THE IDENTIFICATION OF ANEUPLOID LINES AND A STUDY OF SOMATIC ASSOCIATION IN *AVENA SATIVA* L.

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JEAN-PIERRE DUBUC

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ABSTRACT

Several aneuploid lines of common oats, *Avena sativa* L., were studied to identify the chromosomal deficiencies in relation to the standard karyotype. Two ditelosomic, four monosomic and two nullisomic lines were identified.

A line ditelosomic for chromosome 20^L and two isolates ditelosomic for chromosome 21^L were identified by cytological and karyotype analyses respectively. Three previously identified lines monosomic for chromosome 7, 10 and 20 were found to be deficient for the same chromosome, namely 20. The gene for normal vs. abaxial curling of the leaves was associated with the short arm while the diploidisation gene was associated with the long arm of chromosome 20. The gene for normal vs. kinky neck was also confirmed to be on 20^L.

One line monosomic for chromosome 6, one for chromosome 10, two for chromosome 21 and two lines nullisomic for chromosome 15 were identified by cytological and karyotype analyses.

The reciprocal translocation present between the variety Sun II and Garry and Rodney does not involve the following chromsomes 4, 6, 12, 14, 15, 18 and 20.

A study of somatic association of the chromosomes in *Avena sativa* was conducted. Root tip cells were examined at mitotic metaphase, and distances between homologous as well as between non-homologous chromosomes were measured and their frequency distributions compared. Non-homologous chromosomes were scattered at random in the cells studied. In contrast, the mean distance between homologous chromosomes was significantly shorter indicating a tendency for somatic association of homologues in this species.

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INTRODUCTION

Avena sativa L. is an allohexaploid species with $2n = 6x = 42$ chromosomes comprising three genomes, namely A, C and D. Due to the polyploid nature of common oats, the deficiency of a whole chromosome or a pair of homologous chromosomes can be tolerated, and it should be possible to produce a monosomic and nullisomic series of 21 different lines. In wheat, also an allohexaploid, a monosomic series was developed by Sears (1939) from the progeny of a haploid plant and a partially asynaptic line. He also established the related nullisomic and ditelosomic series (Sears, 1954).

In wheat, ditelosomic lines have been used to verify monosomic identification, for linkage mapping (Sears, 1963) and in somatic association studies (Feldman *et al.*, 1966). A telocentric chromosome includes a functional centromere plus one complete arm of a normal chromosome. Telocentrics usually arise by misdivision of unpaired chromosomes at meiosis (Morris and Sears, 1967).

A monotelosomic plant has one arm of a chromosome replacing a pair of homologous chromosomes; its meiotic configuration is 20 bivalents plus one telocentric univalent. Ditelosomics arise in progeny of self-pollinated monotelosomic plants. Because of their meiotic stability and fertility, ditelosomics are useful tools to carry out cytogenetic studies.

Thus far, a total of 19 different monosomic lines have been identified in *Avena* by different authors. Spontaneous and induced aneuploids have been the main sources of monosomics studied. Interline crosses have been used to determine the homology of monosomic lines whose univalent chromosomes appeared similar from critical karyotype analysis. No attempts have been made to obtain ditelosomic lines from the monosomic lines already identified though monotelosomics were obtained and used to associate phenotypic changes with specific arms of chromosomes.

Association of homologous chromosomes in somatic cells has been reported in a number of organisms. Somatic association of homologues can be studied in common oats because a number of chromosomes are readily identifiable. Chromosome pair 21 (shortest of the complement) and a pair of telocentrics other than 21 are ideal material for such a study. By comparing the frequency distributions of the distances between homologous and non-homologous chromosomes, any attraction between homologues can be established.

The present study is concerned with three major phases: (1) the identification of aneuploid lines available at this institution with the view to completing the monosomic series; (2) the identification of ditelosomic lines and the association of known markers with specific arms of chromosomes; and (3) an investigation of somatic association of homologous chromosomes in the species.

LITERATURE REVIEW

The establishment of a monosomic series in hexaploid oats was facilitated by the use of a standard karyotype proposed by Rajhathy (1963). Previous studies consisted of association of phenotypic changes with chromosomal deficiencies, each worker using his own system of nomenclature for the chromosomes involved. No relationship existed between the various systems of nomenclature and the results could not be compared. This review, therefore, will only cover the studies made in relation to the standard karyotype and those which can be related to it through recent findings.

Present status of aneuploid investigations.

A total of 19 monosomic lines have been identified according to the standard karyotype. The chromosomes still awaiting identification are 11 and 16. Table 1 shows the aneuploid characteristics specifically resulting from the loss of one chromosome or a pair of homologous chromosomes. The arm of the chromosome responsible for the characteristic has been identified in some cases. The aneuploid characteristics are described as seen on nullisomic plants except for neck kinkyness, side-panicles and white striations on the leaves where the monosomic plants also demonstrate the phenotype due to the dominance of the deletion in the hemizygous condition.

Other phenotypic effects such as low fertility, shorter straw, lack of vigor, smaller panicles, retarded growth, etc., are produced by the loss of a number of chromosomes or chromosome pairs.

Duplication of genetic functions are common in polyploid species. Several chromosomes in hexaploid oats produced similar phenotypic and

Table 1. Identified monosomics and gene-chromosome association.

*Induced aneuploids.

Chromosome	Arm	Origin	Aneuploid characteristics	Authority
1	-	Sun II	Glaucous Complete asynapsis (control early phases of meiotic pairing)	Hacker & Riley, 1965
2	-	Sun II	Asynapsis Abaxial curling of leaves and Kinky neck	Hacker & Riley, 1965
3	short	Victorgrain* (_A. byzantina_)	Sterility (normal meiotic behavior) Dosage effects for dark grey color of lemmas	Singh & Wallace, 1967
4	-	Garry*	Narrow leaves	Sun, 1965, Lin, 1968
	-	Russel x _A. strigosa_	Albino Homoeologous to 15 and 21	McGinnis _et al._, 1968
5	-	-	---	Rajhathy (personal communication)
6	-	Garry*	None	Sun, 1965.
7		Rodney*	Abaxial curling of the leaves	Gauthier, 1967
	long - -	Rodney*	Kinky neck Complete sterility Reduced chiasma frequency in one genome	Gauthier, 1967
	-	Rodney*	Diploidisation gene Frustated panicles	Gauthier, 1967
	-	Victorgrain*	Complete asynapsis Control the pairing of one genome	Singh and Wallace, 1967

continued.....

Chromosome	Arm	Origin	Aneuploid characteristics	Authority
8	-	Garry*	Large necrotic spots on leaves	Gauthier, 1967
9	-	Garry x Rodney	None	Lin, 1968
10	-	Garry*	None	Gauthier, 1967
11	-	-	No reported aneuploids	-
12	short	Garry*	Non-synchrony in anther and stigma maturity	Gauthier, 1967
13	short	Garry*	Inhibitors of fatuoid phenotype	Gauthier, 1967
	-	Garry	Neck kinkyness	Gauthier, 1967
	short	Victorgrain*	Desynapsis in two genomes	Singh & Wallace, 1967
	short	-	Asynapsis	Huskins & Hearne, 1933
	short	Victory	Fatuoid phenotype	Nishiyama, 1933
	long	Garry*	Asynapsis	Gauthier, 1967
	long	Victorgrain*	Fatuoid phenotype	Singh & Wallace, 1967
	long	Victory	Desynapsis	Nishiyama, 1933
	-	Sun II	Fatuoid phenotype Desynapsis	Hacker & Riley, 1965

continued......

Chromosome	Arm	Origin	Aneuploid characteristics	Authority
14	long	Garry*	Abaxial curling of leaves White striations on leaves Fatuoid expression when part of chromosome 19^S is deleted	Sun, 1965
	long	Sun II	A two gene control of chlorophyll synthesis	Hacker, 1966
	-	Rodney*	Abaxial curling of leaves Secondary florets enclosed in primary lemmas Pubescence on primary lemmas Semi-abscission of the spikelet	Gauthier, 1967
		Garry x Victoria	Partial asynapsis Nuda Multiflorous spikelets Elongated rachilla of the first floret	Lin, 1968
15	-	Garry*	None	Sun, 1965
	-	Sun II	Thick culms	Hacker & Riley, 1965
	-	Sun II x Rodney	None	Lin, 1968
	long	White Russian x Exeter (F_3)	Albinism	McGinnis & Andrews, 1962
	-	Garry	Side panicles Thick stems	McGinnis & Lin, 1966

continued......

Chromosome	Arm	Origin	Aneuploid characteristics	Authority
16	-	-	No reported aneuploids	--
17	-	Victorgrain*	Protandry Awns on primary and secondary florets Green floret base	Singh & Wallace, 1967
18	long	Garry*	Kinky neck Reduce spikelet size Compact and upright spikelets	Sun, 1965
19	short	-	Weak inhibitor of fatuoid expression when chromosome 14 is in nullisomic condition	Sun, 1965
	-	-	---	Rajhathy (personal communication)
20	long	Garry	Kinky neck	Gauthier, 1967
	-	Sun II x Rodney	None	Lin, 1968
		Garry x Victoria	None	Lin, 1968
21	-	Rodney*	Albinism	Gauthier, 1967
	-	Garry*	None	Sun, 1965
	-	R.L. 1574 x Ripon	Albinism	McGinnis & Taylor, 1961
	long	$Rodney^5$ x Exeter	Albinism	McGinnis <u>et</u> <u>al</u>., 1963
	-	Sun II	None	Hacker & Riley, 1965
			Homoeologous to 4 and 15	McGinnis <u>et</u> <u>al</u>., 1968

meiotic changes such as albinism, abaxial curling of the leaves, neck kinkyness and control of pairing.

Albinism.

Nullisomy of chromosome 4, 15 and 21 produces albinism. McGinnis et al. (1968) suggested that these chromosomes comprise a homoeologous series carrying the V_3, V_2 and V_1 genes respectively responsible for chlorophyll production.

Abaxial curling of the leaves and neck kinkyness.

Abaxial curling of the leaves and neck kinkyness are two other phenotypic changes associated with the loss of several chromosome pairs. These phenotypic changes are also expressed mildly in monosomics. Abaxial curling of the leaves was associated with chromosome 2 (Hacker and Riley, 1965), 14 (Sun, 1965), 7 (Gauthier, 1967). Neck kinkyness was associated with chromosome 2 (Hacker and Riley, 1965), 18 (Sun, 1965), 7 and 13 (Gauthier, 1967) and 20 (Gauthier and McGinnis, 1965). Gauthier and McGinnis (1965) associated the genetic factor for normal neck-shape on the long arm of chromosome 20.

Synapsis.

Synapsis is influenced by several chromosomes. Nishiyama (1933) located a synaptic factor on the long arm of the "C" chromosome while Huskins and Hearne (1933) located it on the short arm. Gauthier (1967) working with a fatuoid line in the variety Garry tentatively identified the "C" chromosome as 13 and located an asynaptic factor on the long arm. Singh and Wallace (1967) also working with an aneuploid line expressing the fatuoid phenotype in the variety Victorgrain located a desynaptic factor on the short arm of chromosome 12 or 13. They concluded that the nullisomy of chromosome 12 or 13 resulted in desynapsis of two genomes by controlling the later steps in the pairing processes and movements of the chromosomes belonging to the C and D genomes.

Hacker and Riley (1965) working with naturally occurring aneuploids in the variety Sun II identified two monosomic lines for chromosome 1 and 2. Nullisomy of chromosome 1 resulted in complete asynapsis with 40 univalents regularly seen at metaphase I and nullisomy of chromosome 2 resulted in partial asynapsis leading to a mean pairing of 4 bivalents and 32 univalents at metaphase I of meiosis.

Singh and Wallace (1967) reported that nullisomy of chromosome 7 caused asynapsis. Chromosome 7 controls the pairing of all chromosomes but more specifically the 14 chromosomes belonging to the A genome. Gauthier (1967) working with irradiated material from Avena sativa var. Rodney identified two isolates monosomic for chromosome 7. This chromosome also had an influence on the pairing of the chromosomes of the genome it belongs to by reducing the chiasma frequency in 6 bivalents. In a nulli-haploid plant, the absence of chromosome 7 resulted in a high number of multivalents which could be explained by the presence of a diploidisation gene on chromosome 7 (Gauthier and McGinnis, 1968).

Schulenburg (1965), in an aneuploid line for a chromosome in the group 16 - 20, found that nullisomy of the chromosome resulted in partial asynapsis (7^{II} and 26^{I}) at metaphase I of meiosis.

Somatic Association.

At mitotic interphase the nucleus appears as a mass of chromatin without any form of organisation. Yet, at the onset of cell division, the chromatin arranges itself into chromosomes which separate to opposite poles of the cell with great precision. At meiosis, the homologues regularly pair together, form chiasmata and segregate to opposite poles. Such precision is more understandable if the homologues are actually lying close to each other in the interphase nucleus.

Association of homologous chromosomes in somatic cells has been reported in a number of organisms, namely Crepis capillaris (Kitani, 1963), Dipterans (Metz, 1916), Man (Schneiderman and Smith, 1962), maize (Maquire, 1967), and wheat (Feldman et al., 1966).

Feldman et al., (1966) demonstrated that the homologues in wheat tend to lie closer to each other than expected by chance alone by measuring the distances between members of pairs of homologous and between non-homologous chromosomes in root tip cells and comparing the frequency distributions. They also demonstrated that in wheat the centromere was mainly responsible for the attraction by measuring distances between telocentrics of opposite arms of the same chromosome. Feldman (1966) studying the effect of chromosome 5B on meiotic pairing in wheat,found that homologous chromosomes are associated before meiosis begins. This and the somatic association of homologues in root tip cells suggest that somatic association is present throughout the life of the organism. Feldman (1966) also demonstrated that genes, located on homoeologous chromosomes 5A, 5B and 5D, regulate somatic association.

MATERIALS AND METHODS

The aneuploid lines used in the study were obtained from several sources and are outlined in Table 2. Special attention was given to the screening of monotelosomic plants in the progreny of selfed monosomics previously identified. Ditelosomic lines were derived by selfing monotelosomic plants.

For mitotic investigation seeds from aneuploid lines were placed on a moist blotter, cold-treated at 0 - 2°C for 2 weeks and germinated at 27°C for 24 hours. The young root tips were excised, placed in ice-chilled water (0 - 2°C) for 24 hours to accumulate metaphase figures and then fixed in Farmer's fluid (3 ethyl alcohol:1 acetic acid) for at least two days. After fixation the root tips were hydrolysed in 1N HCl for 10 minutes and stained with reduced basic Fuchsin. The stained meristematic tip was squashed between a slide and cover slip in either 1% acetocarmine or 45% acetic acid and the chromosomes counted. In the later steps of the study, acetic acid (45%) was used to improve the spreading of chromosomes at mitotic metaphase, thus facilitating karyotype analysis. Karyotype analysis was made on the best fresh preparations, both by direct examination and by photomicrographs where the chromosomes could be cut out and organized according to the standard karyotype of Rajhathy (1963).

For meiotic studies young panicles, used to study pollen mother cells (PMC), were collected, fixed in Carnoy's fluid (6 ethyl alcohol: 3 chloroform:1 acetic acid) and squashed in 2% acetocarmine. The chromosome number determined from root tip counts was confirmed and in the case of mono and ditelosomic plants their pairing behavior was particularly noted.

Interline crosses were designed to check the homology of monosomic chromosomes. Lines monosomic for the same chromosome will give rise to

Table 2. List of aneuploid lines used in the study.

Line	Chromosome	Variety (X-ray dose)	Authority
Mono VIII	2	Sun II (spontaneous)	Hacker & Riley, 1963
M-3	3	Victorgrain (?)	Singh & Wallace, 1967
R-355	4	Garry (300r)	Sun, 1965
R-524	6	Garry (300r)	Sun, 1965
Rx - 742	7	Rodney (150r)	Gauthier, 1967
ST-7	7	Victorgrain (?)	Singh & Wallace, 1967
GR-27-106	9	Garry x Rodney	Lin, 1968
21-120	10	Garry (spontaneous)	McGinnis, unpublished
S-214	12	Garry (sonic vibrations, 20 min.)	Gauthier, 1967
R-176	14	Garry (300r)	Sun, 1965
18-81	15	Garry (spontaneous)	McGinnis & Lin, 1966
Rx - 40	15	Rodney (75r)	McGinnis, unpublished
ST-17	17	Victorgrain (?)	Singh & Wallace, 1967
R-364	18	Garry (300r)	Sun, 1965
4-27	20	Garry (spontaneous)	Gauthier, 1967
Gx - 26	-	Garry (75r)	Andrews & McGinnis, 1964
Gx - 58	-	Garry (300r)	" " "
Gx - 69	-	Garry (300r)	" " "
Gx - 99	-	Garry (600r)	" " "
Gx - 154	-	Garry (600r)	" " "
Gx - 164	-	Garry (600r)	" " "
Gx - 240	-	Garry (600r)	" " "
Gx - 438	-	Garry (300r)	" " "
Gx - 623	-	Garry (150r)	" " "
R-127	-	Garry (300r)	" " "
R-365	-	Garry (300r)	" " "
Ditelo 3	-	Sun II (spontaneous)	Hacker & Riley, 1963
Ditelo 4/2	-	" "	" " "
Ditelo 21	-	" "	" " "
Ditelo 101	-	" "	" " "
Ditelo 102	-	" "	" " "
Ditelo 105	-	" "	" " "
Mono 8/1	-	" "	" " "
Mono 8/2	-	" "	" " "

20 bivalents in the 40-chromosome F_1 hybrids whereas if the monosomics are different the meiotic configuration will be 19 bivalents plus 2 univalents. In F_1, 40 + t-chromosome hybrids (monosomic x telosomic aneuploid), 20 bivalents plus a telocentric univalent indicate that the telocentric is one arm of the monosome under investigation; a heteromorphic bivalent and a full univalent plus 19 bivalents demonstrate that the telocentric is not homologous to the monosome used as female parent (Figure 1).

Hybrids between different aneuploids were produced by the introduction of yellowish anthers into florets emasculated one to five days prior to hand-pollination. Emasculated florets were bagged to prevent outcrossing. In interline crosses involving monosomics, the male parent was chosen on the basis of percent of nullisomic plants in its progeny. The higher the percent, the greater the chance of having 40-chromosome plants in the progeny of interline crosses. In crossing monosomic by telosomic aneuploids, telosomic plants were used as the male parent. The presence of a telocentric chromosome in the male gamete increases the frequency of the desired type of hybrids (40 + t-chromosome), thus reducing the effort of producing hybrid seeds.

The number of micronuclei per tetrad was scored to calculate the frequency of normal gametes produced. It was assumed that in tetrads where no micronuclei were present four 20 + t-chromosome pollen grains would be produced, where one micronucleus occurred the ratio would be 3 normal to 1 deficient gametes and where two micronuclei were found, two gametes would be deficient, etc.

To determine whether somatic association of homologues was present in common oats, a ditelosomic line (Ditelo 3) was used. The telocentrics

Figure 1.

Identification of ditelosomic lines

Identified monosomic gametes (20 21) x Ditelosomic line (20 + t)

41 + t (rejected)

40 + t (retained)

at meiosis, two (2) pairing patterns are possible:

1) $19^{II} + 1^{II}$ hetero $+ 1^{I}$
(<u>not</u> the monosomic used)

2) $20^{II} + 1^{I}$ telo
(<u>is</u> the monosomic used)