other ones transmitted to next generation together with all the A and B chromosomes. Since the plants are predominantly self-pollinating the ultimate result, after a number of generations, would theoretically be a multitude of plants with different chromosome constitutions, ranging from pure hexaploid triticales to typical bread wheats with the genome formula AABBRR and AABBDD, respectively.

However in the present findings dominance of univalents were observed in all the crosses. It may be said based on above mentioned statement that most of the univalents found in the present study are from triticale and thereby from *Secale cereale* having the genomic sign 'R'.

In present results of meiosis in hybrid of *Triticum aestivum*×triticale BAT-1 (12 bivalents + 26 univalents) were found. On the other hand, 9 bivalents and 18 univalents were found in the F_1 of *T. durum* and triticale.

May and Appels (1982) found univalents in hybrid line of triticale×*Triticum aestivum.* They described that the F_1 plants would have shown a maximum pairing at meiosis of 15 bivalents + 12 univalents which support the present results.

SUMMARY

The present investigation deals the cytological characters of triticale and *Triticum* species and their hybrids. Three lines of triticale such as triticale BAT-1, triticale BAT-2, and triticale WRF and two *Triticum* species viz. *Triticum aestivum* and *Triticum durum* were studied. Triticale BAT-1 and triticale BAT-2 are octoploid (2n=56), triticale WRF is hexaploid (2n=42). *Triticum aestivum* is hexaploid (2n=42) and *Triticum durum* is tetraploid (2n=28).

Triticale lines are hybrid, so cytologically their chromosome pairing are different. Cytological studies in triticale are of great interest because of its partial sterile nature. Chromosome number in triticale should be 56, twice the total gametic sum of parents. The cells at meiosis would contain 28 different chromosomes of which 21 would be wheat and 7 would be rye. In present study of triticale BAT-1 and triticale BAT-2 are derivatives of *Triticum aestivum* and *Secale cereale*, triticale WRF of *Triticum durum* and *Secale cereale*. The number is different for different triticale lines and thus, the gametes after meiosis show anomalous chromosome number.

Interphase nuclear phenotype of triticale and *Triticum* species were studied in present investigation. Nuclear volume (NV) and Interphase chromosome volume (ICV) were found to range from 35.99 (triticale BAT-2) to 26.89 (triticale WRF). Similarly in *Triticum* species the values were 30.75 (*Triticum aestivum*) and 25.12 (*Triticum durum*).

To identify the chromosomes through somatic karyotype, root tips of all the triticale lines and *Triticum* species were collected. Root tips of 1.5cm length were appropriate for obtaining maximum number of metaphase plates. At least three well spread metaphase plates were observed for this investigation. Somatic chromosomes were measured from photomicrographs. For making quantitative karyotypic analysis the method proposed by Ahmed *et al*. (1983) was adopted on the basis of scatter diagram of total chromatin lengths (TCL) and arm ratios (AR) of all chromosomes in a number of cells. The cells with well spread metaphase chromosomes having more or less distinct morphology are presented. Measurement for the lengths and ratios of representative complement of root tip chromosomes in three lines of triticale and two lines of *Triticum* species were taken. Data were taken at the desirable stage for each of the three cells and were plotted in separate scatter diagrams and combined scattered diagrams.

The average values of total length and arm ratio were calculated constituting the haploid complement of that cells. Then the chromosome of haploid complements were numbered in decreasing order of total chromatin length and increasing order of arm ratios with in

the same length. Total haploid complement lengths and chromosome distribution of triticale lines and *Triticum* species were studied. Highest CV% was (2.18) found in triticale BAT-1 and the lowest value (0.612) was found in *Triticum aestivum*. CV% values were 0.96 (triticale BAT-2), 1.94 (triticale WRF) and 1.36 (*Triticum durum*). χ^2 values were also estimated. Here highest value was 10.081 in triticale BAT-1 and lowest value was 0.044 in triticale BAT-2. Other χ^2 values were 0.13 (triticale WRF), 16.60 (*Triticum aestivum*), 0.095 (*Triticum durum*) are observed.

The unidentified chromosomes were distributed to the various morphological categories using probabilistic inferences, especially on the chromosome frequency in a given class per haploid set (L=Large, M=Medium, S_1=relatively short, and S_2=short). The morphological features of the haploid complement in triticale lines and *Triticum* species were also determined here. The standard karyotypes were proposed for triticale lines and *Triticum* species on the basis of centromeric formula, range and average chromatin length per chromosome. Data on chromosome morphology, i.e., length, arm ratio, relative length, TCL%, TF% and chromosome type were also determined.

Chromosome association and chaisma frequency in different genotypes in the present investigation were found to vary. Frequency of normal bivalent was very low and that of univalent was very high in all triticale lines. The results reveals that the presence of univalent was highest in triticale WRF (1.41%) and the lowest value in triticale BAT-2 (0.99%), trivalent was found in all triticale lines. The highest value (0.29%) was found for triticale BAT-2 and the lowest value (0.13%) was found in triticale BAT-1. The highest value of quadrivalent (0.36%) was found to be triticale WRF and the lowest value (0.23%) was found in triticale BAT-1. The highest value for chaismata per bivalent were found in triticale BAT-1 and in triticale WRF. On the other hand, chiasma frequency in *Triticum* species were compared with triticale. The value of univalent is 0 in *Triticum aestivum* and the value of univalent was only 2 in *Triticum durum*. Similarly trivalent and quadrivalent was not present in *Triticum aestivum* but in *Triticum durum* only 2%, quadrivalent was observed. The chaismata per bivalent was found to be 1.85 in *Triticum aestivum* which is highest than all triticale line and also *Triticum durum*.

Different meiotic irregularities were found in triticale lines and *Triticum* species. In triticale lines meiotic irregularities like laggards, fragment, bridges, unequal distribution were found frequently. The total percentages of irregularities varied from 11.45 (triticale BAT-2) to 9.46 (triticale BAT-1). Similarly the meiotic irregularities were found in *Triticum aestivum* and *Triticum durum* which were very negligible in number and not effectible. Fertility of triticale lines were low.

The pollen grain abnormalities like mononuclei binuclei, trinuclei, tetranuclie were frequently found. The highest percentage of this abnormality was 43.14% in triticale BAT-1. The lowest value (34.55) was found in triticale BAT-2. Similar type of pollen grain abnormalities were also found in *Triticum* species but the frequency were very low than that of triticale. These were caused due to chromosomal anomalies and caused the plants directly sterile.

Hexaploid wheat (*Triticum aestivum*) and Rye (*Secale cereale*) chromosomes made the meiotic pairing in all triticale lines. Table 41 indicates that there were 1560 ring and 930 open bivalent chromosome of wheat and 290 ring, 136 (OL-102 & OS-34) open bivalent chromosome of Rye (*Secale cereale*) present in triticale BAT-1.

There are 10I+2III+3IV in wheat chromosome and 8I+2III+2IV in rye chromosome abnormalities which are present in triticale BAT-1. In triticale BAT-2 there are 1414 (Ring), 928 open bivalent of wheat and 305 ring, 130 (OL-105, OS-25) open bivalent chromosome of rye present in this lines. Here hexaploid wheat (*Triticum aestivum*) and Rye (*Secale cereale*) chromosomes make the meiotic pairing in all triticale lines. There are 10I+2III+3IV in wheat chromosome and 8I+2III+2IV in rye chromosome abnormalities which are present in triticale BAT-1. In triticale BAT-2 there are 1414 (Ring), 928 open bivalent of wheat and 305 ring, 130 (OL-105, OS-25) open bivalent chromosome of rye present in this lines.

Crosses were made to produce hybrids from triticale×hexaploid wheat and triticale× tetraploid wheat and their reciprocal. To obtain these intergeneric hybrid attempts were made in several ways. But results were very poor. Present result represented a few total seed set, poor percentages of seed set, seed germination, seed germination and seedling viabilities.

Frequency of normal bivalents was very low and that of univalent was very high in all the hybrids compared to that of their parents. The value obtained for chromosome association and chaisma frequency have been presented in this investigation.

The percentage of univalent was found to be 59.14 in *Triticum aestivum*×triticale BAT-1 and that value was found to be 65.71 in *Triticum durum*×triticale WRF. The result revealed that the percentage of univalent was higher than their parents.

Present results and discussion reveals that triticale lines were semi-sterile. The chromosomes of *Secale cereale* and *Triticum* species were present obviously in triticale, but they cannot combine always during meiosis and thus, normal gametes are not formed.

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