CHIASMA TERMINALIZATION AND GENETIC RECOMBINATION IN TERTIARY TRISOMICS OF BARLEY

by

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STATEMENT BY AUTHOR

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TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

This experiment was conducted at Tucson, Arizona, at the Campbell Avenue Farm during 1966 and 1967 to determine the frequencies of cytological configurations at metaphase I and how the chromosomes disjoin, the per cent of lagging chromosomes and micronuclei at later meiotic stages, and to explain the 65:35 diploid to trisomic ratio in a balanced tertiary trisomic of barley.

Balanced tertiary trisomic plants carrying dominant alleles were selfed, and also crossed onto diploid recessive plants in the field. The progeny of selfed plants and of test cross plants were grown in the greenhouse. Cytological material was collected from balanced tertiary trisomic plants in the field.

Examination of metaphase configurations indicated an excess of alternate disjunction. In addition, there was a high frequency of cells with a univalent chromosome.

Data from the counts made at meiosis indicated that pairing of the ends of the chromosomes was preferential and not random.

The frequency of lagging chromosomes did not explain the deviation in the 65:35 diploid to trisomic ratio.

INTRODUCTION

Tertiary trisomics of barley (Hordeum vulgare L.) can be used to study the relation of chiasma terminalization and chromosome disjunction to genetic recombination. Ramage (1963) used a balanced tertiary trisomic of barley to explain the relation of genetic recombination to chromosome disjunction in meiosis.

A balanced tertiary trisomic is best defined by explaining each term separately. A trisomic individual has one extra chromosome. In a tertiary trisomic, the extra chromosome is an interchanged one composed of parts of two non-homologous chromosomes. Balanced refers to the breeding system, the self progeny produced being about 70% recessive diploids and 30% dominant tertiary trisomics. The balanced tertiary trisomics, when selfed, again produce recessive diploids and dominant tertiary trisomics in a 70:30 ratio.

This thesis is an analysis of chiasma terminalization and genetic recombination in a balanced tertiary trisomic of barley. The objectives of this thesis are: (1) to determine the frequency of chromosome configurations at metaphase I, (2) to determine how the chromosome configurations disjoin, (3) to determine the frequency of

lagging chromosomes, and (4) to explain why the ratio of diploids to trisomics deviates from 50:50.

LITERATURE REVIEW

Belling and Blakeslee (1926) working with Datura, first described a tertiary trisomic. They hypothesized the formation of the quinquevalent (chain of five chromosomes) because one of the chromosomes had a segmental interchange.

Burnham (1930) found tertiary trisomics in semi-sterile Zea mays L. In 1934, Burnham described the possible origin of a tertiary trisomic from either a cross of the trisomic interchange heterozygote onto a normal or by 3:1 disjunction of the ring of four chromosomes in a plant heterozygous for the interchange.

The progeny of selfed trisomics of Pisum sativum L. and the origin of trisomics from interchange configurations of four chromosomes was discussed by Sutton (1939). She also diagrammed all possible metaphase I configurations expected from the original tertiary trisomic and trisomic interchange heterozygote.

Ramage (1960) expanded Sutton's work and described the eight trisomics expected from 3:1 disjunction of a configuration of four chromosomes in the progeny of a selfed interchange heterozygote in barley. Hagberg (1954) first hypothesized that the different trisomics occur in the progeny of an interchange heterozygote as a result of a kind of orientation of the four chromosomes in which two

of the chromosomes are oriented and two are not oriented in the metaphase I spindle. The normal chromosomes may be oriented or the interchanged chromosomes may be orientated. If the two normal chromosomes are orientated, 3:1 disjunction of the ring gives an n+1 gamete with one normal chromosome and two interchanged ones. If the two interchanged chromosomes are orientated, 3:1 disjunction gives an n+1 gamete with two normal chromosomes and one interchanged chromosome. Eight different trisomics are expected in the self progeny of one interchange heterozygote. The eight trisomics are produced from four different n+1 eggs and two different n pollen grains. The n+1 gametes are not transmitted through the pollen, therefore, only the n pollen grains are functional. Two of the trisomics are tertiary trisomics (Ramage, 1960).

The breeding behavior of a particular tertiary trisomic in barley was described by Ramage (1955); in 1957[1] he termed this trisomic a balanced tertiary trisomic.

The balanced tertiary trisomic may have metaphase chromosome configurations which are seven pairs and a univalent, six pairs and a chain of three, and five pairs

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1. R. T. Ramage, The use of tertiary trisomics to assign genes to chromosomes. Paper presented at the Third Barley Research Conference, Fort Collins, Colorado, February, 1957.

and a chain of five.[2] The extra translocated chromosome is a univalent, one of the end chromosomes of a chain of three, or the middle chromosome of a chain of five.

Balanced tertiary trisomics with properly linked genetic recessive male sterile markers can provide the female parent for commercial production of hybrid seed (Ramage, 1965). The translocated chromosome carries the dominant marker which is male fertile. The trisomic produces all of the pollen for pollination of the male sterile diploid.

According to Ramage (1963) other uses of a balanced tertiary trisomic are: (1) orienting linkage maps, (2) maintaining lines of lethal and sterile genes, and (3) studying the relation of genetic recombination to chromosome disjunction in meiosis. A balanced tertiary trisomic was set up using the translocated chromosome 2_d^7 (Ramage[3]) to obtain information about the third use of a balanced tertiary trisomic mentioned. Ramage concluded that there was a high degree of preferential pairing in this tertiary trisomic and that at least some of the genetic recombination took place before zygotene pairing.

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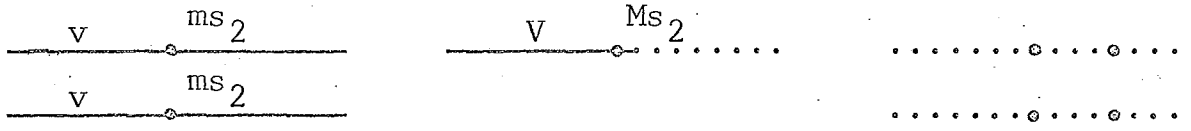
2. R. T. Ramage, Genetic mechanisms for the production of male sterile plants. Paper presented at the Hybrid Barley Conference, Minneapolis, Minnesota, January, 1966.

3. R. T. Ramage, Genetic recombination and chromosome disjunction in a balanced tertiary trisomic. Paper presented at the XI International Congress of Genetics, The Hague, The Netherlands, September, 1963.

MATERIALS AND METHODS

The balanced tertiary trisomic used in this experiment was developed by Ramage[4] with the interchanged chromosome 2_d^7 as the extra chromosome. The break-point is close to the centromere of chromosome 2, and the marker gene Ms_2 (Ms_2 produces male fertility and ms_2 results in male sterility) is tightly linked with the break-point and the centromere. The V locus (V produces two row and v results in six row) is carried on the 2 arm of the interchanged chromosome as illustrated below. In normal stocks,



the V locus shows about 26% recombination with Ms_2.

Self seed of a balanced tertiary trisomic was planted in the field. The types of plants expected with no crossing over were (1) balanced tertiary trisomics with the two row and male fertile alleles on the extra chromosome and the six row and male sterile alleles on the two normal chromosomes, and (2) diploid, six row male steriles. The balanced tertiary trisomic was crossed as male onto the male sterile diploids. Only haploid pollen was expected to

4. R. T. Ramage, Genetic recombination and chromosome disjunction in a balanced tertiary trisomic. Paper presented at the XI International Congress of Genetics, The Hague, The Netherlands, September, 1963.

function because microgametophytes with an extra chromosome do not develop normally. Heads from the male parent plants in the cross were bagged for self seed.

Progeny of thirty crosses and of three selfs were grown in the greenhouse. Phenotypes were recorded at heading. The per cent of genetic recombination was calculated from the frequency of diploids heterozygous for the two row locus in the progeny of the crosses.

Material for cytological study was collected in the field from eight balanced tertiary trisomic plants. The time of collection was from 9:00 to 11:00 A.M. The sporocytes were placed in glassine bags and killed and fixed in a 3:1 solution of 95% alcohol and glacial acetic acid. After 48 hours at room temperature, the sporocytes were stored in the fixative at 5 C. The plants used for cytological study were among those used as male parents in crosses. In addition, sporocytes from one plant were collected from the next generation of self progeny grown in the greenhouse.

Various stages of meiosis were prepared for examination by the acetocarmine squash technique. Counts were made of about 1000 cells at each stage of late diakinesis or early metaphase I, telophase I, prophase II, and quartets of microspores. Every cell was counted, where possible, on each slide. The frequency of the different configurations was determined at late diakinesis or

metaphase I. At the other three stages, the frequency of lagging chromosomes and of micronuclei was determined. The microscope powers for determining the configurations were 430X and 970X.

A chromosome that wasn't included in the nucleus became a micronucleus in the cytoplasm of the cell. It was believed that lagging chromosomes formed micronuclei unless they were included in the nucleus or were reabsorbed by the cytoplasm.

RESULTS AND DISCUSSION

Chiasma terminalization was studied by observing the kinds and frequency of orientation of chromosome configurations at late diakinesis or early metaphase I. The frequency of the different chromosome configurations are given in Table 1.

The ( )-shaped chain of three chromosomes was observed in 52.64% of the classifiable metaphase I cells. This chromosome configuration has alternate disjunction of the chromosomes producing n and n+1 gametes. One of the end chromosomes is the interchanged chromosome, therefore, the n+1 gamete must contain the interchanged chromosome.

The (, , ,)-shaped chains of five were observed in 5.17% of the classifiable metaphase I cells. These chromosome configurations also produce n and n+1 gametes. The middle chromosome of the chain of five is the interchanged chromosome, therefore, the n+1 gamete must contain the interchanged chromosome.

A univalent chromosome was observed in 23.63% of the metaphase I cells. A univalent chromosome in the microspores of a trisomic individual is the interchanged chromosome. The univalent chromosome may move to either pole with equal frequency, so the resulting gametes contain

Table 1. Frequency of chromosome configurations observed at metaphase I in a balanced tertiary trisomic.

Configurations	Number of Cells	Per Cent of Cells
Chain of three		
	548	52.64
	13	1.25
	49	4.71
	111	10.66
	8	0.77
Total	729	70.03
Chain of five		
	26	2.49
	25	2.40
	2	0.19
	3	0.29
	2	0.19
	6	0.58
	1	0.09
	1	0.09
Total	66	6.32
Univalent	246	23.63
Total	1041	
Unclassifiable	407	

n and n+1 chromosomes. The n+1 gametes contain the interchanged chromosome.

The rest of the configurations at metaphase I do not disjoin alternately. Three of the configurations of three chromosomes (J, I, J) disjoin so that two adjacent chromosomes move to the same pole. Since the interchanged chromosome is one of the end chromosomes of a chain of three, this chromosome and the one adjacent to it move to one pole, and the third chromosome moves to the opposite pole. The interchanged chromosome does not go by itself to one pole leaving the two normal chromosomes to move to the opposite pole. If this happened, 16.62% of the self progeny should have been primary trisomics. In similar studies less than 1% were recovered.

Table 2 shows the number and frequency of cells with and without lagging chromosomes or micronuclei. Lagging chromosomes were found in 8.44% of the telophase I cells. Laggards were observed in 3.64% of the prophase II cells and micronuclei were observed in 0.93% of them. Micronuclei were found in only 2.37% of the quartets of microspores. The per cent of laggards decreased from telophase I to the quartet stage. This indicates that lagging chromosomes do not persist throughout all cell divisions to quartet stage. It is possible that the laggards could have been reabsorbed by the cytoplasm or included in the nucleus at a later stage. In barley,

Table 2. Number and frequency of cells with and without lagging chromosomes and micronuclei at telophase I, prophase II, and quartets of microspores in tertiary trisomics of barley.

	Telophase I		Prophase II		Quartets	
	Number	Per Cent	Number	Per Cent	Number	Per Cent
No laggers	1031	91.56	1491	95.33	1113	97.56
Precocious laggers	53	4.71	29	1.85		
Non-precocious laggers	42	3.73	28	1.79		
1 micronuclei			11	0.70	17	1.49
2 micronuclei			5	0.23	9	0.79
2 micronuclei in one spore					1	0.09
Total	1126		1564		1140	
Unclassifiable	42		70		25	

pairing of the chromosomes begins at or near the ends--not at the centromere (Kasha and Burnham, 1965). Basec on the assumption that all chromosome ends pair and that trisomic ends pair at random, the probability of obtaining a univalent is 1/9, a chain of three is 4/9, and a chain of five is 4/9. The actual frequencies observed in this study were: univalent 23.63%, chain of three 70.03%, and chain of five 6.32%. When the expected frequencies are compared with the observed, it is evident that the pairing of the ends of the chromosomes is preferential and not random.

Genetic recombination was measured in the progeny of crosses of the balanced tertiary trisomic as male onto recessive females and in the progeny of selfed balanced tertiary trisomics. The diploid test cross progeny consisted of 6.64% two row (Vv) and 03.36% six row (vv) male sterile plants (Table 3). This means that 6.64% of the pollen grains carried the dominant allele, V. The test cross progeny contained 6.64% genetic recombination. For a pollen grain to carry a V allele, genetic recombination must have occurred in the gene-centromere region between one of the normal chromosomes and the interchanged chromosome.

A trisomic configuration with no genetic recombination taking place between the gene V and the centromere will produce two kinds of pollen grains, v and Vv. A

Table 3. The observed and expected[a] phenotypes of the progeny of selfed balanced tertiary trisomics and phenotypes of the progeny of crosses using a balanced tertiary trisomic as male onto recessive females.

	Self (Observed)		Self (Expected)	Test Cross		
Phenotype	Number	Per Cent	Per Cent	Number	Per Cent	Per Cent 2n
Diploid						
VV msms	4	0.63	0.68			
Vv msms	78	12.18	11.94	73	6.59	6.64
vv msms	335	52.34	52.12	1026	92.59	93.36
Trisomic						
VV- Ms--	11	1.72	3.21			
Vvv Ms--	189	29.53	28.44	5	0.45	
vvv Ms--	23	3.59	3.21	2	0.18	
Vvv msmsms				1	0.09	
	640			1107		

[a]Based on certain assumptions as explained in the text.

trisomic configuration with genetic recombination occurring between the gene V and the centromere will produce four kinds of pollen grains, V, v, Vv, and vv. The gametes with an extra chromosome are not transmitted through the pollen because of slower growth of the pollen tube. Only two kinds of pollen, V and v, were available to fertilize the eggs.

If the chromosome configurations disjoin in the early meiotic stages as mentioned above, then an expected population can be formulated. The expected population was formulated in the following way:

1. The diploid portion of the self population was used to calculate a recombination value for the V to Ms_2 region in the interchanged chromosome. Based on the expansion of the binomial, about 10% recombination was observed. This means that a cross over occurred in the V-Ms_2 region between the interchanged chromosome and one of the normal chromosomes, in about 20% of the spore mother cells.
2. It was assumed that cross over chromosomes went to opposite poles in configurations with alternate disjunction.
3. It was also assumed that no crossing over occurred in the rest of the configurations and that non-cross over chromosomes could move to the same pole.

4. Based on these assumptions, an array of expected gametes was calculated.
5. This array of gametes was adjusted to account for the observed discrimination against gametes carrying an extra chromosome.
6. It was assumed that the female produced n and n+1 gametes, and that the male produced only n gametes.
7. Then, an expected population was formulated from these kinds and frequencies of gametes.

This population was compared to the observed self data in Table 3. The observed data showed a very good fit with the expected. The Chi-Square value was 0.79 (df = 5, P = 97.5).

The ratio of diploids to trisomics in the self progeny is 65:35. The expected ratio would be 50:50, because the egg has a 50:50 chance of receiving or not receiving the extra chromosome. It could be that the n and n+1 gametes are not formed in equal numbers or that all of the gametes are formed in equal numbers, but the n+1 gametes are discriminated against.

About 24% of the cells contained a univalent chromosome. If all of the univalents were lost in cell division, these cells would produce only n gametes. Then the ratio of diploids to trisomics would be 62:38.

The per cent of lagging chromosomes at telophase I was 8.44%. The laggards do not account for the deviation in the expected 50:50 diploid to trisomic ratio, because the ratio obtained from losing only the laggards is 54:46.

If all of the laggards and some of the univalents were lost, this could account for the skewed ratio of diploids to trisomics.

SUMMARY

1. There was an excess of alternate disjunction of the metaphase chromosome configurations.
2. A univalent chromosome occurs in 23.63% of the cells; this could mean preferential pairing of the two normal chromosomes or a dissolving of loose chiasmata.
3. Pairing of the ends of the chromosomes was preferential and not random.
4. There was a tendency away from complex multivalent chromosome configurations.
5. The lagging chromosomes did not account for the deviation in the 50:50 ratio of diploids to trisomics.
6. The lagging chromosomes did not persist throughout all cell divisions to the quartet stage.
7. Crossing over was common since at least 50% of the cells had two chiasma in the chromosomes involved in the trisomic. Genetic recombination was uncommon as seen by only 6.64% recoverable recombination between the gene and the centromere.
8. The frequency of the chromosome configurations presented here were true only for the balanced

tertiary trisomic with the 2^{7}_{d} interchange as the extra chromosome.

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