THE DURATION OF MEIOSIS IN TRITICALE (X TRITICOSECALE WITTMACK) AND ITS PARENTAL SPECIES

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Demetrios George Roupakias

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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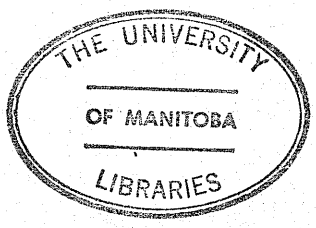

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ABSTRACT

Roupakias, Demetrios G. Ph.D., The University of Manitoba, February, 1978. The Duration of Meiosis in Triticale (X Triticosecale Wittmack) and Its Parental Species. Major Professor; P. J. Kaltsikes.

Utilizing the developmental gradients of the cereal inflorescence and the synchronous meiotic development of the three anthers within each floret, the duration of meiosis (and its stages) and that of pollen maturation at 20 °C and continuous illumination were determined for the following cereal species: (1) Tetraploid wheat (Triticum turgidum L.); (2) hexaploid wheat (Triticum aestivum L. em. Thell.); (3) tetraploid, hexaploid, and octoploid triticale (X Triticosecale Wittmack); and (4) diploid rye (secale cereale L.). Meiosis lasted from 52.6 to 58.7 h in the tetraploid, from 44.1 to 53.3 h in the hexaploid, and 43.6 h in the octoploid triticales. These estimates are longer than others previously reported for hexaploid (34-37 h) and octoploid triticale (21-22 h) grown under the same conditions. Pollen maturation took from 13.5 to 14.0 days in tetraploids, from 9.5 to 11.2 days in hexaploids, and from 10.2 to 10.9 days in octoploids. Apart from possible genotypic differences, and subjective judgments as to the beginning of meiosis, the present estimates include the stage of nucleolar fusion (average duration: 6.9 h in tetraploids; 6.0 h in hexaploids and 8.0 h in octoploid triticales) as part of meiotic prophase.

The number of univalents per PMC ranged from 0.37 to 1.89 in tetraploids, from 0.67 to 4.89 in hexaploids and from 2.78 to 5.21 in octoploid triticales. Pairing failure occurred mostly among chromosomes of the rye genome. Telomeric heterochromatin of rye chromosomes affected chromosome pairing in hexaploid triticale. Thus, in Rosner triticale, plants lacking most of the telomeric heterochromatic band on the short arm of chromosome 6R($6R^S$) had significantly higher chromosome pairing than plants carrying the telomeric band.

In tetraploid triticales the duration of meiosis and chromosome pairing were significantly affected by various chromosome combinations. Lack of chromosome 5B in a tetraploid triticale increased the duration of meiosis and resulted in poorer pairing. The origin of the cytoplasm (from tetraploid or hexaploid wheat) had no significant effect on chromosome pairing or meiotic duration of hexaploid triticales. No relationship could be demonstrated between the total duration of meiosis, or that of zygotene plus pachytene, and chromosome pairing in any of the ploidy levels (tetraploid, hexaploid, and octoploid) studied.

The duration of meiosis in several genotypes sharing the same AB, D and R genomes was from 32.8 to 44.5 h in the AABBDD; 44.1 to 44.6 h in the AABB; 46.4 to 51.3 h in the AABBRR; 43.6 h in the AABBDDRR; 44.5 h in the AABBD; 51.6 to 52.7 h in the ABRR and 52.6 h in the RR genotype. Addition of the D genome to the AABB and AABBRR genotypes resulted in (1) elongation of the stage of nucleolar fusion (2) shortening of the combined duration of zygotene and pachytene and (3) reduction of the total duration of meiosis in AABBRR while it had no effect on AABB. Addition of the R genome to AABB resulted in the elongation of the

total duration of meiosis and its stages while it had no significant effect when added to AABBDD. Thus the variation observed in the duration of meiosis and its stages among the various cereal genotypes studied was more likely due to genes carried by the D and R genomes rather than to simple changes in ploidy level or DNA content.

No constant difference in meiotic development between primary and secondary florets were found. The rate of anther elongation was approximately 0.02 mm/h in all hexaploid, and octoploid triticales, 0.01 mm/h in tetraploid and hexaploid wheat and 0.03 mm/h in diploid rye. Although, on the average, the stage of the meiocytes in an anther could be ascertained with certain degree of accuracy by measuring anther length, the degree of overlapping between successive meiotic stages mitigates against the use of this criterion for meiotic stage determinations in biochemical studies of meiosis.

FOREWORD

This thesis has been written in manuscript format. It consists of a literature review, six manuscripts, a general discussion and a summary. Manuscript I, "The effect of wheat cytoplasm on meiosis of hexaploid triticale"; Manuscript II, "Independence of duration of meiosis and chromosome pairing in hexaploid triticale"; Manuscript III, "Genomic effects on the duration of meiosis in triticale and its parental species" and Manuscript V, "The effect of telomeric heterochromatin on chromosome pairing of hexaploid triticale"; have been published in the Canadian Journal Genetics and Cytology. Manuscript IV "The meiotic cycle of tetraploid triticale"; will be presented at the Fifth Wheat Genetics Symposium. Manuscript VI "Anther length and meiotic development in triticale and its parental species" has been published in z. Pflanzenzüchtg.

INTRODUCTION

INTRODUCTION

The synthetic amphiploid triticale (X Triticosecale Wittmack) shows a number of unpaired chromosomes at first meiotic metaphase (Müntzing, 1957; Weimarck, 1974; Merker 1973a; Scoles and Kaltsikes, 1974). Darlington (1940) theorised that variation in the number and position of chiasmata may be caused by differences in the time available for chromosome pairing and chiasma formation. Pursuing the above suggestion further Bennett et al. (1971) and Bennett and Kaltsikes (1973) studied the duration of meiosis in wheat, rye, and triticale. They found that rye required a considerably longer time in which to complete meiosis than either wheat or triticale. They suggested, therefore, that insufficient time for normal pairing of rye chromosomes may be the cause of meiotic abnormalities in triticale.

Larter and Hsam (1973) and Rimpau et al. (1973) reported that the cytoplasm affected chromosome pairing in triticale and wheat - rye addition lines. Furthermore Kaltsikes (1974a) noted "it is possible that the cytoplasm affects the duration of meiosis and especially those stages connected with pairing". By extension, therefore, it could also affect chromosome pairing and meiotic irregularities.

Bennett et al. (1974) utilizing several wheat genotypes, concluded that in wheat no relationship existed between meiotic duration and the level of chromosome pairing observed at MI. In addition, on the basis of the differences in pairing observed between diakinesis and MI in

triticale, Lelley (1974) expressed doubts as to the influence of meiotic duration on chromosome pairing.

Bennett (1972, 1973) found that Triticum monococcum had a shorter duration of meiosis than Secale cereale; and Bennett and Smith (1972, 1973) found that hexaploid wheat and octoploid triticale had shorter durations of meiosis than tetraploid wheat and hexaploid triticale, respectively. They attributed these differences in meiotic duration to the amount of DNA content and the ploidy level per se.

Thomas and Kaltsikes (1972), working with hexaploid triticale, have shown that the unpaired chromosomes observed as univalents at MI mostly belonged to the rye genome. In addition, Thomas and Kaltsikes (1974b, 1976b) found that rye chromosomes with large heterochromatic bands at both telomeres fail to pair more often than those which have only one large telomeric band.

Erickson (1948) working with Lilium established a high correlation between bud length and meiotic stage. This relationship has been extensively used to determine the meiotic stage of the meiocytes contained within a bud by measuring the length of the bud and checking it against a standard curve (Hotta and Stern, 1961a and b, 1963a and b).

With these findings in mind the present series of experiments were conducted to investigate; (1) whether the source of cytoplasm affects chromosome pairing in triticale by affecting the duration of meiosis; (2) the relationship between duration of meiosis and chromosome pairing by studying both events in several triticales; (3) the variation in the duration of meiosis within a ploidy level and a species; (4) the effect of the addition of a new genome on the duration of meiosis;

(5) the effect of telomeric heterochromatin on chromosome pairing; and (6) the relationship between anther length and meiotic stage of the meiocytes included within the anther.

LITERATURE REVIEW

LITERATURE REVIEW

1. Duration of Meiosis

1.1 Duration of meiosis in cereals

Little was known about the duration of meiosis in cereals till Lindgren et al. (1969) attempted to time meiosis and its stages in barley. They found that the total duration of the stages from pachytene to quartets was not more than three days and that the duration of the shortest stage lasted less than 1 h. More information on this topic, however, was obtained from the studies of the duration of meiosis in cereals conducted by Bennett et al. (1971), Bennett and Finch (1971), Bennett and Kaltsikes (1973) and Finch and Bennett (1972). They found that, at 20 ± 1 °C and continuous illumination, meiosis lasted about 39 h in barley, 51 h in rye, 42 h in Triticum monococcum, 30 h in tetraploid wheat, 24 h in hexaploid wheat, 37 h in hexaploid triticale and 21 h in octoploid triticale. Because of the differences found in the duration of meiosis between wheat, rye and triticale Bennett et al. (1971) and Bennett and Kaltsikes (1973) suggested that the meiotic abnormalities observed in triticale (Müntzing, 1939; O'Mara, 1953; Riley and Miller, 1970; and Scoles and Kaltsikes, 1974) may be due to insufficient time for normal pairing and chiasma formation on the part of the rye chromosomes. They made this suggestion on the basis of Darlington's time-limit theory (Darlington, 1940) and their findings on the duration of meiosis in cereal genotypes. They did not however, study both events

(i.e. duration of meiosis and meiotic abnormalities) jointly in several triticales to determine how they are related to each other. For this purpose the present study was undertaken.

1.2 Factors affecting the duration of meiosis

Due to the importance of the meiotic duration, a number of studies have been conducted to clarify the factors which affect it. As a consequence of these studies the importance of DNA content, ploidy level, genetic factors and environmental conditions was realized.

1.2.1 DNA content

Bennett (1971, 1973) found a significant positive correlation ($P \leq 0.001$) between the duration of meiosis and nuclear DNA content when diploid species alone (or tetraploid species alone) were compared. Furuta et al. (1974) reported no differences in DNA content per nucleus among the hexaploid wheat strains they studied. Furthermore, no intraspecific variation in DNA content was evident in barley (Bennett and Smith, 1971) and in rice (Katayama, 1967). Bennett and Smith (1972) and Furuta et al. (1974) considered that the DNA content of a cereal nucleus was equal to the total DNA of the genomes contributing to it. Within a ploidy level therefore, all strains of a cereal species should have equal amounts of DNA.

If (1) within each ploidy level of wheat or triticale all varieties have the same DNA content, and (2) DNA content is the major factor which determines the duration of meiosis, then there should not be significant differences in the duration of meiosis among strains within a ploidy level of a species. The data available, however, indicate that this is

not always the case. Thus Bennett and Smith (1972) and Bennett and Kaltsikes (1973) reported that there was no difference in the duration of meiosis between two rye varieties (i.e. Prolific, and Petkus Spring). In contrast, Bennett and Kaltsikes (1973) found that in hexaploid triticale 6A190 meiosis lasted longer than in Rosner (Bennett and Smith, 1972). Further investigation is warranted, therefore, for a better clarification of the effect of the DNA content on the duration of meiosis.

1.2.2 Ploidy level

Bennett and Smith (1972, 1973) observed that in related genotypes the duration of meiosis was significantly reduced as the ploidy level was increased; they attributed this faster rate of development entirely to the ploidy level *per se*. The question arising, however, is: Is the increase in the rate of development which is observed when the ploidy level increases, always attributable to the change of the ploidy level *per se*, or to genetic factors introduced by the new genome(s)? Part of this study is addressed to this question.

1.2.3 Genetic factors

Bennett and Smith (1973) and Bennett *et al.* (1974) found that in hexaploid wheat, addition or subtraction of individual chromosomes had significant effects on the duration of meiosis. Furthermore Klein (1972) reported variation in the duration of meiosis even between mutant lines of *Pisum sativum*. These data indicate that individual chromosomes or even individual genes have major effects on the duration of meiosis. Further work, however, is needed for a better clarification of the genetic control of the duration of meiosis.

1.2.4 Environmental conditions

The effect of temperature on the duration of meiosis is the only environmental factor studied so far. Thus Sax (1938) observed that in Tradescantia the duration of meiosis was longer in winter than in summer. Furthermore Wilson (1959) and Bennett et al. (1972) found that the duration of meiosis decreased as the temperature increased. Thus, if results on the duration of meiosis from different studies are to be comparable they must be obtained under a constant temperature.

1.3 Meiotic development in cereals

In writing this thesis the view favored was that crossing over follows and is dependent upon, chromosome synapsis which is thought to occur during zygotene and pachytene.

1.3.1 Onset of meiosis

Bennett et al. (1973) in describing the sequence of meiotic development in one variety of hexaploid wheat (Triticum aestivum L.) adopted the classical scheme of meiosis. Thus they considered that meiosis began when the single nucleolus started protruding out of the nuclear surface, tacitly assuming that nucleolar fusion (NF) occurs prior to leptotene. In contrast, Darvey (1972), Darvey et al. (1973), and Thomas and Kaltsikes (1976a) concluded that nucleolar fusion occurs during leptotene in hexaploid wheat and in rye (Secale cereale L.).

Taylor (1959) repeatedly demonstrated that the replication of DNA occurs only during the premeiotic interphase. From his data, however, DNA synthesis seems to extend throughout preleptotene while Callan and Taylor (1968) thought that it extended even into leptotene. In cereals

it has been shown that DNA synthesis is almost complete by the time of nucleolar fusion (about 1.5 nucleoli per nucleus; Thomas and Kaltsikes, 1977a). It is reasonable therefore to adopt the idea that nucleolar fusion coincides with the onset of meiosis in cereal genotypes.

1.3.2 Synaptonemal complex formation

Moens (1968, 1969) reported that the synaptonemal complex is first visible as short segments during the zygotene stage of meiotic prophase. The dogma that the complex appears only during the zygotene and pachytene is so well accepted that its presence is considered sufficient to identify these stages. There are indications, however, that the formation of the synaptonemal complex starts much earlier than zygotene. Thus studies conducted by Grell and Chandley (1965), Koch et al. (1967) and Grell (1969) indicated that, in Drosophila melanogaster, DNA synthesis, genetic exchange, and synaptonemal complex formation were parallel events. Furthermore direct evidence has been reported recently which shows that synaptonemal complex formation occurs simultaneously with DNA synthesis (Day and Grell, 1976). In this study, however, the classical theory of the time of complex formation is adopted because the reports that the synaptonemal complex appears prior to the onset of classical meiotic prophase are few and deal mostly with Drosophila oocytes.

1.3.3 Synchronous development of meiocytes

Within an anther or among anthers of the same floret meiocytes show a high degree of synchronous development. This has been observed in Antirrhinum majus (Ernst, 1938), in Tulbaghia violacea (Taylor, 1953),

in Tradescantia paludosa (Steinitz, 1944), in Endymion nonscriptus (Wilson, 1959), in barley (Bennett and Finch, 1971) and in wheat, rye and triticale (Bennett et al., 1971; Bennett and Kaltsikes, 1973). This attribute has been extensively used in timing meiosis and its stages in cereal genotypes (Bennett, 1971).

1.3.4 Synchronous division of tapetal cells during meiosis

Iijima (1962) reported that during the first meiotic prophase, tapetal nuclei show one or more highly synchronous divisions. Two synchronous tapetal divisions (STD) have been reported by Taylor and MacMaster (1954) in Lilium longiflorum, by Taylor (1950) in Tradescantia paludosa and Bennett et al. (1971) in rye. One STD was observed in wheat, triticale and barley (Bennett et al. 1971; Bennett and Finch, 1971).

The importance of the synchronized division of the tapetal cells for normal meiosis is not clear yet. Thus Taylor (1950) suggested that maintenance of the synchronized mitosis of the tapetal layer is necessary for normal meiosis. In contrast, Ito and Stern (1967) showed that the tapetum does not play a direct role in meiotic development. Lately, however, it has been noted by Whyte (1975) that there is little doubt that the tapetum is of great importance to the development of the meiocytes, while Christensen and Horner (1974) suggested that an association of sporogenous cells with the tapetum is essential for viable pollen to develop in grasses. Further work however is needed for better understanding of the importance of the tapetum to normal development of the meiocytes.

1.3.5 Anther length and meiotic stage

Erickson (1948) working with Lilium established a high correlation between bud length and the stage of the meiocytes included therein. Bennett et al. (1973) also observed a constant rate of increase in anther length during meiosis of wheat. Vasil (1967), however, doubted the idea that the stage of the meiocytes of a bud can be determined by measuring its length and checking it against a standard curve. The question which arises here is: Is the stage of the meiocytes within an anther of a cereal genotype directly related to the anther's length? If so, could this relationship be used for a precise determination of the stage of the meiocytes included within an anther by simply measuring the length of the anther? To this effect part of this study was undertaken.

2. Meiotic Abnormalities

Meiotic abnormalities have been observed in triticale from the very early cytological studies (Levitsky and Benetzkaja, 1931; Lindschau and Oehler, 1935; Müntzing, 1939, 1957; O'Mara, 1953). The genomic origin of the chromosomes involved has been investigated by several workers who concluded that the unpaired chromosomes belonged either predominantly to rye (Müntzing, 1957; Larter et al., 1968; Sanchez-Monge, 1958; Peiritz, 1970; Tsuchiya, 1970; Shkutina and Khvostova, 1971; Thomas and Kaltsikes, 1972, 1974b and 1976b; Lelley, 1975b) or to wheat and rye (Larter and Shigenaga, 1971; Shigenaga et al. 1971; Merker, 1973b, and Weimarck, 1974).

A number of theories have been advanced over the years regarding the causes of the abnormalities in triticale. Kaltsikes (1974a) classified these theories in three major groups: Genotypic theories (inbreeding depression, deleterious genes, genome ratio and ploidy barrier); Cytological theories (allocycly and precocious chromosome separation); and Cytoplasmic effect theories.

2.1 Genotypic Theories

Triticale of all ploidy levels have a combination of chromosomes from two or more genomes of the wheat complement (A,B,D) and the rye genome chromosomes. Therefore when wheat and rye chromosomes are present in the same nucleus several situations may arise: (1) genes of the wheat genome(s) may affect pairing of the rye chromosomes; (2) genes of the rye chromosomes may affect pairing of the wheat chromosomes; (3) interaction between genes of wheat and rye chromosomes may affect the pairing of both wheat and rye chromosomes and (4) the relative

number of wheat and rye genomes included in the same nucleus (genome ratio) may affect pairing of both wheat and rye chromosomes. One or more cases for each of these situations have been reported in the literature. They will be reviewed one by one.

2.1.1 Wheat genes affecting rye chromosomes

Krolow (1966) suggested that meiotic abnormalities in octoploid triticale may be related to genes carried on the D genome chromosomes which may have an effect on chiasma formation in the rye genome. Kaltsikes (1974a) however thought that these genes may not necessarily reside on the D genome chromosomes. Thus Riley and Miller (1970) at the octoploid level and Lelley (1976b) in the combination Nullisomic 5A/Tetrasomic 5B ABDRR found that the 5B system of wheat affects the pairing of rye chromosomes. In contrast, although Thomas and Kaltsikes (1971) considered that the $5B^L$ system may have an effect on the pairing of rye chromosome sets, the ancestral affinities of which have been weakened by isolation, they doubted the importance of the $5B^L$ system for the pairing failure found in disomic triticales.

Darvey and Larter (1973) reported a dosage effect of chromosome 1B on meiotic stability of triticale. Furthermore, Thomas and Kaltsikes (1977b) found that chromosomes 1B and 6B inhibited chromosome pairing in some hexaploid triticales. Both 1B and 6B are involved in the formation of nucleoli in hexaploid triticale. Thus Thomas and Kaltsikes (1977b) suggested that the size and position of wheat nucleoli may interfere with the pairing of rye chromosomes.

2.1.2 Rye genes affecting wheat chromosomes

The possibility that rye genes may affect pairing of wheat chromosomes was first suggested by Pieritz (1966). Following this suggestion, however, Riley et al. (1973) have shown that the dosage of the rye chromosome $5R^{S}$, when added to wheat, influences the homoeologous pairing of wheat chromosomes. In addition Lelley (1976a) reported that genes of the rye genome induced homoeologous pairing in wheat chromosomes by suppressing the action of the gene on $5B^{L}$. These data indicate that when rye and wheat chromosomes are together in the same nucleus then rye genes affect pairing of wheat chromosomes. Further work, however, may reveal more about the effect of individual rye chromosomes or even particular genes on pairing of wheat chromosomes.

2.1.3 Interaction between wheat and rye genes

Riley (1960) observed that the addition of single pairs of rye chromosomes to the full complement of wheat chromosomes resulted in a less regular meiosis. He attributed this effect to disorganizing interactions between the wheat and rye chromosomes. Kaltsikes (1974a) suggested that interaction between wheat and rye genes may contribute to meiotic abnormalities in triticale. In addition, Lelley (1974) found that interactions between wheat and rye genomes affected chromosome pairing in several triticale genotypes. Finally Lelley (1975a) proposed that the system which polygenically controls the pairing of chromosomes in rye (Rees, 1955; Rees and Thompson, 1956) is also functional in triticale. He concluded therefore that the actual meiotic behavior of each triticale plant depends on the actual combination of controlling genes within the rye genome. These genes may produce their effect either

directly or in interaction with the wheat genome.

2.1.4 Genome ratio

It has been mentioned before that chromosome pairing in triticale was affected even by a single chromosome. Therefore, a whole genome may also affect chromosome pairing in triticale. Thus, Müntzing (1957), based on the observation that the octoploid triticales were more unstable than the hexaploid, suggested that the ratio of wheat to rye genomes, which is highest in octoploid triticale, may be related to the meiotic instability of triticales. Furthermore, Miller and Riley (1972) found that reduction of the dose of wheat genomes in triticale increased the homoeologous pairing between rye chromosomes, and simultaneously the increased relative number of rye genomes promoted homoeologous pairing in the wheat complement.

From the data reported so far it becomes clear that meiotic irregularities in triticale are of genetic origin. Further work, however, is needed for a better understanding of the genetic control of meiotic disturbances in triticale.

2.2 Cytological theories

Darlington (1940) theorized that meiotic abnormalities observed in higher organisms may be due to insufficient time for chromosome pairing. Stutz (1962) suggested that the asynchronous meiotic rhythm of the parental chromosomes was the principal factor contributing to meiotic abnormalities in triticale. Following these theories, Bennett et al. (1971) and Bennett and Kaltsikes (1973) proposed that insufficient time for pairing of the rye chromosomes during meiosis in triticale might be

the cause of the meiotic abnormalities. In contrast, Bennett et al. (1974) found that among several wheat genotypes there was no difference in duration of meiosis although they displayed significant differences in chromosome pairing. In addition, Larter et al. (1968), Tsuchiya (1970) and Lelley (1974) found that meiotic pairing was reduced as meiosis progressed from diplotene-diakinesis to first metaphase and suggested desynapsis as the cause of the univalents in triticale. Thus the concept of insufficient time for normal pairing of the rye chromosomes in triticale is now in doubt. Further work, however, is needed to determine just how these two variables (i.e. duration of meiosis and chromosome pairing) are related to each other.

Recent studies of meiosis with the Giemsa staining technique have implicated heterochromatin among the causes of meiotic abnormalities. Thomas and Kaltsikes (1972) have shown that it is the rye chromosomes that do not pair in triticale. Furthermore Thomas and Kaltsikes (1974b, 1976b) reported 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. In addition, Merker (1976) concluded that telomeric heterochromatin affects at least the pairing of the chromosomes which carry it. This relationship between heterochromatin and chromosome pairing has also been observed in organisms other than triticale. Thus Miklos and Nankivell (1976) found that, in Australian grasshoppers, the higher the amount of heterochromatin, either centric or telocentric, the lower the chiasma frequency. In addition Pathak and Hsu (1976) working with Mus did not find complete pairing of heterochromatic telomeres during early stages of meiotic prophase. More work

is needed, however, for a better understanding of the effect of heterochromatin on chromosome pairing.

2.3 Cytoplasmic Theories

The effect of the cytoplasm on univalent formation was first suggested by Sisodia and McGinnis (1970). Thomas and Kaltsikes (1972) found that triticale, the wheat parent of which was the extracted AABB component of hexaploid wheat, had a more regular meiosis than durum x rye triticale. Larter and Hsam (1973) reported that triticale genotypes had a more stable meiosis if the nucleus was placed in hexaploid wheat cytoplasm rather than in tetraploid wheat cytoplasm. Rimpau et al. (1973) also reported that in addition lines, the source of cytoplasm (wheat or rye) affected chromosome pairing. In contrast, Kiss and Tréfás (1973) and Lelley (1975a) did not find significant differences in the number of univalents per PMC, even between lines carrying wheat or rye cytoplasm. Similarly, Merker (1973a) in reciprocal crosses between six different hexaploid triticales having three types of cytoplasm, could not find a significant effect of the cytoplasm on the meiotic irregularities. Finally, Chen et al. (1975) suggested that tetraploid and hexaploid wheat may have a common donor of cytoplasm. Thus the data reported so far on the effect of cytoplasm on chromosome pairing are contradictory and more work is needed to determine just how these two variables (i.e. source of cytoplasm and chromosome pairing) are related to each other.

2.4 Environmental Effects

Although chromosome pairing seems to be genetically controlled, the effect of the external environment can not be overlooked. A number of

studies conducted by Bennett and Rees (1970), Fedak (1973) and Dhesi et al. (1975) have shown that higher rates of phosphate and potash increased the chiasma frequency in desynaptic stocks of rye, barley and pearl millet. Larter et al. (1968) reported that primary triticale strains had more univalents at 28 °C than at 15 °C. The opposite observation however, has been reported by Boyd et al. (1970). Bayliss and Riley (1972) reported that wheat plants nullisomic for chromosome 5D showed varying degrees of asynapsis at the high and low temperatures. Similar results have been reported by Henderson (1962), Izhar (1975), Utkhete and Jain (1974) and Luomajoki (1977). Lately Whyte (1975) attributed the effect of environmental conditions on meiotic abnormalities to physiological, morphological and biochemical factors which may govern the degree to which the genotype is permitted to express itself in the production of normal or abnormal gametes. Therefore, if meiotic instability of various triticale strains is to be compared, the triticales must be grown under constant environmental conditions.

MANUSCRIPTS

MANUSCRIPT I

THE EFFECT OF WHEAT CYTOPLASM ON MEIOSIS OF HEXAPLOID TRITICALE

Abstract

The interrelationships among source of cytoplasm, chromosome pairing and the duration of meiosis were studied in eight combinations of hexaploid triticale (X Triticosecale Wittmack) grown at 20°C under continuous illumination. The number of paired chromosome arms and univalents per pollen mother cell at MI ranged from 32.32 and 4.89 to 37.26 and 1.37, respectively. Meiosis lasted from 44.14 to 49.35 hours. A significant positive correlation ($r = 0.92$) was found between total duration of meiosis and the combined duration of zygotene and pachytene, the stages during which chromosome pairing is thought to occur. The origin of the cytoplasm (from tetraploid or hexaploid wheat) had no significant effect on chromosome pairing or meiotic duration. No relationship was found between total duration of meiosis, or that of zygotene and pachytene, and chromosome pairing. It was concluded that lack of sufficient time for homologues to pair cannot account for the presence of rye chromosomes as univalents in triticale.

Introduction

The synthetic amphiploid triticale (X Triticosecale Wittmack) shows a number of unpaired chromosomes at first meiotic metaphase (Kaltsikes, 1974). Chromosome pairing can be affected by chromosome homologies, genetic factors and the cellular environment during meiosis (Hossain and Moore, 1975). These in turn can also be influenced by the external environment (Bennett and Rees, 1970; Bennett et al., 1972; Bayliss and Riley, 1972; Utkhede and Jain, 1974; Izhar, 1975). Various

investigators therefore have tried to study the relationship between these factors and univalency in triticale. Thus, following Darlington's (1940) theory that variation in the number and position of chiasmata may be caused by differences in the time available for chromosome pairing and chiasma formation, Bennett et al. (1971), Bennett and Smith (1972), and Bennett and Kaltsikes (1973) studied the duration of meiosis and its constituent stages in wheat, rye and triticale. They found that rye required a considerably longer time in which to complete meiosis than either triticale or wheat. They suggested, therefore, that insufficient time for normal pairing of rye chromosomes in the triticale nucleus, may be the cause of the partial failure of chromosome pairing which is evidenced as univalents at MI.

The cytoplasm, as the major component of the cellular environment, has been shown to affect univalency in triticale and wheat-rye addition lines (Larter and Hsam, 1973; Rimpau et al., 1973). Kaltsikes (1974) noted "it is possible that the cytoplasm affects the duration of meiosis and especially those stages connected with pairing". By inference, therefore, it could also affect chromosome pairing and meiotic abnormalities.

The present study was undertaken to investigate the relationship between the source of cytoplasm, duration of meiosis, and chromosome pairing in triticale.

Materials and Methods

Seeds of four pairs of triticale lines, each pair genotypically identical but differing in the source of their cytoplasm, were kindly

supplied by Dr. E. N. Larter (Table I). Due to the outbreeding of the rye parent there was a possibility that the lines of each pair were not strictly identical with respect to the genotype. To eliminate this possible source of error, the lines of each pair were crossed reciprocally to produce F_1 combinations which constituted the material utilized in this study.

Seeds of all genotypes were germinated on moist filter paper, at room temperature. Root-tips were collected and, one day later, the seedlings were transplanted, one per 9 cm pot, and grown in the greenhouse. When leading tillers were judged to be about a week prior to the onset of meiosis they were transferred to a growth cabinet maintained at $20 \pm 1°C$, $71.5 \pm 0.6\%$ relative humidity, and continuous illumination. Root-tip chromosome counts were obtained by standard procedures.

Squash preparations of PMC's were made in acetocarmine (2%) by standard procedures. Chromosome arms paired and configurations at MI, lagging chromosomes at first and second anaphase and micronuclei at the quartet stage were scored in eight euploid plants per line utilizing 25 PMC's in each plant.

The duration of meiosis and its stages were estimated firstly, by the removal and fixation, at sampling time, of three spikelets on one side of the spike axis and secondly, by the anther sampling method. Both methods have been previously described (Bennett et al., 1971 ; Bennett and Smith, 1972).

Twelve stages of meiosis were identified as follows: (1) Nucleolar fusion, considered to begin when the average number of nucleoli per PMC was 1.5, (S_1), (2) Lepotene (S_2), (3) Zygotene to synchronous tapetal

Table I

Pedigree and designation of eight triticale hybrids produced by crossing genomically identical lines which differed in the origin of their cytoplasm

Parentage[1]	Genotypic designation	Cytoplasmic origin	Generation
[Pitic X Turgidum (F_3)] X Centeno rye	A_1A_1	6x wheat	C_1
[Turgidum X Pitic (F_3)] X Centeno rye	A_2A_2	4x "	C_1
	A_1A_2	6x "	F_1
	A_1A_2	4x "	F_1
[Manitou X Turgidum (F_3)] X Centeno rye	A_3A_3	6x "	C_1
[Turgidum X Manitou (F_3)] X Centeno rye	A_4A_4	4x "	C_1
	A_3A_4	6x "	F_1
	A_3A_4	4x "	F_1
[Pitic X Durum (F_3)] X Centeno rye	A_5A_5	6x "	C_1
[Durum X Pitic (F_3)] X Centeno rye	A_6A_6	4x "	C_1
	A_5A_6	6x "	F_1
	A_5A_6	4x "	F_1
[Manitou X Orientale (F_3)] X Centeno rye	A_7A_7	6x "	C_1
[Orientale X Manitou (F_3)] X Centeno rye	A_8A_8	4x "	C_1
	A_7A_8	6x "	F_1
	A_7A_8	4x "	F_1

[1] From Larter and Hsam (1973).

division (STD), (S_3), (4) STD to end of zygotene (S_4), (5) Pachytene (S_5), (6) Diplotene-Diakinesis (S_6), (7) MI (S_7), (8) AI to dyads (S_8), (9) Dyads (S_9), (10) MII (S_{10}), (11) AII to quartets (S_{11}), and (12) Quartets (S_{12}). To reduce the error of estimation each meiotic stage was further subdivided into early, mid and late substages.

The examination of a spike yielded information on the progress of PMC's through meiosis in a given time interval. If, for example, in 13 hours (h) a spikelet had progressed from mid-pachytene to the beginning of anaphase II (AII), then the following equation was set up:

$$1/2S_5 + S_6 + S_7 + S_8 + S_9 + S_{10} = 13$$

Analogous equations were set up for the other intervals studied. As a result a system of linear equations were obtained for each line as follows:

$$b_{11}S_1 + b_{12}S_2 + \cdots\cdots + b_{112}S_{12} = Y = \text{meiotic interval}$$

$$b_{j1}S_1 + b_{j2}S_2 + \cdots\cdots + b_{j12}S_{12} = Y_j$$

The value of the coefficient (b_j) varied between zero (0) and one (1), depending on the part of the meiotic stage estimated to be included in the interval.

The above system of equations can be represented in a matrix notation as follows:

$$[B]\ [S] = [Y]$$

the solution vector being:

$$\hat{S} = [B'\ B]^{-1}[B]'[Y]$$

where B represents the matrix of coefficients (b), S the vector of

unknowns (i.e. duration of the various meiotic stages), and Y the observed time intervals. A computer program written by Lee and Kaltsikes (1972) was utilized to obtain estimates of the duration of the various meiotic stages and the standard errors associated with them.

Results

Developmental Gradients Along the Inflorescence

As in the other cereals (Bennett et al., 1971), examination of several whole spikes showed that the middle spikelets were more advanced with respect to meiosis than those above or below them. The difference in meiotic development between successive spikelets (on the same side of the spike) was about 2-3 h for spikelets in the middle of the spike, 5-6 h for those immediately above or below the middle,and 10-12 h for those at the apex and base of the spike. Furthermore, the meiotic stage of a spikelet was almost intermediate to those which were just above and below it, but on the opposite side of the spike. Best results were obtained from spikelets sampled around the middle of the spike, close to the spikelet of the most advanced meiosis but not including it. If the oldest spikelet was to be included, there would be a danger of under-estimating the duration of a particular meiotic interval. This would be due to the possibility that the two spikelets immediately above and below the oldest spikelet, but on the opposite side, could be at a stage earlier than the oldest one. On the basis of our findings, it would have been assumed that the oldest spikelet was in a stage similar to that of the two sampled spikelets, when in effect, it would have been at a later stage.

In all genotypes examined, one synchronous division of tapetal nuclei was observed. It yielded binucleate tapetal cells and occurred at early zygotene (Tables II, III). Necrotic meiocytes, with a peak around the synchronous tapetal division, were observed in all genotypes. However, $A_3A_4$6x, $A_3A_4$4x and $A_7A_8$6x were characterized by a higher number of necrotic meiocytes and an asynchronous development of meiocytes among anthers of the same floret, and within meiocytes of the same anther. $A_5A_6$4x and $A_7A_8$4x had a low degree of asynchrony, while $A_1A_2$6x, $A_1A_2$4x and $A_5A_6$6x showed the highest degree of synchrony. In all lines, asynchrony was more pronounced after MI so that anthers containing a mixture of meiocytes at AI, TI and dyads were not rare. However, only data from florets in which all three anthers contained PMC's with a high degree of synchrony were used for timing meiosis.

Duration of Meiosis

Within each of the four pairs of crosses examined (Tables II and III), no significant differences in the total duration of meiosis were found ($A_1A_2t = 0.387$; $A_3A_4t = 0.025$; $A_5A_6t = 0.743$; $A_7A_8t = 0.188$). However, there were significant differences among pairs of genotypes ($P \leq 0.01$). As far as the individual meiotic stages are concerned highly significant differences were found only at leptotene of A_3A_4 and A_5A_6 ($P \leq 0.01$), and at STD to pachytene stage of A_5A_6 ($P \leq 0.01$). The duration of quartets and pollen maturation was essentially the same in all four pairs examined. Hexaploid wheat cytoplasm induced a somewhat shorter duration of meiosis in genotypes A_1A_2 and A_3A_4 while it had no effect on genotypes A_5A_6 and A_7A_8. First prophase accounted for a minimum of 82.88% ($A_5A_6$6x) and a maximum of 86.88% ($A_3A_4$4x) of total meiotic

Table II

Duration of meiosis (h), quartets (h) and pollen maturation (days) at 20 °C in two pairs of genomically identical triticale hybrids which differed in the origin of their cytoplasm

Stage of meiosis	Nuclear genotype							
	A_1A_2				A_3A_4			
	Cytoplasm				Cytoplasm			
	6x		4x		6x		4x	
Nucleolar fusion	5.02±0.32	(11.08)[1]	6.81±0.93	(14.38)	5.98±0.63	(12.12)	5.43±0.37	(11.02)
Leptotene	11.31±0.30	(24.97)	10.68±0.92	(22.87)	10.54±0.59	(21.36)	12.31±0.38	(24.98)
Zygotene to STD[2]	2.87±0.29	(6.33)	2.20±0.70	(4.71)	1.34±0.44	(2.71)	1.03±0.35	(2.09)
STD to pachytene	8.26±0.27	(18.24)	9.52±0.63	(20.38)	13.09±0.52	(26.52)	13.12±0.38	(26.62)
Pachytene	8.67±0.32	(19.14)	7.41±0.79	(15.87)	9.19±0.58	(18.62)	8.42±0.41	(17.09)
Diplotene & Diakinesis	2.26±0.31	(4.99)	3.62±0.77	(7.75)	2.21±0.68	(4.48)	2.45±0.45	(4.97)
FIRST PROPHASE	38.39±0.74	(84.76)	40.24±1.93	(86.16)	42.35±1.42	(85.81)	42.76±0.96	(86.77)
Metaphase I	1.91±0.65	(4.22)	1.85±0.69	(3.96)	2.03±0.71	(4.11)	1.90±0.67	(3.86)
Anaphase I to dyads	1.04±0.83	(2.30)	0.96±0.88	(2.06)	1.04±0.82	(2.11)	0.96±0.81	(1.95)
Dyads	1.78±0.93	(3.93)	1.65±0.99	(3.53)	1.78±0.94	(3.61)	1.67±0.74	(3.39)
M_{II}	1.25±0.80	(2.76)	1.16±0.88	(2.48)	1.27±0.82	(2.58)	1.18±0.80	(2.39)
A_{II} to tetrads	0.92±0.69	(2.03)	0.84±0.77	(1.81)	0.88±0.70	(1.78)	0.81±0.71	(1.64)
M_I - T_{II} INCLUSIVE	6.90±1.76	(15.24)	6.46±1.90	(13.84)	7.00±1.80	(14.19)	6.52±1.67	(13.23)
TOTAL MEIOTIC TIME	45.29±1.91	(100.00)	46.70±2.71	(100.00)	49.35±2.29	(100.00)	49.28±1.93	(100.00)
Quartet stage (h)	7.77±0.57		7.95±1.04		7.23±0.46		7.65±0.44	
Pollen maturation (d)	9.69±0.61		9.87±0.08		10.46±0.75		10.40±0.59	
No. plants examined	24		30		36		42	
No. spikes examined	46		52		50		49	
No. anthers examined	1380		1560		1500		1470	

[1] Figures in brackets indicate the duration of a stage as a percentage of total meiotic duration.

[2] STD = Synchronous tapetal division.

Table III

Duration of meiosis (h), quartets (h) and pollen maturation (days) at 20 °C in two pairs of genomically identical triticale hybrids which differed in the origin of their cytoplasm

Stage of meiosis	Nuclear genotype							
	A_5A_6				A_7A_8			
	Cytoplasm				Cytoplasm			
	6x		4x		6x		4x	
Nucleolar fusion	6.31±0.49	(14.29)[1]	5.26±0.66	(11.36)	6.13±0.67	(12.95)	6.41±0.85	(13.35)
Leptotene	9.84±0.38	(22.29)	11.32±0.58	(24.43)	11.06±0.47	(23.36)	11.34±0.59	(23.63)
Zygotene to STD[2]	2.52±0.35	(5.71)	1.62±0.51	(3.49)	1.50±0.49	(3.17)	0.62±0.56	(1.29)
STD to Pachytene	7.99±0.33	(18.10)	10.16±0.45	(21.92)	10.32±0.38	(21.80)	11.59±0.51	(24.15)
Pachytene	7.57±0.28	(17.16)	8.21±0.38	(17.72)	8.39±0.40	(17.73)	9.32±0.56	(19.42)
Diplotene & Diakinesis	2.35±0.30	(5.32)	2.31±0.48	(4.98)	2.44±0.53	(5.15)	1.98±0.57	(4.12)
FIRST PROPHASE	36.58±0.89	(82.87)	38.88±1.27	(83.90)	39.84±1.22	(84.16)	41.26±1.51	(85.96)
Metaphase I	2.20±0.66	(4.98)	2.15±0.69	(4.64)	2.19±0.69	(4.63)	1.94±0.66	(4.04)
Anaphase I to dyads	1.12±0.86	(2.54)	1.12±0.84	(2.42)	1.09±0.83	(2.30)	1.02±0.85	(2.12)
Dyads	1.94±0.95	(4.40)	1.92±0.94	(4.14)	1.95±0.92	(4.12)	1.72±0.97	(3.58)
M_{II}	1.36±0.79	(3.08)	1.34±0.84	(2.89)	1.37±0.84	(2.89)	1.22±0.85	(2.54)
A_{II} to tetrads	0.94±0.70	(2.13)	0.93±0.75	(2.01)	0.90±0.75	(1.90)	0.84±0.76	(1.76)
M_I - T_{II} INCLUSIVE	7.56±1.79	(17.13)	7.46±1.85	(16.10)	7.50±1.81	(15.84)	6.74±1.84	(14.04)
TOTAL MEIOTIC TIME	44.14±1.99	(100.00)	46.34±2.23	(100.00)	47.34±2.18	(100.00)	48.00±2.38	(100.00)
Quartet stage (h)	7.20±0.42		7.17±0.76		6.24±0.57		7.00±0.64	
Pollen maturation (d)	9.75±0.08		10.88±0.65		10.75±0.38		11.00±0.40	
No. plants examined	29		22		18		19	
No. spikes examined	43		37		32		35	
No. anthers examined	1290		1110		960		1050	

[1] Figures in brackets indicate the duration of a stage as a percentage of total meiotic duration.

[2] STD = Synchronous tapetal division.

duration. There was a linear correlation between the total duration of meiosis and the combined duration of zygotene and pachytene (Fig. 1), the stages during which pairing is thought to take place. These stages combined took from 40.96% ($A_5A_6$6x) to 47.8% ($A_3A_4$6x) of total meiotic duration.

No consistent differences were found between the duration of the combined stages zygotene + pachytene and the source of cytoplasm in the present materials. Thus, although in $A_1A_2$6x zygotene + pachytene lasted longer than in their counterparts having the tetraploid wheat cytoplasm, the situation was reversed in $A_3A_4$6x and $A_7A_8$6x (Tables II and III). It can be concluded therefore that, in triticale, cytoplasm derived from either tetraploid or hexaploid wheat has no consistent differential effects on the duration of meiosis and its constituent stages.

Meiotic Chromosome Pairing

In all combinations studied no significant differences were found between genotypically identical lines, irrespective of the cytoplasmic source, regarding number of chromosome arms paired and univalents per PMC, laggards at AI (exception A_7A_8, P = 0.05) and AII, and micronuclei per quartet (exception A_5A_6, P = 0.05) (Table IV). Analysis of the differences among pairs showed that some of the differences were highly significant. Thus, combination A_1A_2 had significantly more paired arms and fewer univalents per PMC, than the rest of the lines examined. These significant differences were not accompanied by different durations of total meiosis or zygotene + pachytene. For example, although pairs A_1A_2 and A_5A_6 differed by more than two chromosome arms paired per PMC their meiotic duration was essentially the same. Over all lines, no significant correlation was found between duration of meiosis and

Figure 1. The relationship between duration of meiosis and of zygotene and pachytene in eight triticale combinations (numbers refer to the order of the lines in Table IV).

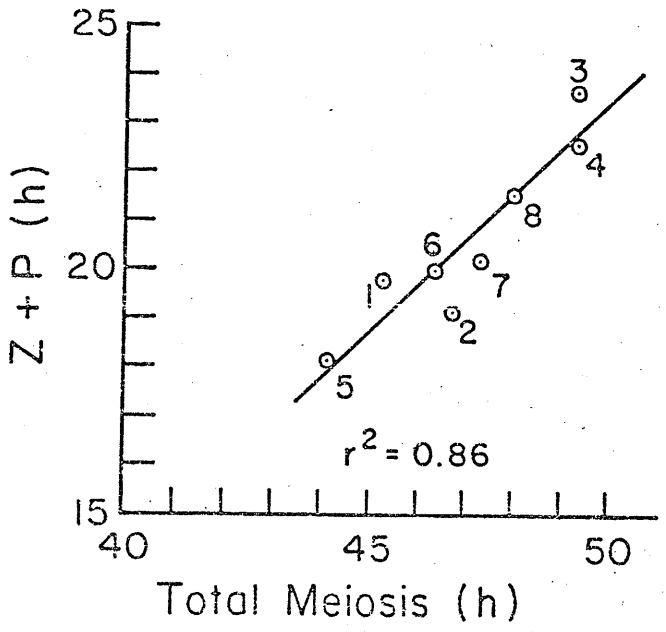

25
20
15
Z + P (h)
40
45
50
Total Meiosis (h)
$r^2 = 0.86$
1
2
3
4
5
6
7
8

Table IV

Meiotic characteristics (duration and chromosome pairing attributes) in four pairs of triticale hybrids each consisting of two genotypically identical lines but differing in the origin of their cytoplasm

Genotype	Cytoplasm	Duration (h)		Paired arms per PMC	Univalents per PMC		Laggard chromosomes at A_I	Laggard chromosomes at A_{II}	Micronuclei per quartet
		Zygotene and Pachytene	Total		Present results	Previous results[1]			
A_1A_2	6x	19.80	45.29	36.09±0.39	1.70±0.10	1.82±0.19	0.94±0.12	2.51±0.20	2.64±0.19
A_1A_2	4x	19.13	46.70	37.26±0.60	1.37±0.21	2.78±0.26	0.94±0.17	2.42±0.40	3.30±0.32
A_3A_4	6x	23.62	49.35	33.48±0.21	3.65±0.19	4.16±0.28	1.85±0.20	4.16±0.26	4.61±0.13
A_3A_4	4x	22.57	49.28	33.80±0.34	3.52±0.25	5.80±0.51	1.43±0.12	4.55±0.36	4.23±0.33
A_5A_6	6x	18.08	44.14	34.48±0.33	2.65±0.34	2.91±0.28	1.50±0.45	2.02±0.27	1.24±0.30
A_5A_6	4x	19.99	46.34	34.78±0.49	2.70±0.33	4.60±0.31	2.05±0.28	-	2.27±0.30*
A_7A_8	6x	20.21	47.34	33.60±0.87	3.58±0.6[illegible]	5.16±0.31	2.22±0.23	3.11±0.35	3.38±0.57
A_7A_8	4x	21.53	48.00	32.32±0.08	4.89±0.01	7.46±0.58	3.40±0.26*	2.71±0.39	2.74±0.24

[1] Larter and Hsam (1973).

* Within pair comparison significant at the 5% level.

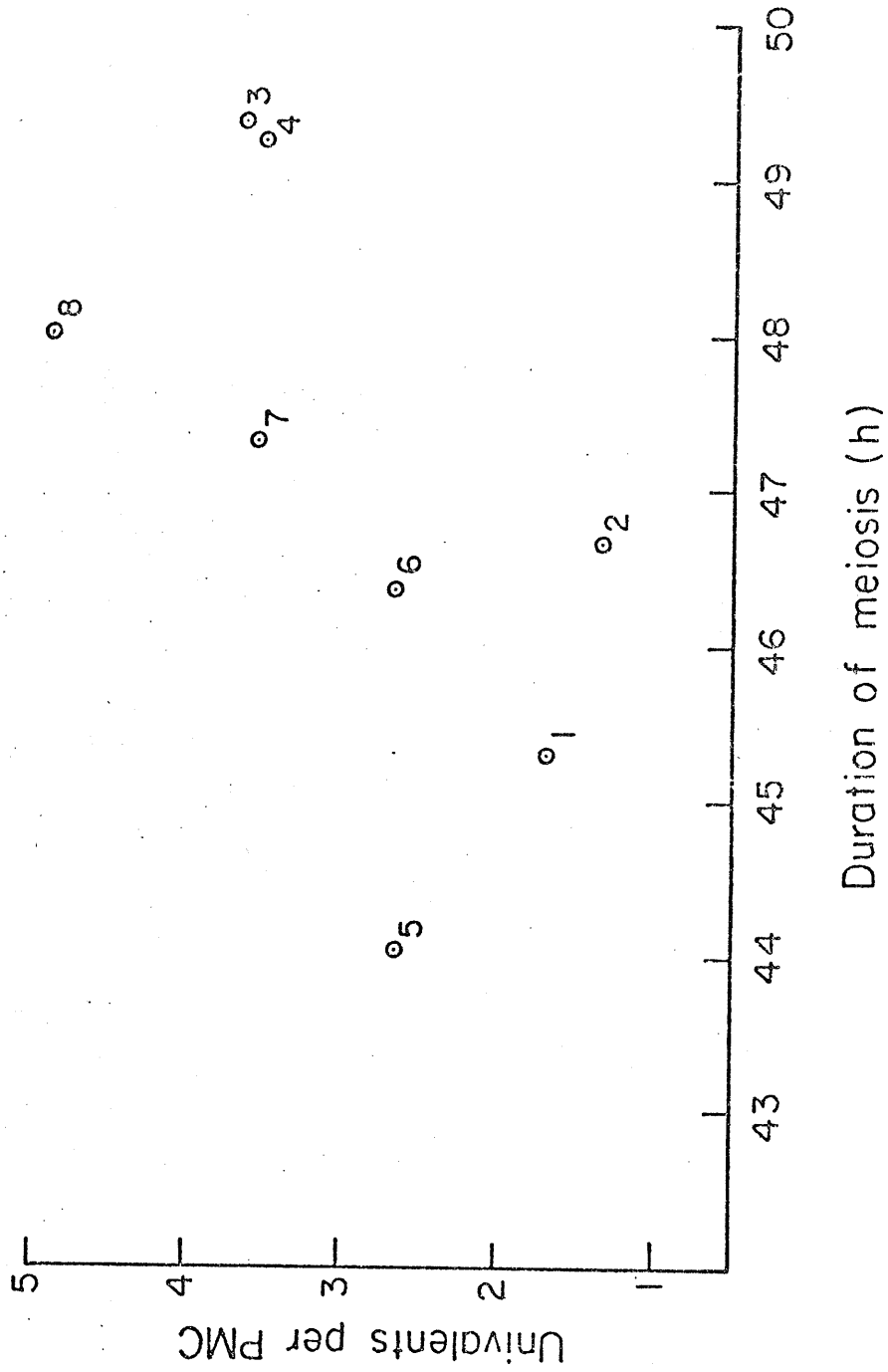
mean number of univalents per PMC at MI (Fig. II). Therefore, the time available for pairing does not seem to be limiting to the pairing process.

Discussion

Nucleolar Fusion and the Beginning of Meiosis

Prior to proceeding with the discussion of the present findings, it is important to establish the morphological criteria which indicate the onset of meiosis. Bennett et al., (1973) reported that in the hexaploid wheat variety Chinese Spring, leptotene began at about the time of the synchronous tapetal division; at 20°C under continuous illumination, this division occurs approximately 10 hours after nucleolar fusion (Roupakias and Kaltsikes, unpublished). At that time each meiocyte carries one nucleolus which protrudes 1/3 to 1/2 of the way out of the nuclear surface. Therefore, according to Bennett et al. (1973) nucleolar fusion occurs prior to the beginning of meiosis. In contrast, Darvey (1972), Darvey et al. (1973) and Thomas and Kaltsikes (1976) concluded that nucleolar fusion occurs during leptotene of hexaploid wheat. Taylor (1959) reported that DNA synthesis extends into preleptotene while Callan and Taylor (1968) thought that it extends even into leptotene. Bennett et al. (1973) reported that DNA synthesis was completed approximately two hours before the onset of what they called leptotene. Thomas and Kaltsikes (1977) found that DNA synthesis was almost completed by the time of nucleolar fusion. Therefore, considering that DNA synthesis extends into preleptotene and leptotene and, that at the time of nucleolar fusion DNA was almost doubled, nucleolar fusion can reasonably be considered as the earliest part of meiosis constituting a part of

Figure 2. The relationship between duration of meiosis and univalents per PMC in eight triticale combinations (numbers refer to the order of the lines as given in Table IV).


Univalents per PMC
5
4
3
2
1
43
44
45
46
47
48
49
50
Duration of meiosis (h)
1
2
3
4
5
6
7
8

preleptotene or even leptotene itself.

Furthermore, the number of nucleoli per PMC can be ascertained objectively while the protrusion of the nucleolus can be changed by differences in the squashing technique employed for the study of the meiocytes. However, to facilitate comparisons with other studies we followed the criteria used by Bennett et al. (1973) (i.e. leptotene begins when the nucleolus starts to project out of the nuclear surface) and retained the stage of nucleolar fusion as a stage distinct from leptotene.

Thomas and Kaltsikes (1976) reported that nucleolar fusion occurred 35-45 h prior to MI in pentaploid triticale hybrids. Their results are in full agreement with the results of the present study (Table II and III).

The Duration of Meiosis

Bennett and Kaltsikes (1973), in a study of the duration of meiosis in a primary hexaploid triticale (6A190), found that first prophase lasted 29.5 h, while Bennett and Smith (1972) obtained a value of 26.5 h in another hexaploid triticale (Rosner). The results of the present study indicate that in hexaploid triticale first prophase can last from 30.27 to 37.33 h (nucleolar fusion excluded). If nucleolar fusion were to be included, first prophase would last from 36.58 to 42.76 h. The differences reported in these three studies are probably due to the different genotypes used. It is also possible that the generation of the material studied may have an effect on the duration of meiosis. F_1 crosses were utilized in this study, while in the previous studies advanced generation lines were used. Tarkowski et al. (1974) found that the number of

univalents in octoploid x hexaploid triticale hybrids was reduced from F_1 to F_6. If the hypothesis of a longer meiosis being associated with better pairing is correct, an increase in the duration of meiosis would be expected as a line becomes stabilized. The opposite was reported in the aforementioned studies. Therefore, the different durations of meiosis obtained with the various hexaploid triticale are possibly due to the different genotypes studied, rather than to some being more stabilized than others.

The Effect of the Cytoplasm on Meiosis

Larter and Hsam (1973) reported that triticale genotypes had a more stable meiosis when the nucleus was placed in hexaploid wheat cytoplasm than in tetraploid wheat cytoplasm. Rimpau et al. (1973) also reported an effect of wheat or rye cytoplasm on chromosome pairing in addition lines. Furthermore, the finding of Thomas and Kaltsikes (1972), that triticale, the wheat parent of which was the extracted AABB component of hexaploid wheat, had a more regular meiosis than durum x rye triticale, may have been due to the influence of the cytoplasm. Kaltsikes (1974) theorized that the cytoplasm probably acts by affecting the duration of meiosis and especially those stages connected with pairing. However, such an effect was not found in the present study. The cytoplasm did not affect the duration of meiosis. Therefore, if the cytoplasm affects chromosome pairing, it must do so through another mechanism.

This study, however, showed no significant differences in the number of univalents and chromosome arms paired per PMC, number of laggards at AI and AII, and number of micronuclei per quartet when identical genotypes were placed in different cytoplasms (Table IV). It could be argued here

that both tetraploid and hexaploid wheat had a common donor of cytoplasm (Chen et al., 1975). Therefore, both lines of each pair examined were similar and accordingly no differences were expected. Kiss and Trefas (1973) and Lelley (1975) did not find significant differences in the number of univalents per PMC, even between lines carrying wheat or rye cytoplasm. Larter and Hsam (1973), who studied material derived from the same parents as in the present study, reported a significant difference in the number of univalents per PMC. In the present study the differences were not significant, but in both studies the genotypes ranked in the same order, the difference being more pronounced within pairs of lines (Table IV). Since chromosome pairing is genetically controlled, the individual outbreeding rye plants used in the crosses may have contributed, to the triticale, different genes for chromosome pairing thus accounting for the differences observed between the two studies. Lelley (1975) proposed that the system which polygenically controls the pairing of chromosome in rye (Rees, 1955; Rees and Thompson, 1956) is also functional in triticale. In other words, the meiotic behavior of a triticale plant is highly dependent on the rye genome involved, which may differ genotypically from plant to plant.

The initial premise of this study was that the improved pairing of triticale with hexaploid wheat cytoplasm, as reported by Larter and Hsam (1973), might be due to an effect of the cytoplasm on the duration of meiosis. The premise was based on the suggestion of Bennett et al. (1971) and Bennett and Kaltsikes (1973) that the rye chromosomes of triticale do not have sufficient time in which to properly pair, since rye requires considerably more time to complete meiosis than either wheat

or triticale. The present results clearly show that, for the materials studied, there was no relationship between duration of meiosis and chromosome pairing (Table IV, Fig. 2), indicating that perhaps these two characteristics of meiosis are independently determined by factors probably other than the cytoplasm. Thus, the hypothesis of insufficient time for normal pairing of rye chromosomes as a cause of meiotic disturbances in triticale cannot account for the present results. Bennett et al. (1974) in a study of the duration of meiosis in wheat genotypes with or without homoeologous meiotic chromosome pairing noted "some doubt must be cast upon the idea that the time available for pairing is limiting to the pairing process". Lelley (1974) also expressed doubts as to this hypothesis when, extending previous observations (see Kaltsikes, 1974 for references), he proposed desynapsis as a cause of univalency in triticale.

Further studies utilizing various other triticale genotypes of advanced generations and of different degrees of stability may provide a better means of testing the possible relationship between duration of meiosis and meiotic instability of triticale. The results of a study related to this question will be reported elsewhere.

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M A N U S C R I P T I I

INDEPENDENCE OF DURATION OF MEIOSIS AND CHROMOSOME PAIRING IN HEXAPLOID TRITICALE

Abstract

The duration of meiosis, its stages, and pollen maturation at 20 °C were determined in six hexaploid triticales (X Triticosecale Wittmack) which differed in the number of univalents per pollen mother cell at metaphase I (0.67 to 2.43). Meiosis lasted from 46.5 to 53.3 h; these estimates are longer than others previously reported for hexaploid triticale (34 - 37 h) grown under the same conditions. The quartet stage lasted from 7.1 to 8.0 h while pollen maturation took from 9.5 to 11.2 days. Apart from possible genotypic differences, and subjective judgements as to the beginning of meiosis, the present estimates include the stage of nucleolar fusion (average duration 6.0 h) as part of meiotic prophase. A strong positive correlation ($r = 0.99$) was found between total duration of meiosis and that of zygotene plus pachytene, the stages during which chromosome pairing is thought to occur. These two stages combined accounted for approximately 46% of the total duration of meiosis; even in the triticale with the shortest meiotic duration these two stages lasted longer than in rye (Secale cereale L.). As no relationship could be demonstrated between the total duration of meiosis, or of zygotene plus pachytene, and the number of univalents per PMC, it is concluded that time is not a limiting factor for chromosome pairing in triticale.

Introduction

Several theories have been advanced (Kaltsikes, 1974) to explain the occurrence of univalents in triticale (X Triticosecale Wittmack), the amphidiploid of wheat and rye. One of these attributed the presence

of univalents at first metaphase (MI) of triticale to the lack of sufficient time for pairing of the rye chromosomes which require a longer duration of zygotene and pachytene than that found in meiosis of triticale (Bennett et al., 1971; Bennett and Kaltsikes, 1973). Recently, however, Bennett et al. (1974) utilizing several wheat genotypes, concluded that in wheat no relationship existed between meiotic duration and the level of chromosome pairing observed at MI. On the basis of the differences in pairing observed between diakinesis and MI in triticale, Lelley (1974) expressed doubts as to the influence of meiotic duration on chromosome pairing. Roupakias and Kaltsikes (1977) examined four pairs of F1 hybrids between triticale strains that were genotypically identical but which differed in the origin of their cytoplasm and concluded that no relationship existed between the duration of zygotene + pachytene (Z + P), or total meiotic duration, and chromosome pairing.

To overcome the difficulties posed by the examination of F1 hybrids, which normally show a high degree of meiotic instability, the present study was undertaken. It utilizes advanced generation triticales which exhibit higher levels of chromosome pairing than those found in F1 hybrids.

Materials and Methods

The following materials were used:

(a) Hexaploid triticale: (1) cv. Rosner; (2) 6A600 (Triticum turgidum L. X Secale cereale L.); (3) 65-4 (T. turgicum var. durum cv. Stewart 63 X S. cereale, U.M. access. OD289); (4) 341-5 (Prelude (AABB) X S. cereale cv. Prolific); (5) R621-5 (Rescue (AABB) X Prolific); (6) 6A301 (Thatcher

(AABB) X Prolific). (b) Tetraploid wheat: T. turgidum var. durum cv. Stewart 63. (c) Diploid rye: Secale cereale OD289. The tetraploid wheats designated as Prelude (AABB), Rescue (AABB), and Thatcher (AABB) were described by Kaltsikes et al. (1969). The conditions of growth and the methodology used were identical to those of a previous study (Roupakias and Kaltsikes, 1977).

Chromosome arms paired and configurations at first metaphase, laggard chromosomes at first (AI) and second anaphase (AII) and micronuclei at the quartet stage were scored in eight euploid plants per strain (exceptions for AII were: Rosner, four plants; R621-5, seven plants; and at AI: R621-5, six plants) utilizing 25 PMC's in each plant. The numbers of plants, spikes and anthers examined per strain are given in Tables I, II, and III. The differences among means for the various attributes studied were tested by either the t-test or the analysis of variance followed by Duncan's new multiple range test.

Results

Meiotic Development

In all genotypes examined various degrees of asynchrony of meiotic development were observed among anthers of the same floret or even within anthers. Asynchrony increased in the following order among the strains examined: Stewart 63, 6A600, Rosner, OD289, 6A301, R621-5, 341-5 and 65-4; asynchrony was easily discernible in anthers containing meiocytes at stages past MI, probably on account of the short duration of those stages. Extreme cases such as the co-existence of MI, dyads (DY), MII and TII in the same anther, were observed in the 341-5 and

65-4 triticales. Among the anthers of the same floret the most frequent type of asynchrony observed was two anthers containing meiocytes at MI and the third at diakinesis or AI. However, extreme cases such as one anther at MI, the second at TI and the third at DY-MII (rye); MI, MII, MI/AI (Stewart 63); and MI, TI/DY, AI/TI (6A600) were occasionally observed.

One synchronous division of tapetal nuclei producing binucleate cells was observed in all genotypes examined, including rye; it took place at early zygotene in triticale, at early to mid-zygotene in wheat, and at the beginning of zygotene in rye (Tables I, II, and III).

Duration of Meiosis

The total duration of meiosis, excluding nucleolar fusion (NF), varied between 40.0 h (341-5) and 47.7 h (Rosner). With NF included, it ranged between 46.5 h (341-5) and 53.3 h (Rosner) (Tables I and II). First prophase occupied from 84.30% (341-5) to 86.73% (6A600) of the total duration of meiosis, while zygotene plus pachytene (Z + P), stages where pairing is thought to occur, accounted for from 45.37% (341-5) to 46.34% (6A600) (Tables I and II). A highly significant linear correlation was found between the duration of Z + P and total duration of meiosis (Fig. 1) ($r^2 = 0.98$, $P \leq 0.01$).

Significant differences were found between pairs of strains as follows:

Total duration of meiosis: Rosner vs. 341-5, 6A600 vs. 341-5 and 341-5 vs. 6A301, all significant at $P \leq 0.05$.

Duration of first prophase: Rosner vs. 65-4 ($P \leq 0.05$), Rosner vs. 341-5 ($P \leq 0.01$), Rosner vs. R621-5 ($P \leq 0.02$), 6A600 vs. 65-4 ($P \leq 0.05$), 6A600

Table I

The duration of meiotic stages (hours) and pollen maturation (days) in two hexaploid triticales grown at 20°C under continuous illumination

Meiotic stages	Rosner		6A600	
Nucleolar fusion	5.6 ± 0.72	(10.50)[2]	5.6 ± 0.50	(10.77)
Leptotene	12.1 ± 0.55	(22.70)	12.4 ± 0.58	(23.85)
Zygotene to STD[1]	1.3 ± 0.52	(2.44)	1.0 ± 0.43	(1.92)
STD to pachytene	12.8 ± 0.42	(24.02)	12.7 ± 0.41	(24.42)
Pachytene	10.5 ± 0.47	(19.70)	10.4 ± 0.46	(20.00)
Diplotene + Diakinesis	3.2 ± 0.48	(6.00)	3.0 ± 0.53	(5.77)
FIRST PROPHASE (total)	45.5 ± 1.31	(85.36)	45.1 ± 1.20	(86.73)
Metaphase I	1.9 ± 0.57	(3.57)	1.6 ± 0.64	(3.08)
AI to dyads	1.2 ± 0.64	(2.25)	1.2 ± 0.81	(2.31)
Dyads	1.8 ± 0.92	(3.38)	1.8 ± 0.82	(3.46)
MII	1.7 ± 0.33	(3.19)	1.4 ± 0.67	(2.69)
AII to quartets	1.2 ± 0.31	(2.25)	0.9 ± 0.68	(1.73)
MI-TII INCLUSIVE	7.8 ± 2.14	(14.64)	6.9 ± 1.63	(13.27)
TOTAL MEIOTIC TIME	53.3 ± 2.51	(100.00)	52.0 ± 2.02	(100.00)
Quartet stage (h)	7.2 ± 0.65		7.8 ± 0.57	
Pollen maturation (d)	9.5 ± 0.23		10.2 ± 0.35	
No. of plants examined	44		44	
No. of spikes examined	55		60	
No. of anthers examined	1650		1800	

[1] STD = Synchronous tapetal division. Not a meiotic stage but an unambiguous landmark.

[2] Figures in brackets indicate the duration of a stage as a percentage of total meiotic duration.

Table II

The duration of meiotic stages (hours) and pollen maturation (days) in four hexaploid triticales grown at 20°C under continuous illumination

Stage of meiosis	65-4		341-5		R621-5		6A301	
Nucleolar fusion	6.3 ± 0.23	(12.65)[2]	6.5 ± 0.55	(13.98)	6.0 ± 0.25	(12.12)	5.8 ± 0.33	(11.31)
Leptotene	11.0 ± 0.17	(22.09)	9.6 ± 0.55	(20.65)	10.8 ± 0.26	(21.81)	12.1 ± 0.33	(23.59)
Zygotene to STD[1]	2.2 ± 0.14	(4.42)	2.7 ± 0.55	(5.81)	2.0 ± 0.22	(4.04)	1.1 ± 0.25	(2.14)
STD to pachytene	11.2 ± 0.13	(22.48)	9.2 ± 0.48	(19.78)	11.2 ± 0.17	(22.63)	12.4 ± 0.22	(24.17)
Pachytene	9.3 ± 0.18	(18.67)	9.2 ± 0.51	(19.78)	9.5 ± 0.19	(19.19)	9.8 ± 0.25	(19.10)
Diplotene + diakinesis	2.3 ± 0.21	(4.62)	2.0 ± 0.66	(4.30)	2.3 ± 0.27	(4.65)	2.4 ± 0.29	(4.68)
FIRST PROPHASE (total)	42.3 ± 0.44	(84.93)	39.2 ± 1.35	(84.30)	41.8 ± 0.56	(84.44)	43.6 ± 0.69	(84.99)
Metaphase I	2.2 ± 0.29	(4.42)	2.0 ± 0.81	(4.30)	2.3 ± 0.37	(4.65	2.1 ± 0.40	(4.09)
AI to dyads	1.1 ± 0.31	(2.21)	1.1 ± 0.39	(2.36)	1.0 ± 0.51	(2.02)	0.9 ± 0.45	(1.75)
Dyads	1.9 ± 0.31	(3.82)	1.9 ± 0.41	(4.09)	1.8 ± 0.59	(3.64)	2.3 ± 0.65	(4.49)
MII	1.4 ± 0.21	(2.81)	1.3 ± 0.31	(2.80)	1.4 ± 0.43	(2.83)	1.4 ± 0.59	(2.73)
AII to quartets	0.9 ± 0.20	(1.81)	1.0 ± 0.29	(2.15)	1.2 ± 0.40	(2.42)	1.0 ± 0.38	(1.95)
MI-TII INCLUSIVE	7.5 ± 0.60	(15.07)	7.3 ± 1.08	(15.70)	7.7 ± 1.05	(15.56)	7.7 ± 1.13	(15.01)
TOTAL MEIOTIC TIME	49.8 ± 0.74	(100.00)	46.5 ± 1.73	(100.00)	49.5 ± 1.19	(100.00)	51.3 ± 1.32	(100.00)
Quartet stage	8.0 ± 0.21		7.1 ± 0.62		7.6 ± 0.33		7.4 ± 0.29	
Pollen maturation	10.2 ± 0.17		10.7 ± 0.13		10.1 ± 0.40		11.2 ± 0.11	
No. plants examined	35		34		29		37	
No. spikes examined	58		45		43		56	
No. anthers examined	1620		1350		1290		1530	

[1] STD = Synchronous tapetal division. Not a meiotic stage but an unambiguous landmark.

[2] Figures in brackets indicate the duration of a stage as a percentage of total meiotic duration.

Figure 1. The relationship between the total duration of meiosis and that of zygotene plus pachytene in six strains of hexaploid triticale.

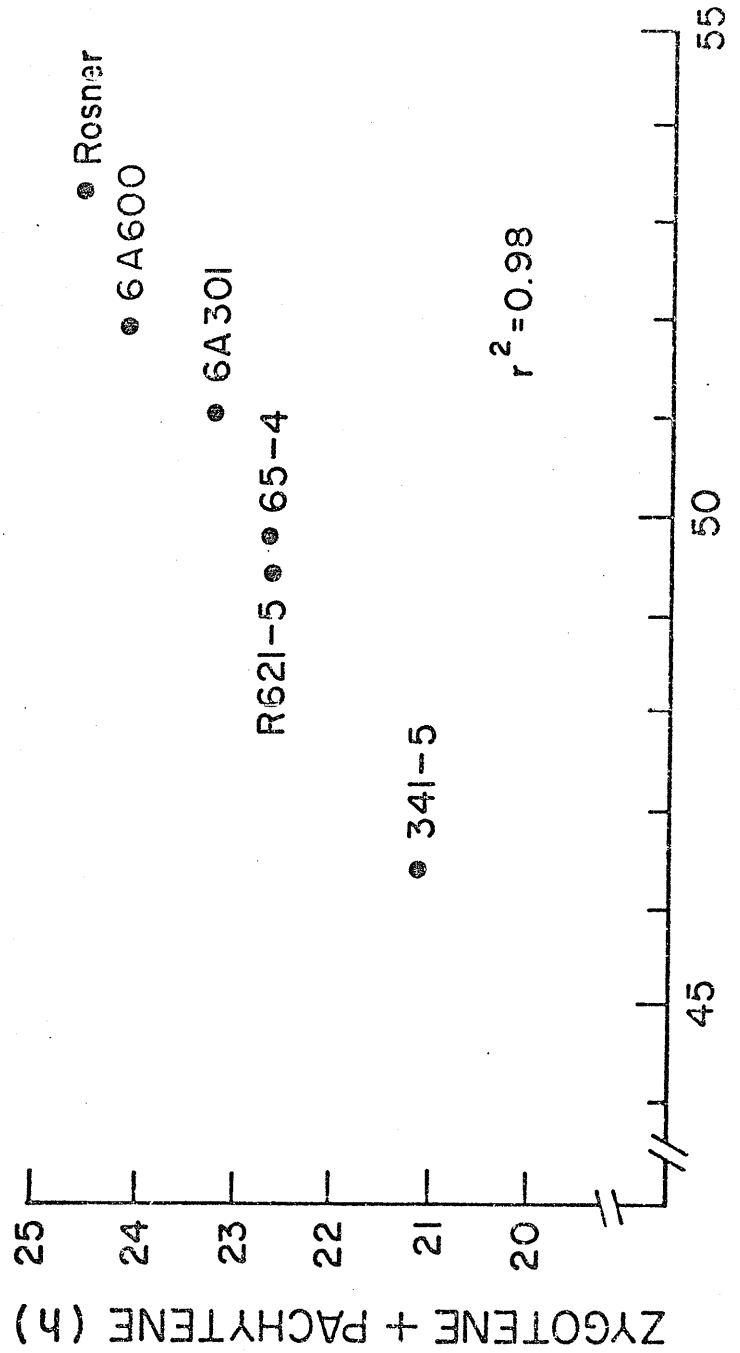

Rosner
6A600
6A301
R621-5
65-4
341-5
r^2=0.98
ZYGOTENE + PACHYTENE (h)
25
24
23
22
21
20
45
50
55
DURATION OF MEIOSIS (h)

Table III

The duration of the stages of meiosis (hours) and pollen maturation (days) in a tetraploid wheat and two diploid ryes grown at 20° C under continuous illumination

Stage of meiosis	Tetraploid wheat		Diploid rye			
	Stewart 63		OD289		Petkus[1] (Spring rye)	
Nucleolar fusion	6.4 ± 0.22	(14.61)[3]	6.6 ± 0.51	(12.48)		
Leptotene	9.4 ± 0.23	(21.46)	15.6 ± 0.35	(29.49)	20.0	(36.06)
Zygotene to STD[2]	3.2 ± 0.20	(7.31)	1.0 ± 0.33	(1.89)	11.4	(22.27)[4]
STD to pachytene	7.7 ± 0.15	(17.58)	12.1 ± 0.31	(22.87)		
Pachytene	7.8 ± 0.20	(17.81)	7.6 ± 0.37	(14.37)	8.0	(15.63)
Diplotene + diakinesis	2.1 ± 0.21	(4.79)	2.8 ± 0.33	(5.29)	1.6	(3.12)
FIRST PROPHASE (total)	36.6 ± 0.50	(83.56)	45.7 ± 0.91	(86.39)	41.0	(80.08)
Metaphase I	2.3 ± 0.27	(5.25)	2.1 ± 0.56	(3.97)	2.0	(3.91)
AI to dyads	0.7 ± 0.36	(1.61)	1.1 ± 0.72	(2.08)	2.0	(3.91)
Dyads	2.1 ± 0.44	(4.79)	1.7 ± 0.39	(3.21)	2.5	(4.88)
MII	1.2 ± 0.63	(2.74)	1.3 ± 0.32	(2.46)	1.7	(3.32)
AII to quartets	0.9 ± 0.50	(2.05)	1.0 ± 0.68	(1.89)	2.0	(3.90)
MI-TII INCLUSIVE	7.2 ± 1.02	(16.44)	7.2 ± 1.96	(13.61)	10.2	(19.92)
TOTAL MEIOTIC TIME	43.8 ± 1.13	(100.00)	52.9 ± 2.16	(100.00)	51.2	(100.00)
Quartet stage	7.7 ± 0.20		8.1 ± 0.37		8.5	
Pollen maturation	10.3 ± 0.28		10.7 ± 0.17		16.0	
No. plnats examined	36		43		-	
No. spikes examined	52		60		-	
No. anthers examined	1380		1650		-	

[1] Bennett et al. (1971)

[2] STD = Synchronous tapetal division. Not a meiotic stage but an unambiguous landmark.

[3] Figures in brackets indicate the duration of a stage as a percentage of total meiotic duration.

[4] Zygotene only

vs. 341-5 ($P \leq 0.01$), 6A600 vs. 621-5 ($P \leq 0.02$), 65-4 vs. 341-5 ($P \leq 0.05$), and 341-5 vs. 6A301 ($P \leq 0.01$).

Duration of Z + P: Rosner vs. 65-4 ($P \leq 0.05$), Rosner vs. 341-5 ($P \leq 0.01$), Rosner vs. R621-5 ($P \leq 0.05$), 6A600 vs. 341-5 ($P \leq 0.02$), and 341-5 vs. 6A301 ($P \leq 0.05$).

Statistical analysis revealed no significant differences in duration of meiotic stages after MI, among wheat, rye, and the triticale (65-4) derived from them (Tables II and III). However, meiosis took longer in rye than triticale and in both it lasted significantly longer ($P \leq 0.001$) than in wheat (Tables II and III). First prophase was significantly longer in rye than in triticale ($P \leq 0.01$), or wheat ($P \leq 0.001$), and also in triticale as compared to wheat ($P \leq 0.001$). The duration of Z + P was significantly longer in triticale than in either wheat ($P \leq 0.001$) or rye ($P \leq 0.01$).

Meiotic Abnormalities

Analysis of variance revealed significant differences in meiotic abnormalities among the triticales studied (univalents $P \leq 0.01$; paired arms $P \leq 0.001$; AI $P \leq 0.001$; AII $P \leq 0.001$; and micronuclei $P \leq 0.01$, Table IV). Further analysis using Duncan's method revealed significant differences ($P \leq 0.05$) between the following pairs:

1. Univalents: Rosner vs. the rest of the strains; 6A600 vs. 65-4 and 341-5; 65-4 vs. R621-5 and 6A301; 341-5 vs. R621-5 and 6A301.
2. Paired arms: Rosner vs. 6A600, 341-5 and 6A301; 6A600 vs. 65-4, 341-5 and R621-5; 65-4 vs. 341-5 and 6A301; 341-5 vs. R621-5 and 6A301; and R621-5 vs. 6A301.

No significant correlation was established between the duration of

Table IV

Meiotic characteristics of hexaploid triticale, tetraploid wheat and diploid rye

Material	Duration (hours)		Meiotic abnormalities				
	Zygotene + pachytene	Total meiosis	Univalents per PMC	Chromosome arms paired	Laggard chromosomes AI	Laggard chromosomes AII	Micronuclei per quartet
Triticale							
Rosner	24.6	53.3	1.62±0.11	35.87±0.30	-	2.35±0.87	1.46±0.58
6A600	24.1	52.0	0.67±0.12	37.72±0.26	0.16±0.40	0.43±0.10	0.23±0.07
65-4	22.7	49.8	2.32±0.14	36.37±0.35	-	2.01±0.22	1.07±0.14
341-5	21.1	46.5	2.43±0.21	33.44±0.45	1.35±0.36	0.99±0.27	1.77±0.24
R621-5	22.7	49.5	1.06±0.12	36.43±0.21	0.52±0.13	1.09±0.17	0.96±0.18
6A301	23.3	51.3	1.06±0.16	37.63±0.23	0.35±0.09	0.57±0.16	0.48±0.10
Wheat							
Stewart 63	18.7	43.8	0.10±0.05	27.32±0.11	0.03±0.03	0.00±0.00	0.00±0.00
Rye							
OD 289	20.7	52.9	0.12±0.08	12.81±0.11	0.03±0.02	0.04±0.03	0.02±0.01

either meiosis or Z + P, and univalents per PMC (Figs. 2 and 3). Furthermore, although the triticale (i.e. 341-5) with the highest number of univalents per PMC had the shortest duration of leptotene or leptotene + NF (Tables I, II and IV), no significant correlation was found between the duration of leptotene (r^2 = 0.50) or leptotene + NF (r^2 = 0.40) and univalents per PMC.

Discussion

Ascertaining the beginning of meiosis and some of its stages is a subjective matter. The present authors have attempted to overcome this subjective bias in two ways. Firstly, by considering nucleolar fusion (NF), a well-defined and objectively ascertainable stage, as the beginning of meiosis. The reasons for this choice were given in Roupakias and Kaltsikes (1977). Secondly, by including in the present study materials (Rosner and Stewart 63) which had already been the subject of similar investigations (Bennett and Smith, 1972; Bennett and Kaltsikes, 1973). The combination of these two factors allows confident comparisons among the strains used by the present authors with the strains used by others.

The present results agree quite well with those of a previous study (Roupakias and Kaltsikes, 1977) in that in both studies first prophase (NF excluded) lasted from 32.7 h (341-5) to 39.9 h (Rosner). Bennett and Smith (1972) found that under the same environmental conditions this interval lasted 26.5 h in Rosner which is approximately two-thirds of the duration found in the present study. If meiotic timing studies are to be comparable they should yield estimates of duration that are not as

Figure 2. The relationship between total duration of meiosis and number of univalents per PMC in six strains of hexaploid triticale.

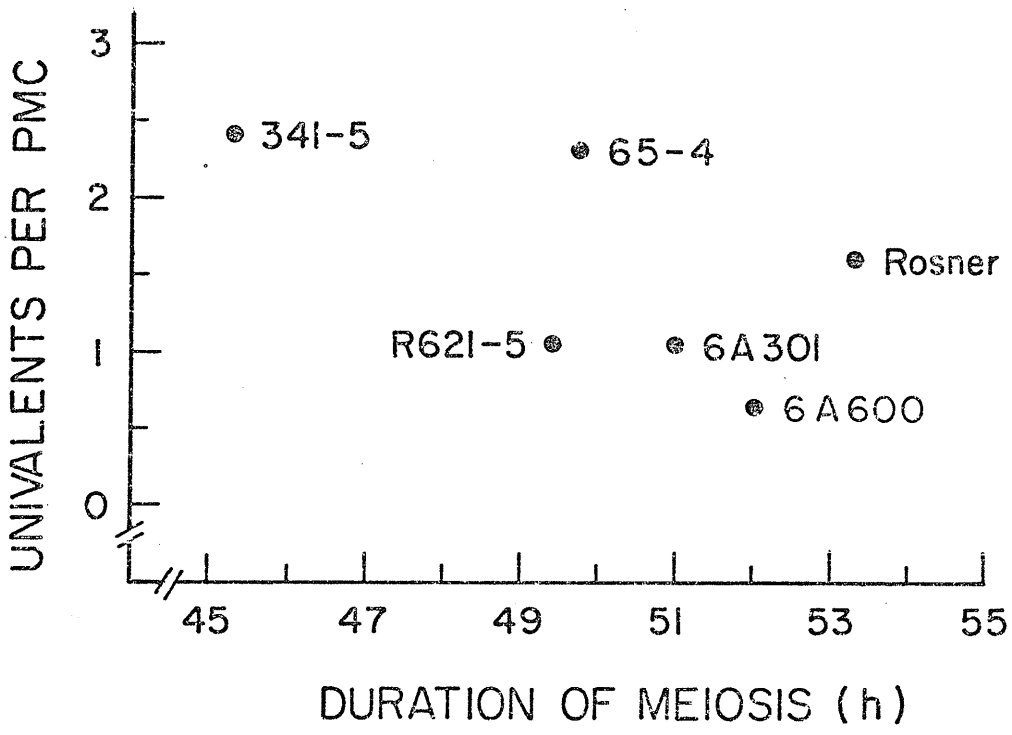
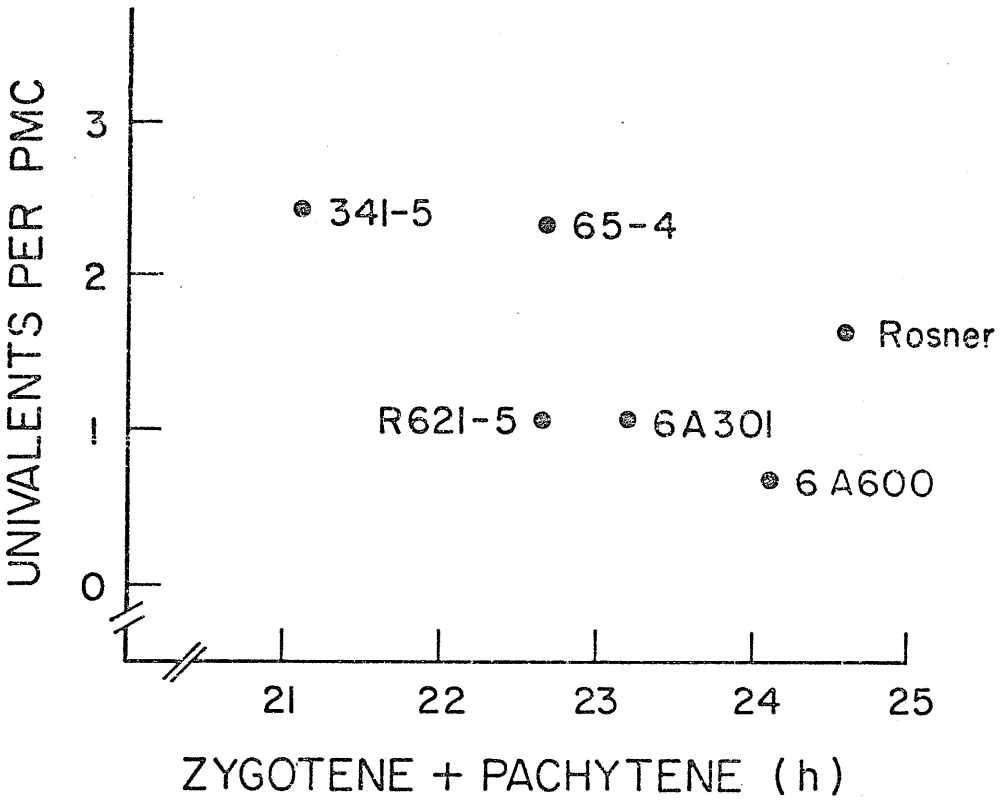

UNIVALENTS PER PMC
3
2
1
0
341-5
65-4
Rosner
R621-5
6A301
6A600
45
47
49
51
53
55
DURATION OF MEIOSIS (h)

Figure 3. The relationship between the duration of zygotene plus pachytene and number of univalents per PMC in six hexaploid triticale strains.


UNIVALENTS PER PMC
3
2
1
0
341-5
65-4
Rosner
R621-5
6A301
6A600
21
22
23
24
25
ZYGOTENE + PACHYTENE (h)

widely different as those indicated above. Fortunately, the unambiguous landmark of the synchronous tapetal division (STD) can help in deciding whether the two studies yielded comparable results. Bennett and Smith (1972) placed STD at the beginning of leptotene and gave the time required for the interval STD to MI as 26.5 h. Our study showed STD to be concurrent with early zygotene; meiocytes took 26.5 h to progress from this point to MI. The difference found in the duration of first prophase between the two studies, therefore, can be attributed to the subjective determination of the beginning of meiosis. It is likely that this difference in judgement is limited to the study of Rosner only, since for Stewart 63 both studies place STD as occurring concurrently with zygotene. In addition, for triticale 65-4, which has the same wheat parent as 6A190 (Stewart 63), both Bennett and Kaltsikes (1973) and the present study place STD as taking place concurrently with early zygotene. Furthermore, Bennett and Kaltsikes (1973) and the present study agree on the occurrence of only one STD in rye, concurrently with zygotene. This indicates a consistent application of subjective morphological criteria for the determination of meiotic stages. It can also be stressed that in all other hexaploid triticales studied STD was found to occur during early zygotene, making it unlikely that Rosner would be very much different in this respect.

In any case, the difference between the two estimates of the duration of first meiotic prophase in Rosner does not affect the main thrust of this investigation, which was to determine the relationship between the duration of Z + P and chromosome pairing in triticale. This study was undertaken to test the hypothesis proposed by Bennett and co-workers

(Bennett et al., 1971; Bennett and Smith, 1972; Bennett and Kaltsikes, 1973) that some rye chromosomes do not pair in triticale because, relative to the wheat chromosome complement, they do not have sufficient time in which to do so. If this premise is accepted then the following should hold true: (1) There should be a positive correlation between the duration of those stages during which chromosome pairing is thought to be taking place (zygotene and pachytene) and the level of pairing. (2) In triticale hybrids, which have a meiotic duration equal to that of their parents, there should be no more meiotic abnormalities than in their parents. (3) There should be little variation with respect to pairing among PMC's of a single anther.

All of these corollaries can be tested. Neither the present study (Figs. 2 and 3) nor a previous one (Roupakias and Kaltsikes, 1977) showed a consistent relationship between the duration of meiosis, or that of the combined stages of zygotene and pachytene, and the level of chromosome pairing. Furthermore, in a hybrid between Rosner and 6A600, which had a meiotic duration similar to the parents (present authors, unpublished) 2.3 and 5.6 times more univalents were observed in the hybrid than in Rosner and 6A600, respectively. Increased univalency, relative to the parents, has been reported many times in triticale hybrids (Larter and Hsam, 1973; Merker, 1973; Roupakias and Kaltsikes, 1977).

Not all PMC's showed the same level of chromosome pairing, since some of them had no univalents, while others had more than four, although the time they spend in Z + P should be the same for all. Furthermore, the present study has shown that the actual duration of Z + P (Table IV) was longer in hexaploid triticale than in rye. Therefore, the hypothesis

that the duration of Z + P determines the amount of rye chromosome pairing in triticale does not seem to account for all the known facts of univalency in triticale. It is possible, however, that the duration of earlier stages (i.e. leptotene) may affect chromosome pairing because the triticale with the shortest leptotene had more univalents than the others (Tables I, II, and IV). However, no significant correlation was detected between duration of leptotene, or leptotene + NF, and univalents per PMC. It becomes necessary, therefore, to examine factors other than duration of meiosis and its stages as causes for this phenomenon.

In established triticale strains the mean number of univalents is rarely more than four and in most cases it is between one and two (Scoles and Kaltsikes, 1974), indicating that on the average only a few chromosomes fail to pair in triticale. It is also known that the majority of the chromosomes that fail to pair belong to the rye genome (Thomas and Kaltsikes, 1974, 1976). Furthermore, among the rye chromosomes, those with prominent Giemsa-staining bands at both telomeres are more likely to be seen as univalents than those having a band at only one of the telomeres. Loss of most, or the entire telomeric band of chromosome arms $6R^S$ (Kaltsikes and Roupakias, 1976) and $7R^L$ (Merker, 1976) improved pairing in triticale. Additionally, Miklos and Nankivell (1976) have shown that in grasshoppers the amount of heterochromatin was inversely correlated with chiasma frequency. The above observations implicate heterochromatin in the control of chromosome pairing even though the precise mechanism of its action remains unknown (see Thomas and Kaltsikes (1974) for a hypothesis on this subject).

The possibility also exists that genetic factors on certain wheat

or rye chromosomes act or interact to regulate chromosome pairing in triticale (Lelley, 1975). It was mentioned above that there was variation with respect to the number of univalents per PMC within an anther although all carry the same chromosome complement. It could well be that these PMC's are not in contact with the tapetum and, therefore, do not receive from, or through it, the factor(s) necessary for normal progression through meiosis (Christensen and Horner, 1974). It could also be that competition may exist among meiocytes for precursors needed for the formation of the synaptonemal complex, reunion of the breaks that occur during crossing-over, or enzymes which affect chromosome movement and homologue recognition. Such a protein, present only in meiotic cells, and with a functional importance in chromosome pairing and recombination, has been reported by Hotta and Stern (1971). Further biochemical studies in various triticales may, therefore, result in a better understanding of the cause of the meiotic abnormalities.

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M A N U S C R I P T III

GENOMIC EFFECTS ON THE DURATION OF MEIOSIS IN TRITICALE AND ITS PARENTAL SPECIES

Abstract

The effect of the D and R genomes on the duration of meiosis and its stages was studied in the following materials: 1. AABBDD (Triticum aestivum L. em. Thell.); 2. AABB (extracted from AABBDD); 3. AABBRR and AABBDDRR (X Triticosecale Wittmack); 4. AABBD; 5. ABRR; and 6. RR (Secale cereale L.). Genomes AB, D and R were the same irrespective of the material in which they were found. At 20 °C and continuous illumination meiosis lasted 32.8 to 44.5 h in the AABBDD; 44.1 to 44.6 h in the AABB; 46.4 to 51.3 h in the AABBRR; 43.6 h in the AABBDDRR; 44.5 h in the AABBD; 51.6 to 52.7 h in the ABRR and 52.6 h in the RR genotype. Addition of the D genome to the AABB and AABBRR genotypes resulted in (1) elongation of the stage of nuclear fusion (2) shortening of the combined duration of zygotene and pachytene and (3) reduction of the total duration of meiosis in AABBRR while it had no effect on AABB. Addition of the R genome to AABB resulted in the elongation of the total duration of meiosis and its stages while it had no significant effect when added to AABBDD. It was concluded that the variation observed in the duration of meiosis and its stages among the various cereal genotypes studied was more likely due to genes carried by the D and R genomes rather than to simple changes in ploidy level or DNA content.

Introduction

With respect to the duration of meiosis at 20 °C the various forms of triticale (X Triticosecale Wittmack) and their wheat (Triticum L.) and rye (Secale L.) parents can be arranged as follows: octoploid triticale

(21-22 h), hexaploid wheat (24 h), tetraploid wheat (31-37 h), hexaploid triticale (34-47 h) and rye (47-51 h) (Bennett et al., 1971, Bennett and Kaltsikes, 1973; Roupakias and Kaltsikes, 1977a, b). Bennett and Smith (1972) attributed these differences in meiotic duration to the amount of nuclear DNA and the ploidy level of the strain involved. Roupakias and Kaltsikes (1977a, b) reported significant differences among hexaploid triticale strains (i.e. same ploidy level and most likely same DNA content) which indicated that genetic factors may have a major effect on the duration of meiosis, while Bennett et al. (1974), in a study of euploid and aneuploid wheat genotypes, found that individual chromosomes (i.e. 5B) have major effects on the duration of meiosis. Law et al. (1976) found that the extreme earliness observed in Chinese Spring hexaploid wheat was due to chromosome 5D. It is possible, therefore, that the much shorter duration of meiosis in hexaploid wheat and octoploid triticale strains was induced by the D genome acting directly or interacting with genes carried by the A, B and R genomes.

The present study was undertaken to investigate the possible effect(s) of the D and R genomes on the duration of meiosis and its constituent stages. It utilized genetically related tetraploid and hexaploid wheat strains, one rye strain and the triticales derived from them.

Materials and Methods

The materials used in this study (genomic formula in brackets) were:

1. Diploid rye: Secale cereale L. 'Prolific' (RR).

2. Tetraploid wheat: Triticum turgidum L. (1) 'Prelude' (AABB); (2) 'Rescue' (AABB); (3) 'Thatcher' (AABB).

3. Hexaploid wheat: T. aestivum L.em. Thell.: (1) 'Prelude' (AABBDD); (2) 'Chinese Spring' (AABBDD).

4. Octoploid triticale: (1) 8A73 (hexaploid Prelude X Prolific); (2) 8A74 (hexaploid Rescue X Prolific); (3) 8A599 (hexaploid Thatcher X Prolific).

5. Hybrids of hexaploid triticale X Prolific: (1) Rosner X Prolific (ABRR); (2) 6A299 (tetraploid Prelude X Prolific) X Prolific (ABRR). Meiotic examination of these hybrids showed that Rosner and 6A299 contributed all 7 chromosomes of the R genome although 2R in Rosner lacks the terminal heterochromatic bands (present authors, unpublished).

6. Hybrids of tetraploid wheat Stewart 63 X hexaploid wheat: (1) Stewart 63 X Rescue (AABBD); (2) Stewart 63 X Thatcher (AABBD).

The tetraploid wheats designated as Prelude (AABB), Rescue (AABB) and Thatcher (AABB), have been extracted from their hexaploid counterpart (Kaltsikes et al., 1969). The duration of meiosis of the hexaploid triticale strains derived from the extracted tetraploid wheats (Prelude, Rescue, and Thatcher) was reported by Roupakias and Kaltsikes (1977b). The conditions of growth and the methodology used have been given previously (Roupakias and Kaltsikes, 1977a). Although this study deals with events taking place in the meiocytes, the tapetum was also studied because the synchronous division of its cells (STD), which occurs concurrently with meiosis, provides a convenient and unambiguous landmark that can be used in comparing meiotic duration data reported by different researchers (see discussion).

The number of plants, spikes and anthers examined for the determination of the meiotic duration are given in Tables I, II, and III. The

t-test was used to calculate differences among means of the various attributes studied.

Results

Meiotic Development

In each floret of the genotypes studied there were three anthers. Within individual anthers, and among anthers of each floret, asynchrony of approximately 1-2 h was observed (see also Roupakias and Kaltsikes, 1977a, b) with the exception of the hybrids Rosner X Prolific and 6A299 X Prolific which showed a higher degree of asynchrony (1-3 h).

One STD, which resulted in binucleate tapetal cells,was observed in all genotypes investigated. It was concurrent with: (a) early zygotene in Prolific rye and in both of its hybrids, Rosner X Prolific and 6A299 X Prolific; (b) early- to mid-zygotene in all tetraploid wheats and in the hybrids Stewart 63 X Rescue (6x) and Stewart 63 X Thatcher (6x); (c) mid- to late-leptotene in octoploid triticale (8A74, 8A73, 8A599); and (d) mid-leptotene in hexaploid wheat Prelude (6x) and Chinese Spring (Tables I, II and III). One STD which occurred while PMC's were at zygotene in Prolific rye and early- to mid-zygotene in tetraploid wheat was also reported by Bennett and Kaltsikes (1973) and Roupakias and Kaltsikes (1977b), respectively. Bennett and Smith (1972) also reported one STD in hexaploid wheat and octoploid triticales. It occurred while PMC's were at: the beginning of leptotene in Chinese Spring; the beginning of zygotene in Holdfast and early leptotene in the octoploid triticale strains.

Duration of Meiosis

Secale cereale cv. 'Prolific': The total duration (which includes the period of nucleolar fusion (NF) to TII unless otherwise indicated) of meiosis in Prolific rye was 52.6 h (Table I). First prophase and zygotene + pachytene (Z + P) occupied 85.4% (44.9 h) and 37.6% (19.8 h) of total meiotic duration respectively. These results are in full agreement with those reported by Roupakias and Kaltsikes (1977b) for OD289 rye (differences for Z + P and total duration of meiosis were not significant). Bennett and Kaltsikes (1973) also reported a total duration of meiosis in Prolific rye equal to 51.2 (NF excluded) and that of Z + P equal to 19.4 h. There is a close agreement between the two studies. The differences observed were restricted mainly to stages following MI, i.e., stages which have no effect on chromosome pairing. Bennett and Kaltsikes (1973) found a duration of 10.2 h for all meiotic stages between MI and TII inclusive, which in the present study was found to be 7.7 h. Quartets lasted about 8.5 h in both studies but pollen maturation was found to require four days less in the present study (Table I).

Tetraploid wheat: The total duration of meiosis in the tetraploid wheats ranged between 44.1 and 44.6 h; the first prophase between 37.0 h (i.e. 83.0% of the total duration of meiosis) and 37.3 h (84.6%); and the duration of Z + P between 18.8 h (43.6%) and 19.6 h (44.4%) (Table I). The differences observed among the tetraploids were not statistically significant. The duration of the quartet stage ranged between 7.3 and 8.0 h; the differences being significant only for the pair, Prelude and Rescue ($P \leq 0.05$). Somewhat greater differences among the three tetraploids examined were observed for the duration of pollen maturation.

Table I

The duration of meiotic stages (hours) and pollen maturation (days) in three tetraploid wheats and Prolific rye grown at 20°C under continuous illumination

Stage of Meiosis	Rye		Tetraploid wheat					
	Prolific (RR)		Prelude (AABB)		Rescue (AABB)		Thatcher (AABB)	
Nucleolar fusion	4.9±0.48	(9.32)[2]	5.9±0.14	(13.38)	5.8±0.21	(13.15)	5.8±0.63	(13.00)
Leptotene	17.6±0.26	(33.46)	9.5±0.17	(21.54)	9.9±0 19	(22.45)	9.8±0.53	(21.97)
Zygotene to STD[1]	1.3±0.23	(2.47)	4.3±0.16	(9.75)	4.3±0.18	(9.75)	4.2±1.62	(9.42)
STD to pachytene	10.6±0.25	(20.15)	6.9±0.14	(15.65)	7.0±0.16	(15.87)	7.4±1.70	(16.59)
Pachytene	7.9±0.34	(15.02)	8.4±0.29	(19.04)	7.5±0.22	(17.01)	7.8±0.39	(17.49)
Diplotene + diakinesis	2.6±0.31	(4.94)	2.2±0.28	(4.99)	2.8±0.23	(6.35)	2.0±0.43	(4.49)
FIRST PROPHASE (total)	44.9±0.79	(85.36)	37.2±0.51	(84.35)	37.3±0.49	(84.58)	37.0±2.56	(82.96)
Metaphase I	2.1±0.28	(3.99)	1.9±0.22	(4.31)	2.3±0.37	(5.22)	2.5±0.51	(5.61)
AI to dyads	1.2±0.34	(2.28)	1.0±0.60	(2.27)	0.9±0.33	(2.04)	1.0±0.75	(2.24)
Dyads	1.8±0.37	(3.43)	1.5±0.61	(3.40)	1.4±0.29	(3.17)	1.8±0.70	(4.03)
MII	1.4±0.45	(2.66)	1.5±0.36	(3.40)	1.2±0.23	(2.72)	1.4±0.44	(3.14)
AII to quartets	1.2±0.47	(2.28)	1.0±0.25	(2.27)	1.0±0.23	(2.27)	0.9±0.69	(2.02)
MI-TII INCLUSIVE	7.7±0.87	(14.64)	6.9±0.99	(15.65)	6.8±0.66	(15.42)	7.6±1.41	(17.04)
TOTAL MEIOTIC TIME	52.6±1.18	(100.00)	44.1±1.11	(100.00)	44.1±0.82	(100.00)	44.6±2.92	(100.00)
Quartet stage	8.4±0.32		8.0±0.19		7.3±0.20		7.5±0.37	
Pollen maturation	12.0±0.21		11.2±0.18		9.9±0.09		11.1±0.31	
No. plants examined	52		35		31		25	
No. spikes examined	59		65		44		33	
No. anthers examined	1590		1170		792		594	

[1] STD = synchronous tapetal division. Not a meiotic stage but an unambiguous landmark.

[2] Figures in brackets refer to the percentage of the total meiotic duration taken up by the meiotic stage.

It ranged between 9.9 (Rescue) and 11.2 days (Prelude). The differences were significant for the pairs, Prelude and Rescue ($P \leq 0.001$) and Rescue and Thatcher ($P \leq 0.01$) (Table I).

Hexaploid wheat: Examination of a limited number of spikes in Prelude, Rescue and Thatcher showed that the duration of meiosis was essentially identical in these three cultivars. This fact coupled with the similarities in meiotic duration in (1) their extracted AABB counterparts (Table I) and (2) the hybrids of Stewart '63 with hexaploid Rescue and Thatcher (Table III) led us to examine in detail only one of these three cultivars, namely Prelude. The results reported (Table II), however, are considered to apply to all three hexaploid cultivars.

The total duration of meiosis in hexaploid Prelude was 44.5 h or approximately the same as in tetraploid Prelude (Tables I and II) (difference not significant). However, the two genotypes differed significantly ($P \leq 0.001$) in the duration of NF and of Z + P. NF and quartets lasted longer in hexaploid than in tetraploid wheat, while Z + P and pollen maturation lasted longer in tetraploid than in hexaploid wheat. Bennett and Smith (1972) reported a much shorter duration of meiosis (24.0 h, NF excluded) for Chinese Spring. Because of the magnitude of the difference (12 h) between the two hexaploid wheats, Prelude and Chinese Spring, we decided to reinvestigate Chinese Spring.

With NF excluded, our estimate of the duration of meiosis in Chinese Spring becomes 27.5 h (Table II) as compared to the 24 h reported by Bennett et al. (1971). The difference can be attributed to the subjective criteria used to ascertain the beginning of meiosis; it is not considered large enough to indicate a real difference between the two estimates.

Table II

The duration of meiotic stages (hours) and pollen maturation (days) in two hexaploid wheat and two octoploid triticales grown at 20°C under continuous illumination

Stage of meiosis	Octoploid triticale				Hexaploid wheat			
	8A74		8A599		Prelude		Chinese Spring	
Nucleolar fusion[1]	8.3±0.26	(18.99)[2]	7.7±0.31	(17.66)	7.9±0.23	(17.75)	5.3±0.20	(16.16)
Leptotene to STD	6.3±0.19	(14.42)	7.3±0.12	(16.75)	6.9±0.16	(15.51)	4.8±0.14	(14.63)
STD to zygotene	4.7±0.22	(10.75)	3.9±0.13	(8.94)	4.0±0.14	(8.99)	4.9±0.17	(14.94)
Zygotene	8.8±0.20	(20.14)	9.1±0.11	(20.87)	9.1±0.13	(20.45)	5.9±0.23	(17.99)
Pachytene	7.7±0.25	(17.62)	7.5±0.12	(17.20)	7.3±0.17	(16.40)	4.0±0.25	(12.19)
Diplotene + diakinesis	1.5±0.27	(3.43)	1.5±0.15	(3.44)	1.8±0.19	(4.04)	1.0±0.21	(3.05)
FIRST PROPHASE (total)	37.3±0.57	(85.35)	37.0±0.42	(84.86)	37.0±0.42	(83.14)	25.9±0.50	(78.96)
Metaphase I	1.9±0.41	(4.34)	1.7±0.17	(3.90)	1.7±0.20	(3.82)	1.8±0.21	(5.49)
AI to dyads	0.8±0.50	(1.83)	1.2±0.24	(2.75)	1.1±0.23	(2.47)	0.8±0.23	(2.44)
Dyads	1.5±0.85	(3.43)	1.4±0.27	(3.22)	1.7±0.55	(3.82)	1.9±0.31	(5.79)
MII	1.4±0.72	(3.21)	1.2±0.23	(2.75)	1.8±0.61	(4.05)	1.3±0.48	(3.96)
AII to quartets	0.8±0.42	(1.83)	1.1±0.20	(2.52)	1.2±0.32	(2.70)	1.1±0.43	(3.36)
MI-TII INCLUSIVE	6.4±1.35	(14.64)	6.6±0.50	(15.14)	7.5±0.94	(16.86)	6.9±0.78	(21.04)
TOTAL MEIOTIC TIME	43.7±1.47	(100.00)	43.6±0.65	(100.00)	44.5±1.03	(100.00)	32.8±0.92	(100.00)
Quartet stage	8.1±0.41		7.9±0.17		8.9±0.29		7.7±0.28	
Pollen maturation	10.2±0.23		10.9±0.25		9.3±0.17		7.9±0.07	
No. plant examined	32		21		33		19	
No. spikes examined	61		49		40		21	
No. anthers examined	1590		1320		720		630	

[1] STD = synchronous tapetal division. Not a meiotic stage but an unambiguous landmark.

[2] Figures in brackets refer to the percentage of the total meiotic duration taken up by the meiotic stage.

The difference, however, in the duration of meiosis between Chinese Spring and Prelude (6x), as obtained in the present study, was significant ($P \leq 0.001$) for the total duration of meiosis, Z + P and pollen maturation and for the quartet stage ($P \leq 0.01$).

Octoploid Triticale: The low number of euploid plants obtained in the 8A73 triticale did not allow a detailed study of the duration of meiosis. However, the few spikes that were examined indicated that 8A73 was essentially similar to 8A74 and 8A599. In both of these octoploids the total duration of meiosis was about 43.6 h., Z + P lasted 16.6 h or 38.1% of the total duration of meiosis,and the quartet stage lasted about 8.0 h (Table II). Pollen maturation lasted 10.2 days in 8A74 and 10.9 days in 8A599 (difference was significant at $P=0.05$).

NF took longer in octoploid triticale 8A74 and 8A599 than in hexaploid triticale R621-5 and 6A301 which had the same A, B and R genomes (difference was significant at $P \leq 0.01$). Z + P and total duration of meiosis were shorter in octoploid than in hexaploid triticale ($P \leq 0.001$), while there was no significant difference in the duration of quartet and pollen maturation stages.

No significant differences were found in the total duration of meiosis, duration of Z + P, and duration of NF between hexaploid wheats and the octoploid triticale strains derived from them, even though the total duration of meiosis was slightly shorter in the triticales than in their wheat parent. A similar situation has been reported by Bennett and Smith (1972) for Chinese Spring and two octoploid triticale strains derived from it.

Hexaploid Triticale Rosner X Prolific (ABRR); The total duration

of meiosis, duration of Z + P, leptotene, and quartet stages were not significantly different from the corresponding stages of the Rosner parent (Tables III and IV). The only significant difference observed between Rosner and its hybrid with Prolific was in the duration of pollen maturation ($P \leq 0.01$) which lasted 9.5 and 10.3 days, respectively. Conversely, a significant difference between the hybrid Rosner X Prolific and the rye parent was observed for the following stages: Z + P ($P \leq 0.05$); leptotene ($P \leq 0.001$) and pollen maturation ($P \leq 0.001$). The hybrid Rosner X Prolific, therefore, resembles more its triticale than its rye parent.

Hexaploid 6A299 X Prolific: The parentage of hexaploid triticale 6A299 is identical to that of 341-5 which was studied by Roupakias and Kaltsikes (1977b) (Table IV). Since examination of the first few spikes showed meiotic durations similar to 341-5, 6A299 was not studied further.

No statistically significant differences were found between 6A299 X Prolific and 341-5 in the total duration of meiosis, duration of Z + P, quartet stage and pollen maturation, although differences of 6.3 h, 1.3 h, 1.3 h and 0.4 h respectively, were observed. The lack of significance was probably due to large standard errors associated with the estimates of meiotic parameters in the hybrid (Table III and IV). However, a highly significant difference between the hybrid 6A299 X Prolific and 341-5 triticale was observed in the duration of leptotene ($P \leq 0.001$), while there was no significant differences between Prolific and the hybrid. With respect to leptotene and the rest of the stages, the hybrid 6A299 X Prolific resembles, therefore, its rye rather than its triticale parent (Tables I, III and IV).

Table III

The duration of meiotic stages (hours) and pollen maturation (days) in ABRR and AABBD hybrids grown at 20°C under continuous illumination

Stage of Meiosis	ABRR				AABBD	
	Rosner x Prolific		6A299 x Prolific		Stewart 63 x Rescue (6x)	Stewart 63 x Thatcher (6x)
Nucleolar fusion	6.8±1.83	(13.18)[1]	5.7±0.29	(10.82)		
Leptotene	10.7±1.06	(20.74)	17.8±0.86	(33.78)	19.0[3]	19.0[3]
Zygotene to STD[2]	0.1±1.09	(0.19)	1.1±0.06	(2.09)		
STD to pachytene	13.1±1.23	(25.39)	10.5±0.93	(19.92)		
Pachytene	11.8±1.23	(22.87)	8.2±0.73	(15.56)	18.5[4]	18.5[4]
Diplotene + diakinesis	1.9±0.46	(3.68)	2.7±0.22	(5.12)		
FIRST PROPHASE (total)	44.4±2.98	(86.05)	46.0±1.51	(87.29)	37.5	37.5
Metaphase I	2.2±0.53	(4.26)	1.7±0.25	(3.22)		
AI to dyads	1.2±0.51	(2.33)	1.0±0.94	(1.90)		
Dyads	1.4±0.66	(2.71)	1.6±2.76	(3.04)		
MII	1.3±0.86	(2.52)	1.3±1.08	(2.47)		
AII to quartets	1.1±0.69	(2.13)	1.1±0.09	(2.08)		
MI-TII INCLUSIVE	7.2±1.48	(13.95)	6.7±3.12	(12.71)	7.0	7.0
TOTAL MEIOTIC TIME	51.6±3.33	(100.00)	52.7±3.47	(100.00)	44.5	44.5
Quartet stage (h)	7.7±1.38		8.4±1.86		8.0	8.0
Pollen maturation (d)	10.3±0.11		11.1±0.84		-	-
No. plants examined	33.0		21.0		6.0	5.0
No. spikes examined	53.0		32.0		15.0	13.0
No. anthers examined	1278.0		229.0		90.0	78.0

[1] Figures in brackets refer to the percentage of the total meiotic duration taken up by the meiotic stage.

[2] STD = synchronous tapetal division. Not a meiotic stage but an unambiguous landmark.

[3] Duration of nucleolar fusion to STD.

[4] Duration of STD to end of diakinesis.

Table IV

The duration of meiotic stages (hours) and pollen maturation (days) in four hexaploid triticales grown at 20°C under continuous illumination[1]

Stage of Meiosis	Rosner	341-5[2]	R621-5[3]	6A301[4]
Nucleolar fusion	5.6±0.72	6.5±0.55	6.0±0.25	5.8±0.33
Leptotene	12.2±0.55	9.6±0.55	10.8±0.26	12.1±0.33
Zygotene to STD[5]	1.3±0.52	2.7±0.55	2.0±0.22	1.1±0.25
STD to pachytene	12.8±0.42	9.2±0.48	11.2±0.17	12.4±0.22
Pachytene	10.5±0.47	9.2±0.51	9.5±0.19	9.8±0.25
Diplotene + diakinesis	3.2±0.48	2.0±0.66	2.3±0.27	2.4±0.29
FIRST PROPHASE (total)	45.6±1.31	39.2±1.35	41.8±0.56	43.6±0.69
Metaphase I	2.0±0.57	2.0±0.81	2.3±0.37	2.1±0.40
AI to dyads	1.2±0.64	1.1±0.39	1.0±0.51	0.9±0.45
Dyads	1.8±0.92	1.8±0.41	1.8±0.59	2.3±0.65
MII	1.7±0.33	1.3±0.31	1.4±0.43	1.4±0.59
AII to quartets	1.2±0.31	1.0±0.29	1.2±0.40	1.0±0.38
MI-TII INCLUSIVE	7.9±2.14	7.2±1.08	7.7±1.05	7.7±1.13
TOTAL MEIOTIC TIME	53.5±2.51	46.4±1.73	49.5±1.19	51.3±1.32
Quartet stage (h)	7.2±0.65	7.1±0.62	7.6±0.33	7.4±0.29
Pollen maturation (d)	9.5±0.23	10.7±0.13	10.1±0.40	11.2±0.11

[1] From Roupakias and Kaltsikes, (1977b).

[2] 341-5 = (Prelude (AABB) x S. cereale cv. Prolific).

[3] R621-5 = (Rescue (AABB) x S. cereale cv. Prolific).

[4] 6A301 = (Thatcher (AABB) x S. cereale cv. Prolific).

[5] STD = Synchronous tapetal division. Not a meiotic stage but an unambiguous landmark.

Stewart 63 X Rescue (6x) and Stewart 63 X Thatcher (6x): The time elapsed from the beginning of NF to STD was 19 h in Stewart 63 (Roupakias and Kaltsikes, 1977b), 19 h in the hybrids Stewart 63 X hexaploid wheat (Table III), and 14.8 h in Prelude hexaploid wheat (Table II). However, the duration of the period from the stage of meiosis where STD occurred to MI was 17.6 h in Stewart 63, 18.5 h in the hybrids and 22.2 h in hexaploid Prelude. STD took place during early- to mid-zygotene in Stewart 63, early zygotene in the hybrids and late leptotene in hexaploid Prelude. The hybrids tetraploid X hexaploid wheat, therefore, resembled more the tetraploid than the hexaploid parent.

Direct and Interactive Effects of the AB and R Genomes

A highly significant difference ($P \leq 0.001$) between Prolific rye and Prelude tetraploid wheat was found in the total duration of meiosis (Table I), whereas no significant difference was detected between the same genotypes in the duration of Z + P (Table I). When the wheat (AABB) and rye (RR) genomes were combined in the triticale nucleus, however, both total duration of meiosis and duration of Z + P increased (Table IV). Backcrossing the hexaploid triticale (female) to Prolific rye (male) gave rise to the ABRR genotypes which included the full rye genome (RR) and half of the wheat complement (AB). As far as the duration of meiotic prophase and its constituent stages is concerned the hybrid Rosner X Prolific resembled more its triticale while 6A299 X Prolific resembled more its rye parent (Tables I, III and IV).

The Effect of the D Genome

Removal of the D genome from three hexaploid wheat varieties

(Prelude, Thatcher and Rescue) resulted in the elongation of Z + P (Fig. 1) (difference was significant between 6x and 4x Prelude and 6x and 4x Rescue, $P \leq 0.001$; not significant between 6x and 4x Thatcher). However, it had no significant effect on the total duration of meiosis (Fig. 2). Substituting the R genome for the D genome of hexaploid wheat resulted in an increased duration of both Z + P (significant in all cases, $P \leq 0.01$) and total duration of meiosis (Rescue and Thatcher significant $P \leq 0.01$; Prelude not significant) (Figs. 1 and 2).

Addition of the R genome to the complement of hexaploid wheat (AABBDD) had no major effect on either the total duration of meiosis or duration of Z + P (Figs. 1 and 2).

Addition of the R genome to that of tetraploid wheat (AABB) resulted in the elongation of both total duration of meiosis (significant in Rescue and Thatcher but not in Prelude) and Z + P (significant in Rescue but not in Thatcher and Prelude) (Figs. 1 and 2). When both D and R genomes were added to AABB, both Z + P and total duration of meiosis became shorter (Z + P significant in Prelude and Rescue $P \leq 0.001$, Thatcher not significant, total duration not significant) (Figs. 1 and 2).

Finally, addition of the D genome to the complement of hexaploid triticale (AABBRR) resulted in the shortening of the duration of Z + P ($P \leq 0.001$) as well as of the total duration of meiosis (significant in Rescue, $P \leq 0.01$ and Thatcher, $P \leq 0.001$, Prelude not significant) (Figs. 1 and 2).

Figure 1. The duration of the combined stages zygotene + pachytene of meiosis (hours) in various combinations of the same AB, D and R genomes. Prelude, Rescue and Thatcher, all hexaploid wheat cultivars, provided the basic genomic complement (AABBDD) from which others were derived. The diploid cultivar Prolific was the donor of the R genome.

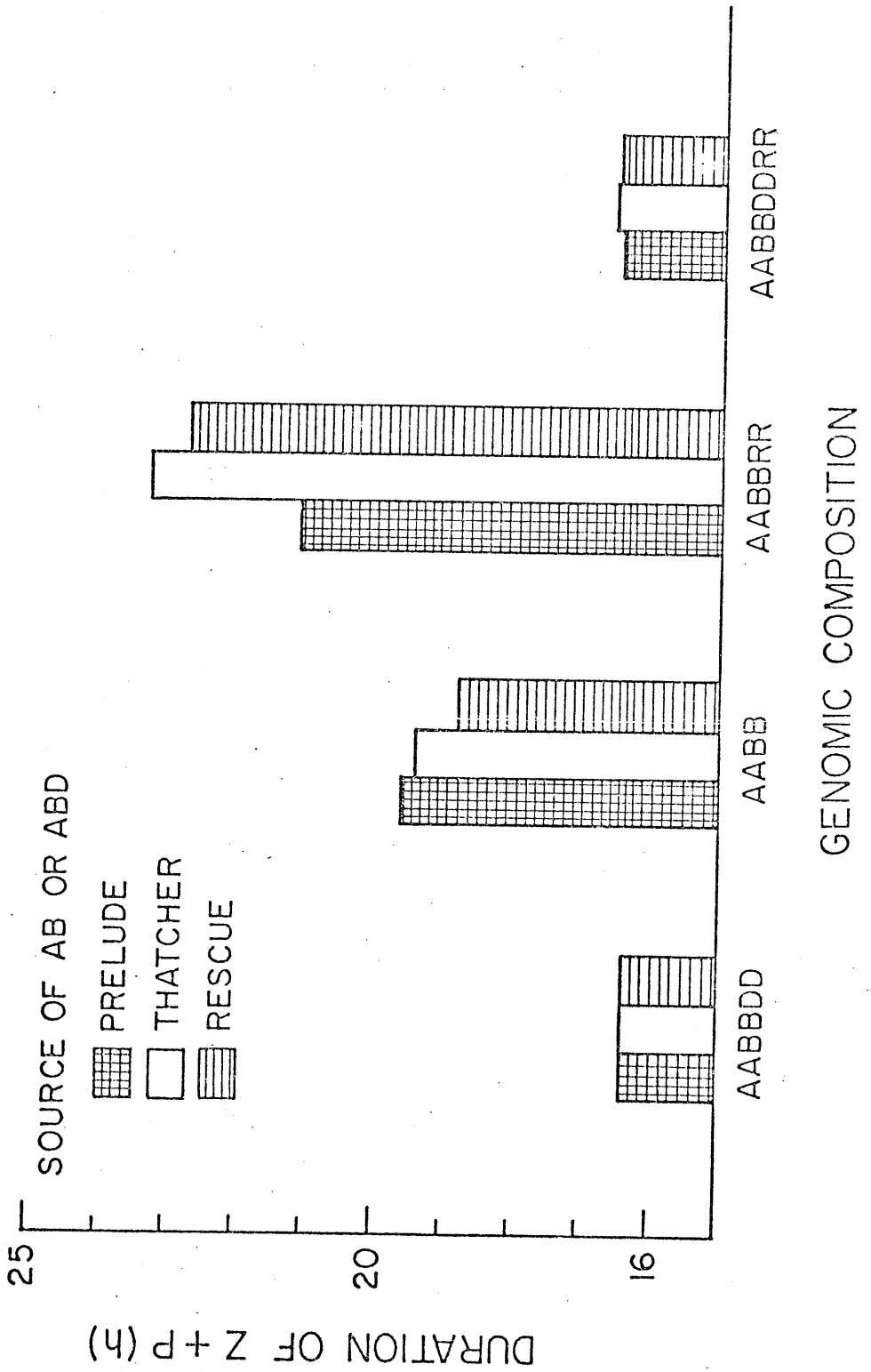
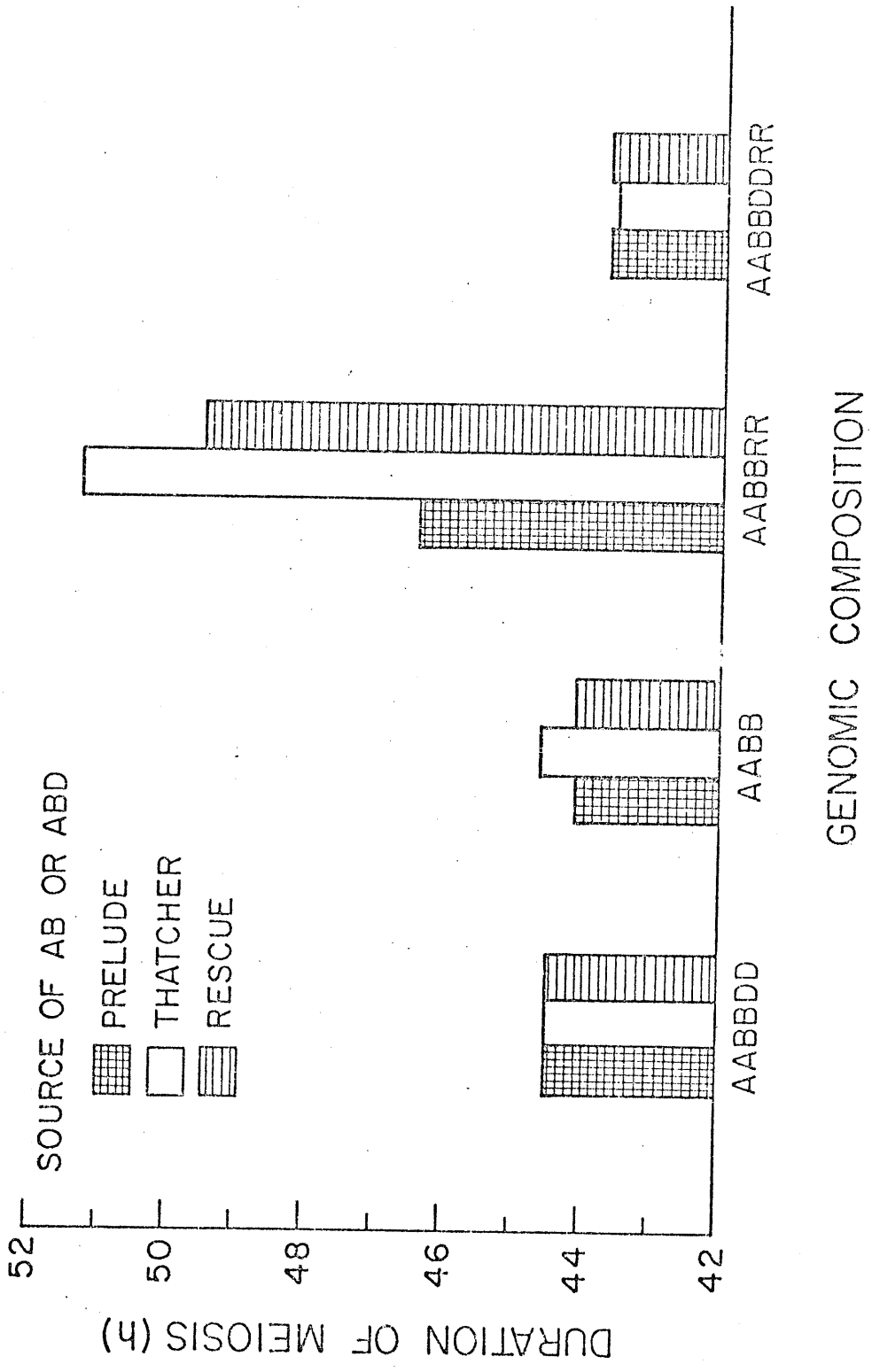

DURATION OF Z + P (h)
25
20
16
SOURCE OF AB OR ABD
PRELUDE
THATCHER
RESCUE
AABBDD
AABB
AABBRR
AABBDDRR
GENOMIC COMPOSITION

Figure 2. The duration of meiosis (hours) in various combinations of the same AB, D and R genomes. Legend as in Fig. 1.


DURATION OF MEIOSIS (h)
52
50
48
46
44
42
SOURCE OF AB OR ABD
PRELUDE
THATCHER
RESCUE
AABBDD
AABB
AABBRR
AABBDDRR
GENOMIC COMPOSITION

Discussion

In the process of meiosis there are some unambiguous landmarks either in the surrounding tapetal layer or in the meiocytes themselves, i.e. MI and stages of second meiotic division, which could be used for testing the consistency of the method used in timing meiosis. The stage for meiocytes during which the tapetal cells undergo synchronized division (STD) varies from species to species but it is constant within a variety. Therefore, only comparisons among estimates of meiotic duration of the same material studied by different investigators could give an indication about the consistency of the method. Bennett et al. (1971), in a study of Chinese Spring hexaploid wheat, reported that meiocytes required 24 h to progress from the stage in which they were while the tapetal cells were undergoing STD, to TII, while we obtained a value of 23 h for the same interval (Table II). Both Bennett and Smith (1972) and Roupakias and Kaltsikes (1977b) estimated the duration of the interval STD to MI in Rosner triticale as 26.5 h. Bennett and Kaltsikes (1973) found a total duration of meiosis in Prolific rye equal to 51.0 h, which is not very different from the results of the present study (Table I). The agreement of the results reported by different investigators strengthens our belief that the method used for meiotic timing provides consistent results.

In producing the strains of each ploidy level studied by combining the D, R, or both genomes with the same tetraploid complement (AABB), three major changes occurred in the nucleus: (1) the level of ploidy increased; (2) the amount of DNA increased; and (3) additional genetic factors carried by the D and R genomes were introduced. The possible

effect(s) of these changes on the duration of meiosis and its stages will be discussed one by one.

Increase of Ploidy Level

The results of the present study indicate that hexaploid wheat and octoploid triticale, compared with tetraploid wheat and hexaploid triticale respectively, had a shorter duration of Z + P (Tables I, II and IV). Can this faster rate of meiotic development be attributed entirely to the change in the ploidy level per se as suggested by Bennett and Smith (1972) and Bennett and Kaltsikes (1973)? If the ploidy level as such had a direct effect on the duration of meiosis then:

1. An increase in this level should always affect the duration of meiosis in the same direction. However, increasing the ploidy level of tetraploid wheat (AABB) by the addition of the R genome resulted in the elongation of the duration of Z + P, while addition of the D genome to the same genotype (AABB) resulted in the reduction of the duration of these stages (Tables I, II, and IV, Fig. 1). Furthermore, the R genome lengthened the duration of meiosis when added to the same tetraploid wheat but had no significant effect when added to hexaploid wheat (Fig. 2).

2. F_1 hybrids between genotypes of different ploidy levels should be different from their parents in the duration of meiosis and its stages. On the contrary, the Rosner X Prolific hybrid (ABRR) resembled the Rosner parent, the 6A299 X Prolific hybrid (ABRR) resembled the Prolific parent, while the tetraploid X hexaploid wheat hybrids (AABBD) resembled their tetraploid parent (Tables I, II, III and IV).

3. If increases in the ploidy level shorten the meiotic cycle

(Bennett and Smith, 1972), they should also affect, in the same way, the duration of the mitotic cycle. However, as Bennett et al. (1975) pointed out, studies of the duration of mitosis showed that the cell cycle in polyploids is longer (Evans et al., 1970), the same as (Yang and Dodson, 1970), or shorter (Gupta, 1969) than in related diploids. Kaltsikes (1973) and Kaltsikes et al. (1975) reported that the duration of the mitotic cycle in the triploid endosperm of the 6A190 hexaploid triticale was about 4-5 h during the first 5 to 6 endosperm divisions; 8-10 h during the next 3 endosperm divisions; and about 18-24 h thereafter, while the mitotic cycle of diploid cells of the same line was 12 h (Kaltsikes, 1971). Similar results obtained by Bennett et al. (1975) led them to suggest that factors other than ploidy level, i.e. developmental conditions, have major effects on the mitotic cycle even within the same tissue where ploidy level and DNA content are similar. Callan (1973) stated that "the enormous variation observed in the duration of the S-phase in cells of the same organism at different developmental stages is due to large differences in the number of initiation points operative for replication rather than to gross diversity in replication rate." Therefore, factors such as differential coiling which increase or decrease the number of initiation points affect the duration of the cell cycle, irrespective of the ploidy level and DNA content. However, when such factors are nonexistent or are weak, ploidy level and DNA content likely exert an influence on the duration of the cell cycle. The shorter duration of Z + P, therefore, in hexaploid wheat and octoploid triticale as compared to tetraploid wheat and hexaploid triticale respectively, cannot be wholly attributed

to simple changes in the ploidy level.

Increase of DNA Content

Roupakias and Kaltsikes (1977a, b) reported significant differences in the duration of meiosis among hexaploid triticale strains. Bennett and Smith (1972) found differences in meiotic development between the hexaploid wheats Chinese Spring and Holdfast, at least with respect to the stage of meiosis during which STD occurred. However, more pronounced differences between Chinese Spring and Prelude hexaploid wheat were found in the present study (Table II). Bennett and Smith (1972) suggested that the differences in the duration of meiosis among strains within a ploidy level were due to DNA content. Considering that the DNA content of a cereal nucleus is equal to the total DNA of the genomes contributing to it (Bennett and Smith, 1972; Kaltsikes, unpublished), then strains of the same genomic constitution should not vary greatly in this respect. Therefore, the differences observed in the duration of meiosis within hexaploid wheat and triticale strains can not be explained on the basis of DNA content alone. However, small differences in DNA content among the triticales cannot be ruled out since polymorphism for Giemsa banding of rye chromosomes in triticale has been found (Weimarck, 1975; present authors, unpublished; Gustafson, unpublished). It is considered unlikely, however, that there were differences of such a magnitude as to influence the duration of meiosis. Furthermore, Nagl (1974) reported an increase in the nuclear DNA content in the genera Anacyclus and Anthemis by the addition of heterochromatin without any lengthening of the mitotic cell cycle.

It could, however, be argued that there was a significant difference

in the DNA content among the various triticale strains examined. This does not seem to be true, because triticale strains derived from wheat parents having the same duration of meiosis and, therefore, DNA content, and a common diploid rye (Prolific) should have the same amount of DNA in their nucleus. Significant differences in the duration of meiosis were observed, however, among hexaploid triticales; such a case was not observed in the octoploid triticale with genetically related or identical parents (Table II; Bennett and Smith, 1972). Another indication that DNA content alone may have little effect on the duration of meiosis is that there was no difference between F_1 hybrids (hexaploid triticale X Prolific) and one or both of their parents (Tables I, III and IV). Such a situation has also been reported with respect to the mitotic cycle by Tanaka (1966), Collins (1968) and Gupta (1969). It seems that the correlation between DNA content and duration of meiosis may not be one of cause - and - effect but is rather due to some other unknown factors.

Introduction of New Genetic Factors

Bennett (1976) reported that, in addition to DNA content and ploidy level, the duration of meiosis is also affected by genotypic and environmental factors. Bennett *et al.* (1974) found that the meiotic duration in the hexaploid wheat Chinese Spring was affected by addition or subtraction of individual chromosomes. Each of the D and R genomes consists of seven chromosome pairs. Addition of whole genome(s), therefore, to tetraploid wheat is more likely to affect the duration of meiosis and its stages than the addition of an individual chromosome. Thus the shorter duration of Z + P in hexaploid wheat (AABBDD) and its longer

duration in hexaploid triticale (AABBRR) compared to their common tetraploid wheat complement (AABB) (Fig. 1) was more likely due to genetic factors introduced by the D or R genomes than to simple changes in ploidy level and DNA content. Such factors may act directly or in interaction with genes carried by the A and B genomes. Similarly, the elongation of the duration of meiosis when the R genome was added to tetraploid wheat and the lack of a significant effect when it was added to hexaploid wheat (Fig. 2) indicate that the D genome, rather than the ploidy level, was the cause of the shorter duration of Z + P in hexaploid wheat and octoploid triticale.

The differences observed in the duration of meiosis between octoploid triticale strains studied by Bennett and Smith (1972) and those of the present study were due to the wheat parent. Chinese Spring, the parent of Bennett's octoploid triticale, had a much shorter duration of meiosis than Prelude (6x), Rescue (6x) and Thatcher (6x), the wheat parents of the octoploid triticales used in this study (Table II).

Normal rye populations, because of their outbreeding behaviour, produce pollen grains which differ genotypically from one another. When rye gametes from one variety unite with gametes from one wheat parent to produce triticale, it is likely that the resulting genotypes are not completely identical. Thus in each case the constituent genes can act and interact in different ways. These actions and interactions may explain some of the differences observed among the hexaploid triticale strains studied. It is likely that the lack of differences among the octoploid strains studied was due to the presence of the D genome which may have suppressed any differential activity of the genes introduced

by the R genome.

In general it can be concluded that the differences observed in the duration of meiosis and its stages between and within the various ploidy levels of the cereals examined in the present study are more likely due to genetic factors carried by the D and R genomes than to simple changes of ploidy level and DNA content.

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MANUSCRIPT IV

THE MEIOTIC CYCLE OF TETRAPLOID TRITICALE

Abstract

The duration of meiosis, chromosome pairing and fertility were studied in three tetraploid triticales (X Triticosecale Wittmack) having eleven chromosome pairs in common (1R to 7R, 1A, 2B, 6A and 7B) and differing with respect to the other three (Trc 4x2 carries 3B, 4B, 5B; Trc 4x3 carries 3A, 4A, 5B; and Trc 4x5 carries 3B, 4A, and 5A). Beginning with nucleolar fusion and ending at telophase II, meiosis lasted from 52.6 to 58.7h, while the number of paired chromosome arms and univalents per PMC ranged from 21.38 to 25.82 and from 1.89 to 0.37, respectively. The duration of meiosis and chromosome pairing were significantly affected by various chromosome combinations. Lack of chromosome 5B in a tetraploid triticale increased the duration of meiosis and resulted in reduced pairing. The ploidy level (ie. tetraploid or hexaploid) had no significant effect on the duration of meiosis which in turn had no discernible effect on chromosome pairing. Pairing failure occurred mostly among chromosomes of the rye genome. Seed fertility ranged from 10.6% to 47%. No relationship between meiotic abnormalities and fertility was detected. It was concluded that the ploidy level and the duration of those meiotic stages following nucleolar fusion are not related to chromosome pairing.

Introduction

Triticale (X Triticosecale Wittmack), the amphiploid combining representatives of the genera Triticum L. and Secale L., can be obtained relatively easily at the octoploid (hexaploid wheat x diploid rye) or the hexaploid levels (tetraploid wheat x diploid rye) by crossing, embryo culture and colchicine treatment of the resulting hybrid (Kaltsikes, 1974). Attempts to produce tetraploid triticale, however, had been unsuccessful (Kiss, 1965; Larter et al. 1968) until Krolow obtained it by crossing hexaploid triticale to diploid rye and selfing the resulting hybrid (Krolow, 1973, 1974). All of Krolow's tetraploid triticales have the full complement of the rye parent but contain various combinations of A- and B-genome chromosomes (Gustafson and Krolow, 1977). In our continuing study of the meiotic cycle in triticale (Roupakias and Kaltsikes, 1977a, b, c) we have studied the effects of the various genomes and the level of ploidy (6x or 8x) on the meiotic cell cycle. This study was undertaken to study the effect(s) of (1) various chromosome combinations and (2) the tetraploid level of ploidy on the meiotic cycle.

Materials and Methods

The three tetraploid triticales, Trc 4x2, Trc 4x3 and Trc 4x5, were obtained from the F_2 generation of a cross between a hexaploid triticale (T. turgidum x S. cereale) and S. cereale (Krolow, 1973). All triticales studied had the full rye complement and seven pairs of wheat chromosomes. Trc 4x2 carries 1A, 2B, 3B, 4B, 5B, 6A, and 7B;

Trc 4x3 carries 1A, 2B, 3A, 4A, 5B, 6A, and 7B, while triticale Trc 4x5 carries 1A, 2B, 3B, 4A, 5A, 6A, and 7B (Gustafson and Krolow, 1977). The conditions of growth and the methodology used have been previously described (Roupakias and Kaltsikes, 1977a).

Meiotic chromosome configurations at first metaphase (MI) were scored in 150 PMC's of Trc 4x2, 194 PMC's of Trc 4x3 and 200 PMC's of Trc 4x5 from seven plants per strain. Laggard chromosomes at first anaphase (AI) were scored in 200 cells from four plants while micronuclei at the quartet stage were scored in 200 cells from three plants of each strain (exceptions for AI were: Trc 4x2, 75 cells from three plants; and for micronuclei: Trc 4x2, 100 cells from three plants).

The Giemsa staining technique used for the study of the distribution of terminal heterochromatin in rod bivalents and univalents has been previously described (Roupakias and Kaltsikes, 1977d). The numbers of plants, spikes and anthers examined per strain are given in Table I. Fertility was calculated as the percentage of primary and secondary florets having seeds at maturity. The t-test was used for statistical analysis.

Results and Discussion

Meiotic Development

Each floret of the triticales examined had three anthers. Within individual anthers, as well as among anthers of each floret, asynchrony of 1-2h was observed in two of the triticales, Trc 4x3 and Trc 4x5. Roupakias and Kaltsikes (1977 a, b, c) also reported a similar degree of asynchrony in triticales of other ploidy levels. The third strain, Trc 4x2, however, showed a higher degree of asynchrony

Table I

Duration of meiosis (h), quartets (h) and pollen maturation (days) at 20°C in three tetraploid triticales

Stage of meiosis	Trc 4 x 3		Trc 4 x 5		Trc 4 x 2
Nucleolar fusion	6.8 ± 0.20	(12.93)[1]	7.0 ± 0.12	(11.93)[1]	23[3]
Leptotene	13.1 ± 0.19	(24.90)	15.0 ± 0.13	(25.55)	
Zygotene to STD[2]	1.2 ± 0.14	(2.28)	4.1 ± 0.10	(6.98)	
STD to pachytene	11.1 ± 0.13	(21.10)	10.0 ± 0.10	(17.04)	25[4]
Pachytene	9.7 ± 0.14	(18.44)	12.1 ± 0.12	(20.61)	
Diplotene & diakinesis	3.1 ± 0.17	(5.90)	3.1 ± 0.12	(5.28)	
FIRST PROPHASE	45.0 ± 0.40	(85.55)	51.3 ± 0.28	(87.39)	
Metaphase I	2.1 ± 0.24	(3.99)	2.0 ± 0.16	(3.41)	
Anaphase I to dyads	1.0 ± 0.28	(1.90)	1.0 ± 0.23	(1.70)	
Dyads	2.1 ± 0.29	(3.99)	1.8 ± 0.28	(3.07)	
M_{II}	1.3 ± 0.23	(2.47)	1.4 ± 0.25	(2.39)	
A_{II} to quartets	1.1 ± 0.20	(2.10)	1.2 ± 0.18	(2.04)	
M_I-T_{II} INCLUSIVE	7.6 ± 0.56	(14.45)	7.4 ± 0.50	(12.61)	7.0
TOTAL MEIOTIC TIME	52.6 ± 0.69	(100.00)	58.7 ± 0.57	(100.00)	55.0
Quartet stage (h)	8.1 ± 0.20		7.8 ± 0.18		8.0
Pollen maturation (d)	13.8 ± 0.21		14.0 ± 0.30		13.5 ± 0.25
No. plants examined	29		24		18
No. spikes examined	47		61		40
No. anthers examined	1026		1514		884

[1] Figures in brackets refer to the percentage of the total meiotic duration taken up by the meiotic stage.

[2] STD = synchronous tapetald division. Not a meiotic stage but an unambiguous landmark.

[3] Duration of nucleolar fusion to STD.

[4] Duration of STD to end of diakinesis.

(8-10h); anthers having meiocytes at pachytene, diplotene, MI and AI; nucleolar fusion, early leptotene and middle leptotene; or early, mid- and late pachytene were often observed. This high degree of asynchrony made it impossible to time the duration of individual meiotic stages in this strain. Therefore, only the intervals from nucleolar fusion (NF) to synchronous tapetal division (STD), STD to MI and MI to TII were roughly estimated (Table I). The cause of the unexpectedly high asynchrony observed in Trc 4x2 triticale remains unknown.

One synchronous division of tapetal nuclei producing binucleate cells was observed in all tetraploid triticales. It occurred concurrently with early zygotene in Trc 4x3 and Trc 4x2 and with early-middle zygotene in Trc 4x5 (Table I). One synchronous division of tapetal nuclei was also observed in hexaploid and octoploid triticale (Bennett et al., 1971; Bennett and Kaltsikes, 1973; Roupakias and Kaltsikes, 1977 a, b, c).

Duration of Meiosis

The total duration of meiosis, excluding nucleolar fusion, was 45.8h in Trc 4x3 and 51.7h in Trc 4x5. With nucleolar fusion included it was 52.6h in Trc 4x3, 55h in Trc 4x2 and 58.7h in Trc 4x5 (Table I). First prophase occupied 85.55% of the total duration of meiosis in Trc 4x3, 87.39% in Trc 4x5 and 87.27% in Trc 4x2; zygotene plus pachytene (Z + P) accounted for 41.83% in Trc 4x3, 41.82% in Trc 4x2 and 44.63% of total meiotic duration in Trc 4x5 (Tables I and II).

The duration of nucleolar fusion in Trc 4x3 was not significantly different from that in Trc 4x5. These two triticales, however, were significantly different ($P \leq 0.001$) with respect to the duration of leptotene, zygotene, pachytene, first prophase and total duration of

Table II

Duration of meiosis (h) and chromosome pairing attributes in three tetraploid triticales

Genotype	Duration		Meiotic configurations			Chromosome arms paired per PMC	Laggard chromosomes at AI	Micro-nuclei per quartet
	Zygotene and pachytene	Total	Univalents per PMC	Open bivalents	Closed bivalents			
Trc 4 x 2	23	55.0	0.37 ± 0.09[1a]	1.82[2] ± 0.10[a]	12.00 ± 0.11[a]	25.82 ± 0.19[a]	0.08 ± 0.04[a]	0.13 ± 0.05[a]
Trc 4 x 3[3]	22.0	52.6	0.80 ± 0.09[b]	2.59 ± 0.11[b]	10.59 ± 0.13[b]	24.49 ± 0.41[b]	0.28 ± 0.06[b]	0.25 ± 0.02
Trc 4 x 5[4]	26.2	58.7	1.89 ± 0.13[c]	4.72 ± 0.10[c]	8.33 ± 0.11[c]	21.38 ± 0.17[c]	0.25 ± 0.06	0.40 ± 0.06[b]

[1]Standard error of the mean

[2]In each column, means followed by different letters are significantly different from one another ($P \leq 0.01$).

[3]This triticale had 0.03 multivalents per cell.

[4]This triticale had 0.005 multivalents per cell.

meiosis. No significant differences between these two triticales were detected in the duration of meiotic stages following first metaphase. The duration of quartets and pollen maturation was similar in all tetraploid triticales (8.0 hours and 14.0 days, respectively, Table 1).

Bennett, Dover and Riley (1974) reported that wheat plants nullisomic for chromosome 5B had a longer duration of meiosis than euploid plants. Trc 4x5, which lacks chromosome 5B (Gustafson and Krolow, 1977), had a longer duration of meiosis than Trc 4x3, most likely due to the absence of 5B.

All three tetraploid triticales studied were produced by crossing a hexaploid triticale (T. turgidum x S. cereale) to diploid rye (S. cereale) and self-pollinating the resulting hybrid. For hexaploid triticale 6A600 (T. turgidum x S. cereale) Roupakias and Kaltsikes (1977 b) reported the following: total duration of meiosis, 52.0h; first prophase, 45.1h; leptotene, 12.4h; Z + P, 24.1h; and MI to TII inclusive, 6.9h. While the duration of the same stages in S. cereale cv. Prolific lasted 52.6, 44.9, 17.6, 19.8 and 7.7h respectively (Roupakias and Kaltsikes, 1977 c). There were no significant differences in the total duration of meiosis, duration of first prophase and duration of MI - TII among hexaploid triticale, tetraploid triticale (Trc 4x3) and diploid rye. The duration of Z + P lasted significantly longer in 1) hexaploid triticale than in both tetraploid triticale and diploid rye and in 2) tetraploid triticale than in rye. The opposite, however, was true for the duration of leptotene; rye had a significantly longer leptotene than both tetraploid and hexaploid triticale. Bennett and Smith (1972) reported that the higher the ploidy level, the shorter the duration

of meiosis. The results of this study, however, indicate that the duration of meiosis in a tetraploid triticale was not longer than that of the hexaploid triticale derived from the same wheat and rye parents. Similar results were also previously reported (Roupakias and Kaltsikes, 1977c). The significantly longer meiotic duration of Trc 4x5 relative to 6A600 is probably due to the absence of 5B rather than to its tetraploid ploidy level.

Meiotic Abnormalities

The t-test revealed significantly different numbers of univalents, open bivalents and closed bivalents per PMC among the triticales studied (Table II). No relationship was discernible between the duration of meiosis or duration of Z + P and univalents per PMC (Table II). Roupakias and Kaltsikes (1977 a, b and unpublished data) also found no relationship between the duration of meiosis and univalents per PMC in hexaploid and octoploid triticale.

Most of the univalents and open bivalents observed in all tetraploid triticales belonged to the rye genome (Table III; Figs. 1, 2, 3). Rye chromosomes having both telomeres heterochromatic were more often observed as univalents than rye chromosomes with one telomeric band. Furthermore, the heterochromatic telomere was unpaired in open bivalents of rye chromosomes having only one telomeric band (Table III; Figs. 1, 2, 3). Similar behavior of rye chromosomes has also been reported in hexaploid triticales (Thomas and Kaltsikes, 1974, 1976), indicating that there probably is a common basis for pairing failure of rye chromosomes in triticale of all ploidy levels.

The results of this study, together with these dealing with hexaploid

Table III

Distribution of terminal heterochromatic bands in rod bivalents and univalents of three tetraploid triticales

Type of chromosome association	Triticale		
	Trc 4 x 2	Trc 4 x 3	Trc 4 x 5
Rod bivalents			
Chromosomes with			
2 terminal bands	34	78	50
1 terminal band heterochromatin free	71	79	100
1 terminal band heterochromatin paired	5	2	11
0 terminal bands	14	13	76
% banded/total	88.7	92.4	67.9
% 2 terminal/banded	27.4	45.3	31.1
% 1 terminal free/banded	57.3	45.9	62.1
% 1 terminal paired/banded	4.0	1.2	6.8
Univalents			
2 terminal bands	24	62	52
1 terminal band	14	32	58
0 terminal bands	12	2	34
% banded/total	76.0	97.9	76.4
% 2 terminal/banded	63.2	66.0	47.3
% 1 terminal/banded	36.8	34.0	52.7
No. of cells examined	75	103	66

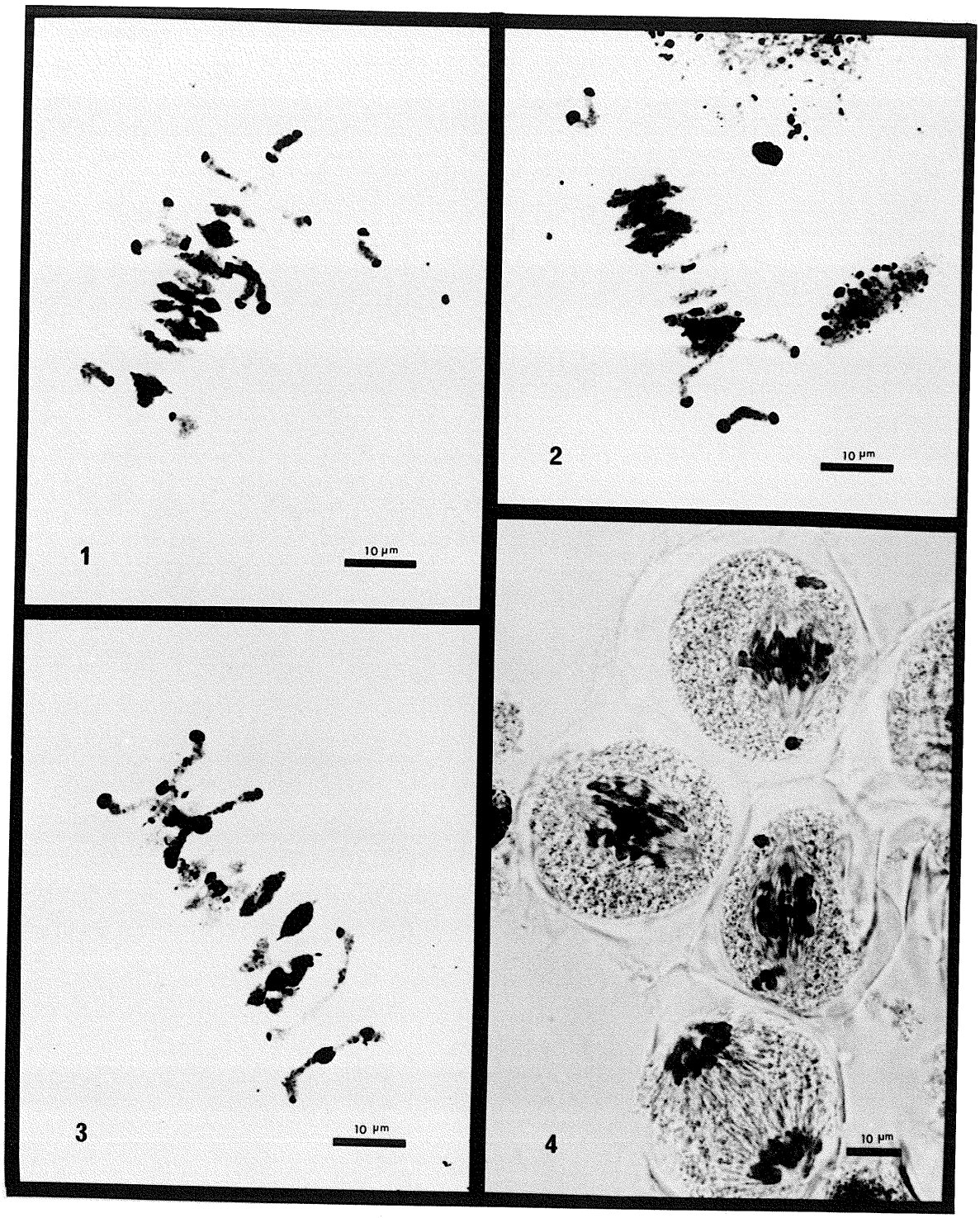
Figure Legends

Figure 1. First meiotic metaphase (after Giemsa staining) of Trc 4x2 with four rye univalents, three rye open bivalents and nine closed bivalents.

Figure 2. First meiotic metaphase (after Giemsa staining) of Trc 4x2 with two rye univalents having heterochromatic bands on both telomeres.

Figure 3. First meiotic metaphase (after Giemsa staining) of Trc 4x2 with four rye open bivalents and ten closed bivalents.

Figure 4. First meiotic metaphase (stained with 2% acetocarmine, but not heated) of Trc 4x5 with the univalents already evenly distributed to the poles.


1
10 µm
2
10 µm
3
10 µm
4
10 µm

and octoploid triticales (Roupakias and Kaltsikes, 1977 a, b, c, and unpublished data) clearly indicate that there is no relationship between the duration of meiosis (measured from the time of nucleolar fusion onwards) and chromosome pairing. It is entirely possible, however, that events relating to the duration of stages prior to the onset of meiosis, such as duration of DNA synthesis or even earlier, may have an effect on chromosome pairing. Thus, synaptonemal complexes have been observed in oocytes of Drosophila as early as premeiotic DNA synthesis (Day and Grell, 1976). If events related to chromosome pairing traditionally thought to occur in meiotic prophase, can be shown to take place as early as meiotic interphase, then the duration of all the stages of meiotic development must be ascertained.

Meiotically, the most stable triticales studied so far are the tetraploids (Table IV). Krolow (1973) reported the occurrence of only 2.5% aneuploids in the progeny of tetraploid triticales as compared to 8.7% reported for hexaploid (Scoles and Kaltsikes, 1974) and 34.4% for octoploid triticales (Weimarck, 1973). Aneuploidy is the result of pairing failure, lagging chromosomes and meiotic elimination of chromosomes in micronuclei. Therefore, the lower frequency of aneuploids found in the progeny of tetraploid triticales was due either to the low frequency of aneuploid gametes or to the inviability of such gametes or zygotes. The former alternative is favored because (1) there was higher frequecy of meiocytes without univalents in the tetraploid as compared to the hexaploid and octoploid triticales (Table IV) and (2) the relative number of micronuclei per univalent was lower in tetraploids (0.25) than in hexaploids (0.91) or octoploids

Table IV

Frequency (in per cent) of meiocytes with various numbers of univalents in Tetraploid, Hexaploid and Octoploid Triticales

Ploidy level	Univalents per PMC							Number of strains examined	Number of PMC's examined
	0	2	4	6	8	>10	Total		
Tetraploid	60.1	29.0	7.6	3.1	0.2	0.0	100.0	3	544
Hexaploid[1]	49.5	32.4	14.3	3.0	0.5	0.3	100.0	6	1162
Octoploid[1]	21.4	25.4	24.7	11.1	7.6	9.8	100.0	2	397

[1] Roupakias and Kaltsikes, unpublished.

(0.95) (Table II, Roupakias and Kaltsikes, 1977 a, b, and unpublished data). It seems, therefore, that in tetraploid triticale the univalents of MI were more often included in the microsporic nucleus than those of hexaploid and octoploid triticales. It was frequently observed that in tetraploid triticales the univalents were already evenly distributed and at the poles while the bivalents were still at the metaphase plate (Fig. 4) thereby ensuring their inclusion in the daughter nuclei. Contrastingly, in hexaploid and octoploid triticales the univalents are often late in arriving at the plate and consequently are not included in the daughter nuclei.

Among the tetraploid triticales studied, there was variation in chromosome pairing. Trc 4x2 had the best pairing, even though it showed the highest degree of asynchrony among meiocytes of the same anther. Of the meiocytes examined, 86% had no univalents (Fig. 3) while 11% had only two univalents per PMC (Fig. 2). This may indicate that chromosome pairing and development of meiocytes are controlled by genes carried on different chromosomes. Trc 4x2 differs from Trc 4x3 in that the former has chromosomes 3B and 4B while the latter has chromosomes 3A and 4A. Kempanna and Riley (1962) reported that chromosome 3B promotes chromosome pairing. The better pairing of Trc 4x2 as compared to Trc 4x3, therefore, was probably due to chromosome 3B. Trc 4x5 was the most unstable among the tetraploid triticales studied. This triticale differs from Trc 4x2 and Trc 4x3 in that it had chromosomes 3B, 4A, and 5A as compared to 3B, 4B, and 5B for Trc 4x2 and 3A, 4A, and 5B for Trc 4x3. Furthermore, it had a longer duration of meiosis than both Trc 4x2 and Trc 4x3. The absence, therefore, of chromosome 5B in Trc 4x5 even though it resulted in the

Table V

Fertility (in per cent) of three tetraploid triticales

Triticale	Number of spikes	Kernels per spike	Kernels per spikelet	Fertility (%) (a+b)
Trc 4 x 2	8	22.1	0.8	47.0
Trc 4 x 3	16	4.0	0.2	10.6
Trc 4 x 5	8	19.8	0.5	32.0

elongation of meiosis, did not result in better pairing of the rye chromosomes (Table III). Thomas and Kaltsikes (1971) considered also unlikely that the $5B^L$ system in the disomic state was supra-optimal the pairing of homologous rye chromosomes and thus responsible for pairing failure in triticale as proposed by Riley and Miller (1970). The higher number of univalents observed in this strain may be due to the action and interaction of genes carried by specific chromosomes. It seems, therefore, that there are "good" and "bad" chromosome combinations which could be included in the nucleus of a tetraploid triticale. Thus, other chromosome combinations may result in meiotically better triticales.

The fertility of tetraploid triticales ranged from 10.6% (0.2 kernels/spikelet) for Trc 4x3 to 47% (0.8 kernels/spikelet) for Trc 4x2 (Table V) which was lower than that reported for hexaploid and octoploid triticale (Merker, 1973 b; Kaltsikes et al., 1975; Weimarck, 1973). There was no relationship between meiotic abnormalities and fertility (Tables II and V). The same result has been reported for hexaploid and octoploid triticales (Weimarck, 1973; Merker, 1971, 1973 a; Hsam and Larter, 1973).

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MANUSCRIPT V

THE EFFECT OF TELOMERIC HETEROCHROMATIN ON CHROMOSOME PAIRING OF HEXAPLOID TRITICALE

Abstract

Plants carrying (++) or lacking (--) most of the telomeric heterochromatic band on the short arm of chromosome 6R ($6R^S$) were isolated from a single-plant progeny of Rosner triticale (X Triticosecale Wittmack) heterozygous for this band. Chromosome pairing at first meiotic metaphase was significantly higher in -- than in ++ or +- plants. The changes in chromosome pairing were likely due to the $6R^S$ telomeric heterochromatin which affected the pairing not only of the arm carrying it but of other chromosomes as well.

Introduction

Among the reproductive abnormalities of triticale (X Triticosecale Wittmack) the incomplete chromosome pairing observed at first meiotic metaphase has been the subject of several studies (Kaltsikes, 1974; Scoles and Kaltsikes, 1974; and Gustafson, 1976). Thomas and Kaltsikes (1972) 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 (Thomas and Kaltsikes, 1974, 1976).

To further study the influence of particular telomeric heterochromatic bands on chromosome pairing, isogenic or nearly isogenic lines are required which either have or lack these particular bands. The present report deals with the isolation and study of such lines in which the large heterochromatic band on the short arm of chromosome 6R of Rosner triticale was either present or absent on one or both homologues.

Materials and Methods

The hexaploid triticale cultivar Rosner was used. Seeds were germinated in petri dishes and one root-tip per seed was collected for chromosome counting by conventional methods. The rest of the root-tips were stained for heterochromatin so that the presence or absence of particular telomeric bands could be established.

The Giemsa staining technique used for the study of mitosis was the same as that described by Gustafson et al. (1976), except for modifications in ETOH treatment, air drying time and the temperature of the 2 x SSC solution. The slides were immersed in 95% ETOH for 105 min. and were then transferred into absolute ETOH for 15 min. Subsequently, the slides were allowed to air-dry overnight at room temperature. Slides in fresh solution of 2 x SSC were placed in an oven so that the temperature of the solution reached 50-52 °C within 30 min., whereupon it was maintained at this level for another 1.5 h.

For the study of meiosis plants were grown in the greenhouse until leading tillers were judged to be approximately one week prior to first meiotic metaphase (MI). They were then transferred into a growth cabinet and maintained at 20 ± 1 °C with continuous illumination. Spikelets close to MI were fixed in 3:1 acetic alcohol. Within 1 h after fixation one of the anthers of each floret was examined so that those florets with anthers having meiocytes at MI could be selected; the remaining two anthers of these florets were maintained in the fixative under refrigeration for 1-3 days. Anthers were macerated and squashed in 45% acetic acid on subbed slides, frozen with CO_2 and the coverslip flipped off. The same procedure as for mitosis was followed for heterochromatin

staining except that the temperature of the 2 x SSC solution was allowed to reach 54-56 °C.

Total (wheat and rye) meiotic chromosome pairing at MI was determined from aceto-carmine squashes, while pairing of rye chromosomes and the identification of individual chromosomes present as univalents were obtained following Giemsa staining. Unpaired 1R and 7R chromosomes were easily identified and thus scored separately from the other rye and wheat chromosomes. The t-test was used for statistical analysis.

Results and Discussion

The Isolation of the Lines

In Rosner, chromosome 6R usually has six interstitial heterochromatic bands on the long arm and one large terminal band on the short arm (Fig. 1). Mitotic analysis of a large number of plants resulted in the identification of one plant, monosomic for an unidentified wheat chromosome, in which most of the large terminal band was missing from one of the 6R chromosomes (Fig. 2). This plant was designated as +- to indicate the presence (+) and absence (-) of the telomeric band on the homologues of 6R and was subsequently selfed to produce the types ++, +-, and -- in the monosomic and ++ and -- in the disomic condition.

Chromosome Pairing

No significant differences in the number of univalents per PMC were found at MI of acetocarmine squashes of ++ and -- monosomic or disomic plants (Table I). In both cases, however, there were significantly (monosomic, $P \leq 0.001$; disomic $P \leq 0.02$) more closed bivalents in the --

Figure legends

Figure 1. Mitotic metaphase of a Rosner plant monosomic for an unidentified wheat chromosome. Both 6R chromosomes have the entire telomeric band on the short arm (arrowheads point at centromere). Figure 2. Mitotic metaphase of a Rosner plant heterozygous for the presence of telomeric heterochromatin on the short arm of chromosome 6R (arrowheads point at centromere). Figure 3. First meiotic metaphase of Rosner with the two unpaired 1R chromosomes (arrowheads point at centromere). The two univalents were identified as 1R because (1) they had large bands on both telomeres in contrast to 7R which in Rosner triticale has a big terminal band on the long arm and a small one on the short arm (Figs. 1 and 4); (2) they were subterminal (in contrast to 3R); and (3) typical 2R chromosomes are absent from Rosner. Figure. 4. First meiotic metaphase of Rosner with the two unpaired 7R chromosomes (arrowheads point at centromere).

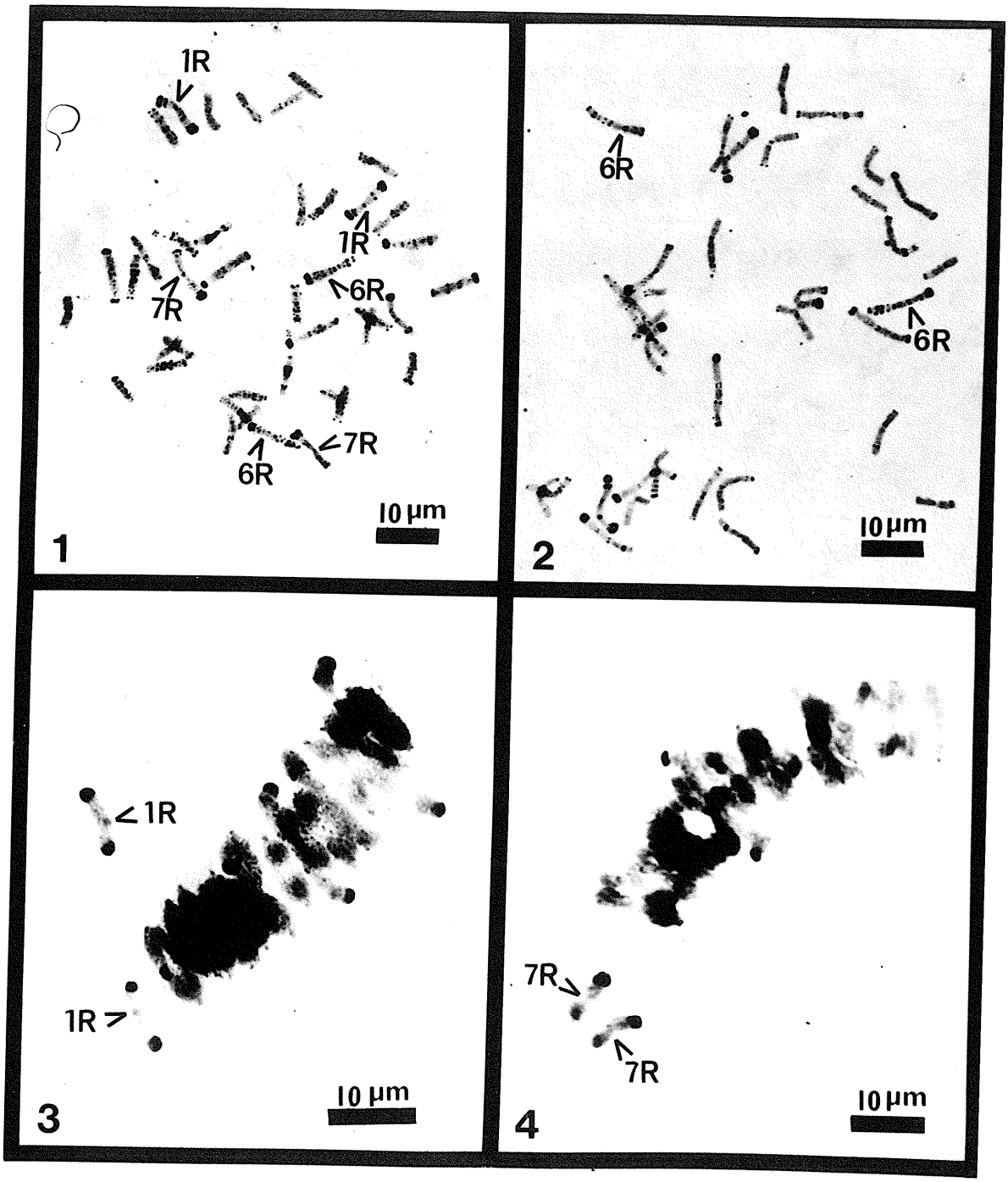
1R
7R
1R
6R
7R
6R
10 µm
1
6R
6R
10 µm
2
1R
1R
10 µm
3
7R
7R
10 µm
4

Table I

Chromosome pairing in disomic and monosomic plants with (++) or Without (--) telomeric heterochromatin on the short arm of chromosome 6R

Chromosome pairing	Type of 6R chromosome	Meiotic configurations			Chromosome arms paired per PMC	Number of cells examined
		Open bivalents	Closed bivalents	Univalents		
Disomic						
Total (wheat and rye)	++	4.56 ± 0.17[1a]	15.93[2] ± 0.17[a]	0.99 ± 0.15[a]	36.44 ± 0.15[a]	75 (1)[5]
"	--	3.91 ± 0.19[b]	16.57 ± 0.19[b]	0.98 ± 0.17[a]	37.04 ± 0.18[b]	67 (1)
Rye	++	3.19 ± 0.13[c]	2.40 ± 0.13[c]	0.83 ± 0.14[b]	7.99 ± 0.16[c]	75 (1)
"	--	2.22 ± 0.21[d]	3.27 ± 0.20[d]	1.02 ± 0.15[b]	8.76 ± 0.22[d]	45 (1)
Monosomic						
Total (wheat and rye)	++[3]	3.67 ± 0.08[a]	15.98 ± 0.10[a]	1.60 ± 0.08[a]	35.72 ± 0.12[a]	242 (3)
"	+-[4]	3.82 ± 0.23[a]	15.84 ± 0.23[a]	1.45 ± 0.16[a]	35.64 ± 0.27[a]	45 (2)
"	--	2.75 ± 0.12[b]	16.98 ± 0.12[b]	1.55 ± 0.07[a]	36.73 ± 0.14[b]	143 (3)

[1] Standard error of the mean.

[2] In each column, means followed by different letters are significantly different from one another ($P \leq 0.001$, with the exception of rye chromosome arms paired of disomic plants, $P \leq 0.01$ and of open bivalents, closed bivalents and chromosome arms paired of disomic plants (total), $P \leq 0.02$).

[3] This type had 0.03 multivalents per cell.

[4] This type had 0.02 multivalents per cell.

[5] Numbers in brackets indicate the number of plants examined.

type than in the ++ type. This resulted in significantly higher number of chromosome arms being paired per PMC in the -- than in the ++ type in both disomic ($P \leq 0.02$) and monosomic ($P \leq 0.001$) plants. Monosomic plants heterozygous +- and homozygous ++ for the presence of the band did not differ in their chromosomal pairing; both of these, however, had significantly ($P \leq 0.001$) fewer chromosome arms paired when compared to the homozygous -- (Table I). This suggests that the presence of the telomeric heterochromatin, even in one of the homologues, reduced chromosome pairing. The loss, therefore, of the terminal heterochromatic band was accompanied by an improvement in chromosome pairing by approximately one chromosome arm in monosomic plants and by 0.6 chromosome arms in disomic plants (Table I).

The improvement in chromosome pairing in plants of the -- type could be due to the better pairing of chromosome 6R and/or of other chromosomes. Giemsa staining of meiocytes of disomic plants revealed that -- plants had significantly ($P \leq 0.001$) more rye chromosomes present as closed bivalents than did ++ plants (Table I). In -- plants the two 6Rs were present as a closed bivalent in about 63% of the meiocytes. This probably accounts for the higher number of rye closed bivalents observed in this type since Lelley (personal communication) found that, in a rye genotype, 6R formed closed bivalents in only 30% of the meiocytes. It is unlikely that the magnitude of the difference in closed bivalent frequency found between the rye parent of Rosner triticale and Lelley's rye (63% vs. 30%) was wholly due to genotypic differences between the two lines. It could, however, account for some of the difference.

No data for the pairing configurations of 6R in ++ plants have been

obtained because of the difficulty in distinguishing 6R bivalents from those of the other rye chromosomes. In both the ++ and -- plants 6R did not frequently appear as a univalent. In -- plants, therefore, the short arm of 6R may be paired more frequently, resulting in more 6R closed bivalents and significantly more chromosome arms being paired per PMC (Table I).

In disomics the loss of the largest part of the terminal heterochromatic band of $6R^S$ was accompanied by an additional 0.60 (37.04-36.44, Table I) chromosome arms being paired, which was approximately two thirds of the better pairing found in rye chromosomes of -- plants (8.76-7.99 = 0.77, Table I). This indicates either an overestimation of 6R closed bivalents or that chromosomes other than 6R were also affected. The latter explanation is favoured because different rye chromosomes did not appear as univalents with equal frequencies. Most of the univalents observed in both ++ and -- plants were other than 6R (Figs. 3 and 4), and predominantly 1R and 7R (Table II). In the ++ type, 56% of the univalents were 1R and 18% were 7R while in the -- plants the situation was reversed with 7R accounting for 45% of the univalents and 1R for 30%. The total frequency of all other univalents (rye and wheat) remained the same irrespective of the presence or absence of the $6R^S$ telomeric band (Table II). It is possible, therefore, that other rye chromosomes may also have been affected by the removal of the telomeric heterochromatin from chromosome 6R.

The telomeric heterochromatin of $6R^S$ is not unique in its effect on chromosome pairing. Thus, Merker (1976), working with what appears to be chromosome 7R of hexaploid triticale, concluded that telomeric

Table II

The frequency (%) of 1R and 7R univalents in disomic Rosner plants with (++) or without (--) telomeric heterochromatin on the short arm of chromosome 6R

Type of 6R chromosome	Chromosome			Number of cells examined
	1R	7R	Other	
++	56	18	26	138
--	30	45	25	180

heterochromatin affects at least the pairing of the chromosomes which carry it. Our results, however, indicate that telomeric heterochromatin of $6R^S$ affected the pairing of other rye chromosomes as well (i.e. 1R and 7R).

Alternatively, the higher frequency of 1R and 7R univalent chromosomes may have been due to the fact that they are the only chromosomes in Rosner that have a large telomeric heterochromatic band on the long arm (Fig. 1). The long arm is usually more often involved in pairing and chiasma formation than the short arm. Thus, if heterochromatin does indeed inhibit pairing it follows that these two chromosomes (i.e. 1R, 7R) would appear as univalents more often than other rye chromosomes.

Interstitial heterochromatin of rye and wheat chromosomes may also affect chromosome pairing. Miklos and Nankivell (1976), in a study of three sibling species of Australian grasshoppers (Atractomorpha australis, A. species-1 and A. similis) found that the higher the amount of heterochromatin, either centric or telomeric, the lower the chiasma frequency and, therefore, the number of chromosome arms paired per PMC. Thus, heterochromatin affected chromosome pairing irrespective of its relative position along the chromosome. The precise mechanism of this effect remains obscure (see Thomas and Kaltsikes, 1974 for a plausible explanation). Further work, however, mainly with stocks having artificially reduced telomeric heterochromatin may result in a better understanding of its effects on chromosome pairing.

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MANUSCRIPT VI

ANTHER LENGTH AND MEIOTIC DEVELOPMENT IN TRITICALE AND ITS PARENTAL SPECIES

Introduction

In cereals each floret has three anthers which contain pollen mother cells that proceed synchronously through meiosis. This synchronous development has been used in determining the duration of meiosis of several cereal genotypes and of the amphidiploid triticale (X Triticosecale Wittmack) (Bennett, Chapman, and Riley, 1971; Bennett and Kaltsikes, 1973; Bennett, Dover and Riley, 1974; Roupakias and Kaltsikes, 1977a, b, and c). The triticale genotypes invariably show a number of univalents at first meiotic metaphase, the causes of which have been the subject of extensive studies (Thomas and Kaltsikes, 1974a and b; Lelley, 1974; Kaltsikes, 1974). These studies, which were mostly cytological, have so far failed to identify the real causes of univalency which may be biochemical in nature (Roupakias and Kaltsikes, 1977b; Bennett, personal communication). A biochemical study of developing meiocytes will require large numbers at particular meiotic stages obtained from intact anthers and florets. Erickson (1948) established a high correlation between bud length and meiotic stage of Lilium, and Hotta and Stern (1961a and b, 1963a and b) used it extensively in biochemical studies. We report in this paper the results of research which utilized several genotypes of triticale, and their parents, to study the possible relationship between anther length and meiotic development as a prelude to further biochemical and ultrastructural studies.

Materials and Methods

The materials used are given in Table I. Their pedigree has been

previously described by Roupakias and Kaltsikes (1977b and c). Plants were grown in the greenhouse until the leading tillers were judged to be approximately one week prior to the onset of meiosis. They were then transferred to a growth cabinet and maintained at 20 ± 1 C, 71.50 ± 0.62% relative humidity and continuous illumination. Spikelets from the middle part of the spikes, estimated to be at the appropriate meiotic stages, were fixed in 1:3 acetic alcohol. Anther length (length of longest loculus) was measured by using a millimetric scale under a stereo microscope. At least 10 anthers from different spikelets were used per each line and per meiotic stage. Squash preparations in aceto-carmine (2%) by standard procedures, were used to determine the meiotic stage of both primary and secondary florets. The estimation of the difference observed in the development of meiocytes between primary and secondary florets was based on the duration of meiotic stages reported by Roupakias and Kaltsikes (1977b and c). Analysis of variance and the t-test comparisons were used to determine the significance of differences in anther length among the various meiotic stages within a line and of the same meiotic stage among the lines examined.

Results

Anther length

Analysis of variance and t-test comparisons revealed significant differences in anther length at various meiotic stages within, as well as among, lines of the same species (Table I). Within each species, lines having shorter anthers at the beginning of meiotic prophase (nucleolar fusion) had, also, shorter anthers at later meiotic stages

Table I

Anther length (mm) at various meiotic stages and anther elongation rate (mm/h) in several cereal genotypes

Line	Meiotic Stage						Growth rate mm/h
	Nucleolar fusion	Synchronous tapetal division[1]	Middle zygotene	Middle pachytene	MI	TII	
Octoploid triticale							
8A74	1.15[2a]	1.54[b]	1.74[c]	1.83[d]	2.02[e]	2.02[e]	0.0199
8A599	1.09[a]	1.31[b]	1.52[c]	1.58[c]	1.79[d]	1.85[e]	0.0174
Hexaploid triticale							
Rosner	1.27[a]	1.67[b]	1.85[c]	1.98[d]	2.08[e]	2.28[f]	0.0189
341-5	1.09[a]	1.49[b]	1.69[c]	1.88[d]	1.97[e]	2.14[f]	0.0227
R621-5	1.31[a]	1.76[b]	1.92[c]	2.01[d]	2.18[e]	2.36[f]	0.0212
6A301	1.21[a]	1.59[b]	1.76[c]	1.86[d]	2.03[e]	2.17[f]	0.0188
Hexaploid wheat							
Prelude (6x)	0.95[a]	1.11[b]	1.20[c]	1.30[d]	1.48[e]	1.47[e]	0.0117
Tetraploid wheat							
Prelude (4x)	0.66[a]	0.89[b]	0.92[b]	0.95[b]	1.06[e]	1.15[d]	0.0111
Rescue (4x)	0.77[a]	0.99[b]	1.00[b]	1.15[c]	1.25[d]	1.28[d]	0.0116
Thatcher (4x)	0.71[a]	0.90[b]	0.96[c]	1.04[d]	1.09[e]	1.20[f]	0.0110
Diploid rye							
Prolific	1.92[a]	2.40[b]	2.73[c]	2.85[c]	3.18[d]	3.42[e]	0.0285

[1] Not a meiotic stage but an unambiguous landmark.

[2] Within each line mean anther lengths followed by the same letter are not significantly different from one another. The standard errors associated with these means are available from the authors upon request.

indicating an approximately similar anther elongation rate (Table I). However, Erickson (1948) reported a fluctuation in growth rate of Lilium longilforum from day to day which he attributed to uncontrolled growth conditions. The shortest anthers, at corresponding meiotic stages, were observed in tetraploid wheat and the longest in rye, with the triticale genotypes being intermediate (Table I), indicating that anther length in triticale is controlled by genes located on both wheat and rye chromosomes.

Correlation between anther length and meiotic stage

A high correlation between anther elongation and meiotic development or meiotic stage was found in all lines examined (Table II). No overlapping of the standard errors of the mean anther length was found between successive stages in most of the lines examined. However, even in zygotene and pachytene, which last for several hours each, there was overlapping with respect to individual anther lengths and the stage of the PMC's contained therein (Fig. 1). The mean value of anther elongation (mm/h) during the development of meiosis (value of b in Table II) was similar in all lines within a species (Tables I and II). It was 0.01 mm/h in all tetraploid and hexaploid wheat lines; 0.02 mm/h in all hexaploid and octoploid triticale lines and 0.03 mm/h in diploid rye.

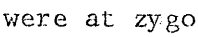
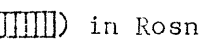
Meiotic development in primary and secondary florets of a spikelet

The difference in meiotic development between the primary and secondary florets of the same spikelet varied from species to species as well as from line to line within a species (Table III). The largest lag in meiotic development of the secondary as compared to primary florets was observed in Prelude tetraploid wheat (21.78 h), and the

Table II

Linear regression (and coefficient of determination) between anther elongation (y) and development of meiosis (x) in various cereal genotypes

Line	Linear regression y = a + bx		Coefficient of determination
	(a) intercept	(b) coefficient	(r^2)
Octoploid triticale			
8A74	1.20	0.02	0.97
8A599	1.07	0.02	0.98
Hexaploid triticale			
Rosner	1.31	0.02	0.98
341-5	1.11	0.02	0.98
R621-5	1.36	0.02	0.98
6A301	1.24	0.02	0.99
Hexaploid wheat			
Prelude (6x)	0.93	0.01	0.97
Tetraploid wheat			
Prelude (4x)	0.67	0.01	0.97
Rescue (4x)	0.76	0.01	0.99
Thatcher (4x)	0.71	0.01	0.99
Diploid rye			
Prolific	1.85	0.03	0.97

Figure 1. Frequency distribution of anther length while the PMC's contained therein were at zygotene () or pachytene () in Rosner triticale. Overlapping area indicates that anthers of similar lengths can contain meiocytes at zygotene or pachytene.

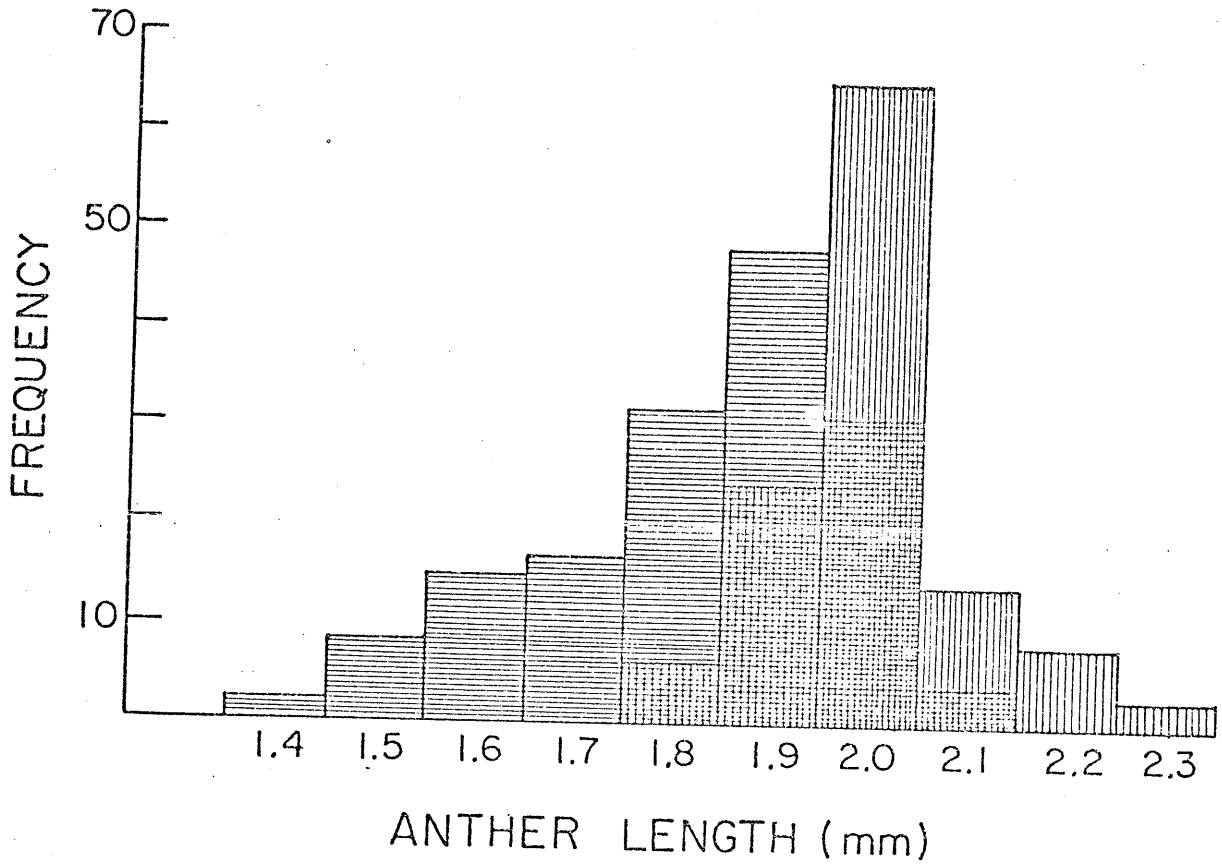

70
50
10
FREQUENCY
1.4
1.5
1.6
1.7
1.8
1.9
2.0
2.1
2.2
2.3
ANTHER LENGTH (mm)

Table III

Mean difference and range, in hours,
of meiotic development between primary and secondary florets
of the same spikelet in various cereal genotypes

Line	mean (h)	Range (h)	No. spikelets examined
Octoploid triticale			
8A74	15.18	9.74 - 22.72	265
8A599	12.68	5.28 - 22.21	220
Hexaploid triticale			
Rosner	20.75	8.75 - 28.50	275
341-5	17.19	9.00 - 23.11	225
R621-5	17.78	10.03 - 25.01	215
6A301	16.40	3.00 - 22.20	255
Hexaploid wheat			
Prelude (6x)	15.23	8.35 - 24.54	120
Tetraploid wheat			
Prelude (4x)	21.78	16.60 - 27.40	195
Rescue (4x)	19.95	9.34 - 25.41	132
Thatcher (4x)	19.86	12.56 - 25.22	99
Diploid rye			
Prolific	14.75	4.50 - 23.75	265

smallest in 8A599 octoploid triticale (12.68 h). Hexaploid wheat (AABBDD) and octoploid triticale (AABBDDRR) showed smaller differences in meiotic development between primary and secondary florets than tetraploid wheat (AABB) and hexaploid triticale (AABBRR), respectively. These differences are probably due to the D genome. However, factors other than the D genome may also have had an effect on meiotic development of primary and secondary florets because significant differences were observed among lines within most species (Table III). It is concluded that the difference in meiotic development between primary and secondary florets of the same spikelet was not constant and, therefore, the meiotic stage of the primary floret cannot be used for the precise determination of the stage of meiocytes carried in the anthers of the secondary floret.

Discussion

Erickson (1948) in a study of Lilium longiflorum Thumb., grown in the greenhouse, reported a high correlation between bud length and stages of meiosis. Furthermore, he believed that, in plants grown under controlled conditions, a particular meiotic stage would be found to occur at a particular bud length with a variation of a few tenths of a millimeter. The variation found in the present study, where anther length was measured, was only a few hundredths of a millimeter, probably due to the shorter length of cereal anthers as compared to Lilium buds (i.e. at MI anther length in cereals = 2.0 mm, bud length in Lilium = 22.5 mm or slightly more than a tenfold difference). Vasil (1967) noted "we recognize the fact of a close correlation between bud length and meiotic

and post-meiotic development of anthers in a large number of plants, but is it really so close and precise that we can determine the exact stage of meiosis just by measuring the length of the bud and checking it against a standard curve?" Considering the two meiotic stages (zygotene and pachytene) during which chromosome pairing is thought to occur, the difficulty in determining the precise meiotic stage of cereal anthers by measuring the anther length and checking it against a standard curve becomes obvious (Fig. 1). However, if one is interested only in obtaining a rough estimate ($\pm$ 4 hours) of the stage of the meiocytes contained within an anther of a given length, then the standard curve can be legitimately used. Vasil (1967) also doubted the results reported by Erickson (1948) and he noticed that the bud length of Lilium should be used only for rough estimation of meiotic stages. For the precise determination of the stage of the meiocytes contained within an anther the known developmental gradients within individual florets and along the inflorescence must be utilized. As a very high degree of synchronous development among the three anthers of a floret exists (Bennett et al., 1971; Roupakias and Kaltsikes, 1977a, b, and c) examination of one anther will provide information about the stages of the meiocytes in the others. But this approach reduces by one third the number of anthers available for further study, a serious limitation when anther culture is contemplated. Alternatively, the florets on one side near the centre of the inflorescence can be used to provide information about the stage of the meiocytes contained in the anthers of the corresponding florets on the other side of the spike. This becomes feasible since it is known (Roupakias and Kaltsikes, 1977a) that florets near the middle of the

spike are intermediate in meiotic development to those directly above and below them but on the opposite side of the rachis. Although this approach requires greater amounts of labor relative to anther examination, it has the advantage that it does not reduce the number of anthers, with meiocytes at a particular stage, which become available for further study.

Summary

The difference in meiotic development between primary and secondary florets of the same spikelet, and the relationship between anther length and the meiotic stage of the meiocytes contained therein were studied in several triticale (X Triticosecale Wittmack) lines and their parents. At 20 °C under continuous illumination, no constant difference in meiotic development between primary and secondary florets was found. The rate of anther elongation was approximately 0.02 mm/h in all triticales, 0.01 mm/h in tetraploid (T. turgidum L.) and hexaploid wheat (T. aestivum L.) and 0.03 mm/h in Secale cereale L. Although, on the average, the stage of the meiocytes in an anther could be ascertained with a certain degree of accuracy by measuring its length, the degree of overlapping observed between successive meiotic stages mitigates against the use of this criterion for precise meiotic stage determinations.

Acknowledgments

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GENERAL DISCUSSION

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1. Duration of Meiosis

During the process of the study of the duration of meiosis in higher plants, under constant environmental conditions, a number of factors affecting the duration of meiosis have been suggested. They are (a) DNA content; (b) ploidy level; (c) cytoplasm and (d) genotype. Each of these factors will be discussed separately.

1.1 DNA Content

Bennett (1972, 1973) found a high positive correlation between the duration of meiosis and nuclear DNA content among species within each ploidy level. He did not study, however, the duration of meiosis in several genotypes within a ploidy level of each species. Thus, it is not known whether there is a variation in the duration of meiosis among the genotypes of a species and within a ploidy level. In contrast, Furuta et al. (1974) measured the DNA content per nucleus in a number of hexaploid wheat strains. They found that there were no differences in DNA content among the strains they studied. In addition, Bennett and Smith (1972) and Furuta et al. (1974) considered that the DNA content of a cereal nucleus was equal to the total DNA of the genomes contributing to it. Therefore, within a ploidy level of a cereal species all strains should have equal amounts of DNA.

The approach taken to the problem of the relationship between duration of meiosis and DNA content was to select a number of strains within

each ploidy level and to study the duration of meiosis under constant environmental conditions. If the amount of DNA per se is the major factor which determines the meiotic duration in cereals, then there should not be significant differences in the duration of meiosis among strains of each species and ploidy level. The information accumulated, however, indicates that there were significant differences in the duration of meiosis among strains within each of the following species and ploidy levels: (a) Triticum aestivum L. em. Thell. and (b) X Triticosecale Wittmack (tetraploid and hexaploid levels). In contrast there were no significant differences in (a) Triticum turgidum L., (b) X Triticosecale Wittmack (Octoploid) and (c) S. cereale. This may indicate that the quality rather than the quantity of DNA is what determines the duration of meiosis in cereal genotypes. Thus,when two strains within a ploidy level have different gene(s) affecting meiotic development ... the durations of their meiosis could be significantly different. If, however, the same or similar genes control the duration of meiosis in both strains, ... meiosis could probably last the same. Such an effect, of individual chromosomes or even gene(s) on the duration of meiosis has been reported in the literature (Bennett and Smith, 1973; Bennett et al., 1974; and Klein, 1972).

1.2 Ploidy Level

Bennett and Smith (1972, 1973) found that hexaploid wheat and octoploid triticale had shorter meiotic duration than tetraploid wheat and hexaploid triticale, respectively. They attributed this difference in meiotic development between hexaploid and tetraploid wheat or hexaploid and octoploid triticale to the ploidy level. In other words they

concluded that the higher the ploidy level the shorter the duration of meiosis.

Bennett et al. (1974) found that addition or subtraction of individual chromosomes from Triticum aestivum (var. Chinese Spring) had as a result the reduction or elongation of the duration of meiosis. Tetraploid wheat and hexaploid triticale differ from hexaploid wheat and octoploid triticale in that they lack the D genome. Therefore, the shorter duration of meiosis observed in hexaploid wheat and octoploid triticale as compared to tetraploid wheat and hexaploid triticale could be due to gene(s) carried by the D genome rather than to simple changes in the ploidy level. Thus, the approach taken to this problem was to select and study the duration of meiosis under constant environmental conditions in a number of genotypes sharing the same AB, D and R genomes but differing in the ploidy level. The information obtained indicates that it is the genotype which affects the duration of meiosis rather than simple changes of the ploidy level. However, if ploidy level is viewed as a factor of the cellular environment, and given that the environment affects the duration of meiosis, then it is possible that the ploidy level has an effect on the duration of meiosis. This effect can be increased or decreased by genetic factor(s).

1.3 Cytoplasm

The duration of meiosis is strongly affected by environmental conditions. The cytoplasm as the major component of the cellular environment, therefore, might have an effect on the duration of meiosis. The approach taken to this problem was to select four pairs of triticale strains and to study the duration of meiosis. The two strains of each

pair were genotypically identical but differed in the source of their cytoplasm (4x or 6x wheat). This study indicated that there were no significant differences in the duration of meiosis between the strains of each pair. The source of cytoplasm (4x or 6x wheat), therefore, has no effect on the duration of meiosis in triticale.

1.4 Genotype

Bennett et al. (1974) found that chromosome 5B had a significant effect on the duration of meiosis in hexaploid wheat. In addition, Klein (1972) found differences in the duration of meiosis between mutant lines of Pisum sativum. Furthermore, this study indicates that there was variation in the duration of meiosis among strains of hexaploid wheat and triticale. Finally, tetraploid triticales which differed in their chromosome composition had significantly different durations of meiosis. It is clear, therefore, that under constant environmental conditions the duration of meiosis is genotypically controlled. Environmental factors, however, such as temperature have major effects on the meiotic development (Wilson, 1959; Bennett et al., 1972). Thus the environmental conditions are seen as governing the degree to which the genotype may be permitted to express itself. As a part, therefore, of the cellular environment the DNA content and the ploidy level may have an effect on the duration of meiosis.

2. Chromosome Pairing

Meiotic irregularities have been observed in triticale even in the very early cytological studies (Levitsky and Benetzkaja, 1931; Muntzing, 1939). Since then it has been established that triticales of all ploidy

levels have a varying number of chromosomes unpaired at first meiotic metaphase (Müntzing, 1957; O'Mara, 1953; Weimarck, 1974; Merker, 1973a; present results).

Darlington (1940) theorized that variation in the number and position of chiasmata can be caused by differences in the time available for chromosome pairing. Following this, Bennett et al. (1971) and Bennett and Kaltsikes (1973) studied the duration of meiosis and its stages in wheat, rye and triticale and found that the duration of meiosis in rye was much longer than in both wheat and triticale. Thus they suggested that insufficient time for normal pairing of rye chromosomes in the triticale nucleus may be the cause of partial failure of chromosome pairing.

In the present study the approach taken to the problem was to select a number of triticales exhibiting varying degrees of meiotic abnormalities and to study the duration of meiosis under constant environmental conditions. The information obtained indicates that there was no relationship between duration of meiosis (nucleolar fusion to TII inclusive) or zygotene plus pachytene (Z + P) and chromosome pairing in any of the three ploidy levels (tetraploid, hexaploid and octoploid) studied. Furthermore, the duration of Z + P, the stages during which chromosome pairing is thought to occur, was found to last longer in hexaploid and tetraploid triticale than in rye. Therefore, incompatibility in the meiotic development between wheat and rye chromosomes does not seem to be the cause of the meiotic abnormalities in triticale.

Larter and Hsam (1973) and Rimpau et al. (1973) have shown that, in triticale and wheat-rye addition lines, the number of univalents per PMC

was affected by the source of cytoplasm. In contrast, Kiss and Trefás (1973) and Lelley (1975a) did not find significant differences in the number of univalents per PMC between strains carrying wheat or rye cytoplasm. The results of the present study support the finding that the origin of the cytoplasm (from tetraploid or hexaploid wheat) has no effect on chromosome pairing. Since the duration of meiosis and the source of cytoplasm have no effect on chromosome pairing other factors have to be investigated.

It has been shown that in all ploidy levels the chromosomes which fail to pair in triticale most likely belong to the rye genome (Pieritz, 1966, 1970; Thomas and Kaltsikes, 1974b, 1976b; present results). It is also known that in hexaploid and in tetraploid triticales (Thomas and Kaltsikes, 1974b, 1976b; present results) rye chromosomes having prominent heterochromatic bands on both telomeres are more often present as univalents. This implicates heterochromatin in chromosome pairing and may indicate that there probably is a common reason for pairing failure of rye chromosomes in triticale of all ploidy levels. Merker (1976) found that loss of telomeric heterochromatin in one of the chromosomes resulted in better chromosome pairing. Additionally, Miklos and Nankivell (1976) reported an inverse correlation between chiasma formation and heterochromatin in Australian grasshoppers. The present study also indicated that loss of telomeric heterochromatin from a rye chromosome resulted in better chromosome pairing.

Müntzing (1957), based on the knowledge that hexaploid triticales had better pairing than octoploids, suggested that meiotic irregularities in triticale may be related to the ratio of rye to wheat genomes. It

has been shown in this study that the tetraploid triticales (as a group) are more stable than the hexaploids, which in turn are more stable than the octoploids. The mean number of univalents per PMC found in each strain within each ploidy level investigated in this study ranged from: 0.37 to 1.89 in tetraploids; 0.67 to 4.89 in hexaploids and 2.78 to 5.21 in octoploids. In other words there were strains from a higher ploidy level which had better chromosome pairing than strains from a lower ploidy level. This may indicate that the ratio of wheat to rye genomes as a whole may have an effect on chromosome pairing. This effect, however, can be drastically modified depending on the genetic factors involved.

One area of investigation which should be pursued in the future is a detailed biochemical study of a number of triticales differing in their degree of meiotic instability. Such a study may result in a better understanding of the cause of the meiotic irregularities in triticale.

A biochemical study of developing meiocytes will require large numbers of particular meiotic stages obtained from intact anthers and florets. Therefore, if a high correlation could be established between the meiotic stage of the meiocytes included in an anther and the anther length, it would be easy for a biochemist to select the meiotic stages he needs. Erickson (1948) established a high correlation between bud length and meiotic stage of Lilium. The resultsof the present study, however, indicate that in cereals the anther length cannot be used for a precise determination of the meiotic stage of the meiocytes therein. Vasil (1967) also doubted the results reported by Erickson (1948) and he suggested the use of the bud length of Lilium only for rough estimation

of meiotic stages. Therefore, for the precise determination of the meiotic stage of the meiocytes included within an intact anther, other alternatives have to be applied. They are either examination of one anther of each floret which will provide information about the stages of the meiocytes in the others; or examination of the anthers of one side of the spike which will provide information about the stages of the meiocytes in the anthers on the other side of the spike.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

From this extensive study of the duration of meiosis and chromosome pairing in several triticales of all ploidy levels (i.e. tetraploid, hexaploid and octoploid) and their wheat and rye parents, the following information was obtained.

(1) The origin of the cytoplasm (from tetraploid and hexaploid wheat) had no significant effect on chromosome pairing or meiotic duration.

(2) No relationship was found, in any of the ploidy levels (tetraploid, hexaploid, and octoploid) studied, between total duration of meiosis, or that of zygotene and pachytene, and chromosome pairing.

(3) A significant intraspecific and interspecific variation was found in the duration of meiosis of cereal genotypes.

(4) The duration of meiosis and chromosome pairing were significantly affected by various chromosome combinations.

(5) Pairing failure occurred mostly among chromosomes of the rye genome.

(6) Tetraploid triticales (as a group) were more stable than hexaploids which in turn were more stable than octoploids.

(7) No relationship between meiotic abnormalities and fertility was detected.

(8) Chromosome pairing at first meiotic metaphase was significantly

higher in plants lacking most of the telomeric heterochromatin on the short arm of chromosome 6R ($6R^S$) than in plants carrying the telomeric band.

(9) There was no constant difference in meiotic development between primary and secondary florets.

(10) A high correlation was found between mean anther length and meiotic stage of the meiocytes contained therein.

From these findings the conclusions which could be drawn are:

(1) Insufficient time (after the stage of nucleolar fusion) for normal pairing of rye chromosomes in the triticale nucleus cannot account for univalency in triticale.

(2) The variation observed in the duration of meiosis and its stages among the various cereal genotypes studied was more likely due to genes carried by the D and R genomes rather than to simple changes in ploidy level and DNA content. This may suggest that ploidy level and DNA content have minor effects on the duration of meiosis.

(3) Telomeric heterochromatin affects chromosome pairing, and

(4) Although, on the average, the stage of the meiocytes in an anther could be ascertained with a certain degree of accuracy by measuring its length, the degree of overlapping observed between successive meiotic stages mitigates against the use of this criterion for precise meiotic stage determination.

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APPENDICES

Appendix 1. Duration of meiosis (h) and meiotic abnormalities in two octoploid triticales.

Material	Duration (hours)		Meiotic data				
	Zygotene + Pachytene	Total	Univalents Per PMC	Chromosome Arms Paired	Range of Univalents Among Plants	No. Cells Examined	No. Plants Examined
8A74	16.55±0.32[1]	43.69±1.47	2.78±0.22	46.60±0.27	1.92 - 3.48	200	8
8A599	16.65±0.16	43.62±0.65	5.21±1.05*	42.70±1.98	2.28 - 8.80	200	8

[1] Standard error of the mean.

* denote significant differences at the 1% level.

Appendix 2-1. Anther length (mm) at various meiotic stages and anther elongation rate (mm/h) in hexaploid triticale

Line	Meiotic Stage						Growth rate mm/h
	Nucleolar fusion	Synchronous tapetal division[1]	Middle zygotene	Middle pachytene	MI	TII	
Rosner	1.27[2] ± 0.033[a]	1.67 ± 0.035[b]	1.85 ± 0.021[c]	1.98 ± 0.013[d]	2.08 ± 0.019[e]	2.28 ± 0.047[f]	0.0189
Triticale turgidum	1.13 ± 0.029[a]	1.52 ± 0.021[b]	1.78 ± 0.026[c]	1.93 ± 0.018[d]	2.06 ± 0.023[e]	2.22 ± 0.029[f]	0.0210
65-4	1.23 ± 0.025[a]	1.59 ± 0.029[b]	1.86 ± 0.034[c]	1.97 ± 0.019[d]	2.11 ± 0.038[e]	2.28 ± 0.034[f]	0.0211
341-5	1.09 ± 0.018[a]	1.49 ± 0.030[b]	1.69 ± 0.037[c]	1.88 ± 0.013[d]	1.97 ± 0.018[e]	2.14 ± 0.032[f]	0.0227
R621-5	1.31 ± 0.027[a]	1.76 ± 0.022[b]	1.92 ± 0.015[c]	2.01 ± 0.020[d]	2.18 ± 0.024[e]	2.36 ± 0.041[f]	0.0212
6A301	1.21 ± 0.016[a]	1.59 ± 0.019[b]	1.76 ± 0.027[c]	1.86 ± 0.017[d]	2.03 ± 0.017[e]	2.17 ± 0.024[f]	0.0188
A_1A_2(6x)[3]	1.42 ± 0.020[a]	1.82 ± 0.027[b]	1.87 ± 0.033[b]	2.03 ± 0.031[c]	2.39 ± 0.031[d]	2.42 ± 0.055[d]	0.0223
A_1A_2(4x)	1.31 ± 0.022[a]	1.75 ± 0.024[b]	1.88 ± 0.033[c]	2.03 ± 0.002[d]	2.24 ± 0.034[e]	2.32 ± 0.043[e]	0.0214
A_3A_4(6x)	1.33 ± 0.018[a]	1.73 ± 0.020[b]	1.95 ± 0.022[c]	2.09 ± 0.027[d]	2.18 ± 0.035[e]	2.34 ± 0.042[f]	0.0204
A_3A_4(4x)	1.21 ± 0.026[a]	1.51 ± 0.038[b]	1.78 ± 0.024[c]	1.92 ± 0.026[d]	2.05 ± 0.030[e]	2.08 ± 0.030[e]	0.0176
A_5A_6(6x)	1.01 ± 0.021[a]	1.46 ± 0.039[b]	1.59 ± 0.063[b]	1.80 ± 0.031[c]	1.97 ± 0.041[d]	2.08 ± 0.049[d]	0.0253
A_5A_6(4x)	0.99 ± 0.013[a]	1.36 ± 0.033[b]	1.45 ± 0.040[b]	1.72 ± 0.030[c]	1.84 ± 0.028[d]	1.99 ± 0.032[e]	0.0211
A_7A_8(6x)	1.02 ± 0.017[a]	1.38 ± 0.024[b]	1.62 ± 0.039[c]	1.76 ± 0.045[d]	1.93 ± 0.026[e]	2.03 ± 0.045[e]	0.0213
A_7A_8(4x)	1.02 ± 0.014[a]	1.30 ± 0.031[b]	1.51 ± 0.034[c]	1.78 ± 0.041[d]	1.91 ± 0.034[e]	2.04 ± 0.046[f]	0.0212

[1] Not a meiotic stage but an unambiguous landmark.

[2] Within each line mean anther lengths followed by the same letter are not significantly different from one another.

[3] Figures in brackets refer to the origin of the cytoplasm: 6x = hexaploid wheat; 4x = tetraploid wheat.

Appendix 2-2. Anther length (mm) at various meiotic stages and anther elongation rate (mm/h) in several cereal genotypes

Line	Meiotic Stage						Growth rate mm/h
	Nucleolar fusion	Synchronous tapetal division[1]	Middle zygotene	Middle pachytene	MI	TII	
Octoploid triticale							
8A74	1.15[2] ± 0.019[a]	1.54 ± 0.024[b]	1.74 ± 0.020[c]	1.83 ± 0.017[d]	2.02 ± 0.011[e]	2.02 ± 0.019[e]	0.0199
8A599	1.09 ± 0.019[a]	1.31 ± 0.012[b]	1.52 ± 0.035[c]	1.58 ± 0.020[c]	1.79 ± 0.018[d]	1.85 ± 0.010[e]	0.0174
Diploid rye							
Prolific	1.92 ± 0.029[a]	2.40 ± 0.043[b]	2.73 ± 0.064[c]	2.85 ± 0.037[c]	3.18 ± 0.056[d]	3.42 ± 0.076[e]	0.0285
OD289	1.68 ± 0.086[a]	2.64 ± 0.041[b]	2.73 ± 0.048[b]	3.03 ± 0.045[c]	3.32 ± 0.053[d]	3.57 ± 0.080[e]	0.0357
Tetraploid wheat							
Stewart 63	0.84 ± 0.020[a]	1.06 ± 0.012[b]	1.11 ± 0.024[b]	1.24 ± 0.019[c]	1.29 ± 0.024[c]	1.41 ± 0.032[d]	0.0130
Prelude (4x)	0.66 ± 0.014[a]	0.89 ± 0.010[b]	0.92 ± 0.015[b]	0.95 ± 0.018[b]	1.06 ± 0.025[c]	1.15 ± 0.016[d]	0.0111
Rescue (4x)	0.77 ± 0.012[a]	0.99 ± 0.005[b]	1.00 ± 0.008[b]	1.15 ± 0.021[c]	1.25 ± 0.016[d]	1.28 ± 0.029[d]	0.0116
Thatcher (4x)	0.71 ± 0.007[a]	0.90 ± 0.015[b]	0.96 ± 0.015[c]	1.04 ± 0.011[d]	1.09 ± 0.016[e]	1.20 ± 0.019[f]	0.0110
Hexaploid wheat							
Prelude (6x)	0.95 ± 0.011[a]	1.11 ± 0.011[b]	1.20 ± 0.037[c]	1.30 ± 0.015[d]	1.48 ± 0.029[e]	1.47 ± 0.031[e]	0.0117
Chinese Spring	0.90 ± 0.011[a]	1.11 ± 0.020[b]	1.14 ± 0.013[b]	1.33 ± 0.032[c]	1.52 ± 0.027[d]	1.56 ± 0.023[d]	0.0201
Hybrids							
6A391 X Prolific	1.52 ± 0.015[a]	2.10 ± 0.086[b]	2.41 ± 0.067[c]	2.50 ± 0.065[c]	2.81 ± 0.051[d]	2.98 ± 0.022[e]	0.0277
Rosner X Prolific	1.36 ± 0.022[a]	1.83 ± 0.016[b]	2.06 ± 0.023[c]	2.31 ± 0.041[d]	2.39 ± 0.097[d]	2.79 ± 0.030[e]	0.0277

[1] Not a meiotic stage but an unambiguous landmark.

[2] Within each line mean anther lengths followed by the same letter are not significantly different fron one another.

Appendix 2-3. Linear regression (and coefficient of determination) between anther elongation (y) and development of meiosis (x) in various cereal genotypes

Line	Linear regression y = a + bX (a) intercept	(b) coefficient	Coefficient of determination (r^2)
Hexaploid triticale			
Triticale turgidum	1.16	0.02	0.98
65-4	1.25	0.02	0.97
341-5	1.11	0.02	0.98
R621-5	1.36	0.02	0.98
6A301	1.24	0.02	0.99
A_1A_2 (6x)	1.39	0.02	0.97
A_1A_2 (4x)	1.33	0.02	1.00
A_3A_4 (6x)	1.37	0.02	0.98
A_3A_4 (4x)	1.22	0.02	0.97
A_5A_6 (6x)	1.02	0.02	1.00
A_5A_6 (4x)	0.98	0.02	1.00
A_7A_8 (6x)	1.03	0.02	0.98
A_7A_8 (4x)	0.98	0.02	0.99
Diploid rye			
OD289	1.72	0.03	0.99
Octoploid triticale			
8A74	1.20	0.02	0.97
8A599	1.07	0.02	0.98
Tetraploid wheat			
Stewart 63	0.83	0.01	0.99
Prelude (4x)	0.67	0.01	0.97
Rescue (4x)	0.76	0.01	0.99
Thatcher (4x)	0.71	0.01	0.99
Hexaploid wheat			
Prelude (6x)	0.93	0.01	0.97
Chinese Spring	0.87	0.02	0.92
ABRR			
Rosner X Prolific	1.38	0.03	0.97
6A299 X Prolific	1.50	0.03	0.98

Appendix 2-4. Mean difference and range, in hours, of meiotic development between primary and secondary florets of the same spikelet in various cereal genotypes

Line	Mean (h)	Range (h)	No. plants examined	No. spikes examined	No. spikelets examined
Hexaploid triticale					
Rosner	20.75	8.75-28.50	44	55	275
Triticale turgidum	17.83	9.70-30.99	44	60	300
65-4	18.36	11.40-26.47	35	58	270
341-5	17.19	9.00-23.11	34	45	225
R621-5	17.78	10.03-25.01	29	43	215
6A301	16.40	3.00-22.20	37	56	255
A_1A_2 (6x)	16.79	8.50-22.44	24	46	230
A_1A_2 (4x)	16.91	9.70-23.49	30	52	260
A_3A_4 (6x)	13.77	5.00-22.62	36	50	250
A_3A_4 (4x)	15.70	5.00-24.99	42	49	245
A_5A_6 (6x)	10.49	3.00-19.68	29	43	215
A_5A_6 (4x)	11.06	3.00-21.52	22	37	185
A_7A_8 (6x)	17.68	9.32-31.01	18	32	160
A_7A_8 (4x)	16.75	6.20-28.35	19	35	175
Octoploid triticale					
8A74	15.18	9.74-22.72	32	61	265
8A599	12.68	5.28-22.21	21	49	220
Diploid rye					
Prolific	14.75	4.50-23.75	52	59	265
OD289	15.24	4.00-24.83	43	60	275
Tetraploid wheat					
Stewart 63	19.70	10.15-28.67	36	52	230
Prelude (4x)	21.78	16.60-27.40	35	65	195
Rescue (4x)	19.95	9.34-25.41	31	44	132
Thatcher (4x)	19.86	12.56-25.22	25	33	99
Hexaploid wheat					
Prelude (6x)	15.23	8.35-24.54	33	40	120
Chinese Spring	7.81	2.00-13.70	19	21	105
ABRR					
Rosner X Prolific	13.46	5.00-28.68	33	53	213
6A299 X Prolific	13.71	5.51-23.15	21	32	380