Review

Aneuploids of wheat and chromosomal localization of genes

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Identification of useful major or minor genes is an important step in crop improvement programs. The chromosome location of such genes is critical for effective utilization and subsequent manipulation. Further, chromosomal localization will lead to the identification of genomic regions responsible for the expression of the trait of interest. DNA markers linked to these traits could be identified and used for marker-assisted breeding. Various cytogenetic stocks and techniques have been previously reported useful in localizing genes on wheat chromosomes. The objective of this paper is to assemble the most commonly used cytogenetic methods for the chromosomal localization of major genes in wheat including Chinese spring (CS) monosomics (*Triticum aestivum*, 2n=6x-1=41) and Langdon durum D-genome disomic substitution lines (*Triticum turgidum*, 2n=4x-2+2=28). The paper reviewed and outlined the use and development of monomosic and substitution lines in a suitable genetic background for genetic analysis in wheat. The information may assist wheat researchers to locate and utilize newly identified genes in breeding programs.

Key words: Aneuploids, Chinese spring, chromosome, cytogenetics, Langdon durum, monosomics, substitution lines.

INTRODUCTION

When novel major or minor genes become available, chromosome localization is necessary for effective utilization and subsequent manipulation in plant breeding programs. Further, chromosome locations help to elucidate the relationship of genes and verify whether the candidate gene is the same or different from previously reported genes. Information about chromosome localization of genes will enable researchers to identify genomic regions responsible for the trait of interest and hence, facilitates the development of molecular markers in marker assisted breeding.

Unlike other common crop species, allopolyploidization

has played a significant role in the evolution of durum wheat (*Triticum turgidum*) or common wheat (*Triticum aestivum*) (Sears, 1952). This genus contains diploids (2n=2x=14) with genomes designated as AA or BB or DD or tetraploids (2n=4x=28; AABB) or hexaploids (2n=6x=42, AABBDD). Reportedly, the progenitors of the A genome is *Triticum monococcum,* the B genome is from a yet unknown species possibly of the genus *Aegilops* and the D genome *Aegilops tauschii* (*Aegilops squarrosa*) (Griffiths et al., 2000). The tetraploid durum wheat (*T. turgidum,* 2n=4x=28, AABB) and the hexaploid common or bread wheat (*T. aestivum,* 2n=6x=42, AABBDD) are cultivated in various regions of the world (Ekboir, 2002).

Bread and durum wheats have seven homoeologous groups of chromosomes. In both species, each chromosome in one genome should be related and homoeologous to a similar chromosome in the other genome(s). Homoeologous chromosomes have similar gene content and can replace each other in suitable combinations (Sears 1952, 1966). During meiosis of durum and bread

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Abbreviations: CS, Chinese spring; **SSRs,** single sequence repeats; **SNPs,** single nucleotide polymorphisms; **LDN,** Langdon.

wheat 14 and 21 chromosome bivalents are formed, respectively. It has been established that any given chromosome has only one specific pairing partner (homologous pairing). In the species *T. turgidum* and *T. aestivum* homoeologous pairing is suppressed by the *Ph1* gene located on the long arm of chromosome 5 of the B genome (Okamoto, 1957; Kimber and Sears, 1987; Gill and Gill, 1991; Feldman, 1993; Dubcovsky et al., 1995). Thus, the *Ph1* gene confers a diploid-like meiotic behavior for these polyploid species.

Various cytogenetic stocks and techniques are available to localize genes on wheat chromosomes. Among the most commonly used cytogenetic stocks are the Chinese spring (CS) monosomics (*T. aestivum*, 2n=6x-1=41) (Sears, 1952, 1954; McIntosh et al., 1998) and Langdon durum (*T. turgidum*, 2n=4x-2+2=28) D-genome disomic substitution lines (Konzak and Joppa, 1988; Joppa and Cantrell, 1990). Chinese spring and other synthetic hexaploid wheat monosomics help to localize genes in both hexaploid and tetraploid wheat germplasm (Sears, 1954; Knott, 1989; Iwaki et al., 2001; Raupp et al., 2001; Singh et al., 2001). The tetraploid aneuploids, Langdon durum D-genome disomic sub-stitution lines, are used to localize genes only in tetraploid wheats (Konzak and Joppa, 1988; Joppa and Cantrell, 1990; Cantrell and Joppa, 1991; Tsunewaki, 1992; Cai et al., 1999; Shimelis et al., 2005).

Detailed and elaborated procedures are limited for chromosomal location and development of potential cytogenetic stocks in desirable genetic background for genetic analysis in wheat. Thus, the objective of this review is to describe the most commonly used cyto-genetic methods in the chromosomal locations of major genes in durum or bread wheat including Chinese spring (CS) monosomics (*T. aestivum*, 2n=6x-1=41) and Langdon durum D-genome disomic substitution aneu-ploids (*T. turgidum*, 2n=4x-2x+2x=28). This paper outlines the detailed procedures on how to use and develop monomosic and substitution lines in a desired genetic background. The information may assist wheat researchers to locate newly identified genes and manipulate these in breeding programs.

ANEUPLOIDS IN WHEAT

Euploid individuals carry the normal chromosome numbers of the species. However, aneuploids show deficiency or excess for a single or even multiple chromosomes. Aneuploids have an important place in genetic research and various breeding programs. In wheat, aneuploids are employed to localize gene(s), transfer specific chromosome(s) from one cultivar or line to another, determine the crossover frequency between a gene and the centromere, study the effect of multiple copies of a gene, assign chromosomes in their respective linkage groups and assess phenotypic effects of

individual chromosomes and other genetic studies (Sears, 1954; Allan and Vogel, 1960; Kuspira and Millis, 1967; Knott, 1989; Marais and du Toit, 1993; Raupp et al., 1993; 2001; Schroeder et al., 1994; Plaschke et al., 1995; Iwaki et al., 2001; Singh et al., 2001, Zeller et al., 2002). However, these individuals are generally less vigorous and less fertile than their euploid counterparts (Joppa and Williams, 1977; Knott, 1989).

Various molecular markers can be applied to locate quantitative trait loci (QTLs) in crop plants. These include restricted fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), micro-satellites or single sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) (Röder et al. 1998; Edwards et al., 2009). In wheat, except SSRs and SNPs, other molecular markers have shown limited numbers of DNA polymorphisms (Plaschke et al. 1995; Smulders et al. 1997; Röder et al. 1998; Edwards et al., 2009). Thus, anepuploid analyses in conjunction with high-throughput and polymorphic DNA markers help to map useful genes in wheat.

Sears (1954) studied and produced the complete sets of aneuploids in the hexaploid common wheat cultivar, Chinese spring (CS). These aneuploids include 21 monosomics (2x-1) which are fertile and stable, 21 nullisomics (2x-2) with low fertility and vigor, 21 trisomics (2x+1) reasonably fertile and stable and 21 tetrasomics (2x+2) that are fertile and stable. The development of 21 monosomic series of CS has provided a tool for considerably circumventing the difficulties of genetic analysis in wheat due to polyploidy. These aneuploids have proved immensely useful in elucidating the cyto-genetic architecture of bread and durum wheats. Sears's aneuploids in CS arose as the progeny of either haploid plants or nullisomic 3B plants (Knott, 1989). Other hexaploid monosomic wheat than developed by Sears (1954) are being used for genetic analysis (McIntosch et al., 1998; Cai et al., 1999; Iwaki et al., 2001, Singh et al., 2001; Tsujimoto, 2001).

As the result of its susceptibility for the naturally occurring wheat rust pathotypes, CS has been widely used for chromosome location of resistance genes in wheat. This has been done from crosses of a resistant parent with sets of CS aneuploids followed by inoculation and analyses of segregating individuals (Sears et al., 1957).

Aneuploids of durum wheat are available for various genetic studies in tetraploid wheats (Joppa and Williams, 1977; Joppa et al., 1987; Joppa and Cantrell, 1990; Joppa, 1993). These aneuploids include monosomics (4x-1=27), D-genome substitution monosomics (4x-1+1=28), monotelodisomics (2x=27+t), ditelomonotelo-somics (2x=27+2t+t), double ditelosomics (2x=27+2t+2t) and D-genome disomic substitutions (4x-2+2=28). Other tetraploid anuploids are being developed and used in genetic analysis of durum wheat (Klindworth et al., 2007).

		Paternal gametes	
		n	n-1
Maternal gametes	n	2n	2n-1
	n-1	2n-1	2n-2

Figure 1. Scheme showing the theoretical progenies of selfed monosomic plants

MONOSOMIC ANALYSIS TO IDENTIFY CHROMOSOMES CARRYING MAJOR GENES IN WHEAT

Monosomics in hexaploid wheat

Among the various aneuploids, monosomics have been used extensively to identify chromosomes carrying candidate genes in wheat and to map these relative to the centromere (Sears, 1954; Knott, 1989; McIntosh et al., 1998; Raupp et al., 2001; Iwaki et al., 2001; Singh et al., 2001). Monosomics are special wheat aneuploids which accommodates individual chromosomes when crossed as maternal parents to other desired paternal lines that may contain a mutant gene, for example, the gene for disease resistance.

During meiosis, the monosomic chromosome lacks a homolog to pair thus, it often fails to move normally to a pole at Meiosis I or II. Consequently, in about 50% of the cells, this chromosome is not included in a nucleus and appears as a micronucleus in the pollen tetrad (Sears, 1954; Knott, 1989). Theoretically, monosomics produce two kinds of gametes during meiosis that is, n (with 21 chromosomes) and n-1 (with 20 chromosomes). Selfing of monosomic plants will lead to the production of disomics (2n), monosomics (2n-1) and nullisomic (2n-2) progenies as indicated in the scheme (Figure 1).

From the scheme, it can be said that there is a 50% chance of recovery of monosomics (2n-1), 25% disomics (2n) and 25% nullisomics (2n-2) after selfing. Nullisomics are recognized by their lack of vigour and narrow leaves. Most nullisomics are almost completely male sterile. However, the Chinese spring nullisomics series 1A, 1D, 3A, 3D, 6A, 6B and 7D are the most fertile and can be maintained and used in crosses (Law and Worland, 1987).

Procedures on chromosome localization of genes through monosomic analysis

The Chinese spring (CS) monosomic lines can be used to localize genes on both tetraploid and hexaploid wheat. A typical procedure of monosomic analysis for both wheat species is summarized in Table 1. The method has been modified from Sears (1954).

Monosomic analysis should be preceded by genetic analysis to determine the pattern of inheritance and number of gene(s) eliciting the mutant phenotype. The analysis will be clear and more efficient if inheritance is governed by one or two major genes. The number of genes involved and the type of gene action for the trait of interest dictates the monosomic analysis (Table 2). In the case of dominant monogenic resistance, the non-critical cross of the F_2 segregants yields 3:1 phenotypic ratio or a proportion less than this. The critical cross, however, gives a higher proportion of phenotypes showing the trait of interest as indicated in Table 2.

Development of monosomic lines in the genetic background of other hexaploid wheat

Monosomic series could be produced should a genetic background other than Chinese spring (CS) be required. The procedure is as outlined in Table 3. Potential problems in producing a new monosomic series include the random occurrence of univalent/monosomic shift while backcrossing and reciprocal translocation in the line that would result to different levels of chromosome configuration (Sears, 1952; Person, 1956; Knott, 1989). This requires continued selection through karyotyping using comparative controls.

SUBSTITUTION MONOSOMIC ANALYSIS TO IDENTIFY CHROMOSOMES CARRYING MAJOR GENES IN TETRAPLOID WHEATS

Monosomics in tetraploid wheat

The first attempt to develop a set of monosomics in durum wheat (*T. turgidum*) was made by Mochizuki (1968, 1970). The monosomics lacked vigour, had low seed set and averaged only 27% transmission of the monosomic condition when compared with CS. This is because of the inability of the species to tolerate the loss of one or more chromosomes or part of a chromosome compared with hexaploid wheat. Joppa et al. (1987) have developed, characterized and discussed the uses of different aneuploid stocks of the durum cultivar Langdon in genetic analysis of durum wheat. These stocks include: double-ditelosomics, dimonotelosmics, D-genome substitution-monosomics, D-genome disomic substitutions, intercultivar chromosome substitution lines and homozygous recombinant lines. These aneuploids were more vigorous and fertile than the monosomics described by Mochizuki (1968), because of the compensation of the D-genome chromosomes (Joppa and Williams, 1977; Salazar and Joppa, 1981).

Table 1. Procedures and activities during monosomic analyses of bread or durum wheat.

Procedure	Action
1	Cross sets of CS monosomic lines (1A, 2A, 3A, 4A, 5A, 6A, 7A, 1B, 2B, 3B, 4B, 5B, 6B, 7B, 1D, 2D, 3D, 4D, 5D, 6D, and 7D) as females with the mutant parent that contains the gene(s) under investigation.
2	The chromosome numbers of the F_1 progenies are analyzed from pollen mother cells (PMC) during meiosis or from root tips during mitosis.
	If cytogenetic analysis of PMC is to be carried out spikes are collected from F_1 plants when the peduncles lengths are 1 cm. Spikes are fixed in Carnoy's fluid (6 parts 95% ethanol: 3 parts chloroform: 1 part acetic acid). After 48 h at 24°C, heads have to be transferred to 70% ethanol and stored at 2 to 4°C until cytogenetic examination. If analysis is required from mitotic chromosomes, seeds should be sampled and surface sterilized in 70% Ethanol for 1 min and 30% household bleach (JIK®, South Africa) for 5 min followed by rinsing three times in distilled water. Transfer 10 seeds per petri dish and germinate on moist filter paper for 2 to 3 days in a germination chamber at 20-21°C. Petri dishes could be sheathed using clean plastic bags to retain the moisture content of the filter paper. When 1-1.5 cm long root tips should be harvested and pre-treated in cold distilled water for 18 hr in icebox kept in a refrigerator. Fix root tips in 45% acetic acid for 3 h at 4°C and store in 70% Ethanol until analyzed. Chromosomes can be analyzed by observing under phase contrast microscope. Slides should be prepared according to an established method (Belling, 1921).
3	The F_1 progenies with monosomic chromosomes are advanced to F_2 for further tests and/or segregation analysis.
4	At the F_2 generation, the critical and non-critical crosses should be decided and the chromosome location of the gene(s) declared from a chi-square goodness of fit procedure on the proportion of segregants.
	The F_2 progenies of F_1 monosomic plants are classified according to the expected segregation ratio. If the phenotypic ratio at the F_2 is the same as the expected ratio or if the proportion of the desired phenotypic group is less than this proportion this cross will be regarded as non-critical cross, otherwise it is critical. All the F_2 progenies of F_1 plants in the critical cross have the dominant phenotype greater than the expected ratio (Table 2).

Table 2. Number of genes conditioning inheritance for a trait and F_2 phenotypic segregation ratios of non-critical and critical crosses.

Number of genes	F_2 segregation ratio	
	Non-critical crosses	Critical crosses
One dominant gene	≤3 : 1	>3
Two independent dominant genes	≤15:1	>15
Two dominant complementary genes	≤9:7	>9
One dominant and one recessive gene	≤13:3	>13
Three dominant independent genes	≤63:1	>63

Furthermore, univalent shift was less of a problem in these lines than in the hexaploid monosomics. Theoretically, the A- and B-genomes of CS monosomics developed by Sears (1954) may be used to determine the chromosomal location of genes in tetraploid wheat. However, the use of a set of tetraploid wheat aneuploids was considered more efficient and eliminated the confounding effect from the D-genome chromosomes (Joppa et al., 1987).

The Langdon substitution monosomics have been used to determine the chromosomal location of genes in Langdon durum (Salazar and Joppa, 1981). The limitations of the substitution monosomics for use in genetic analysis include lower rate of transmission (28%) than hexaploid common wheat monosomics, the necessity for careful cytogenetic analysis to prevent translocations between the A and D or between B and D homoeologous chromosomes, the existence of considerable morphological variation

Table 3. Development of monosomic lines in hexaploid wheat in different genetic background from cultivar Chinese spring.

Procedure	Action
1	Cross the 21 Chinese Spring monosomics (1A, 2A, 3A, 4A, 5A, 6A, 7A, 1B, 2B, 3B, 4B, 5B, 6B, 7B, 1D, 2D, 3D, 4D, 5D, 6D, and 7D) as females with the cultivars of interest as males.
2	Select only monosomic plants through chromosome counts and backcross up to five generations using the desired cultivar as a recurrent parent.
3	Check the presence of genes of the recurrent lines by selfing these monosomic plants to produce disomic lines and comparing the lines with the recurrent parent.

Table 4. Procedures in substitution monosomic analysis of durum wheat.

Procedure	Action
1	Cross a durum line (carrying a dominant homozygous gene) with each of the substitution monosomics.
2	Plant out F_1 seeds and self by covering each spike with a glassine bag. Select plants with $13_{II} + 2_I$ and 14_{II} from pollen mother cells (PMCs).
3	Analyze the F_2 progenies. The F_2 progenies of F_1 plants with chromosomal configurations of $13_{II} + 2_I$ or 14_{II} are tested or checked for segregation. Progenies of F_1 substitution monosomic plants of non-critical crosses should segregate as the expected phenotypic segregation ratio or if the proportion of the desired phenotypic group is less than this proportion this cross will be regarded as non-critical cross. All F_2 progeny of F_2 substitution-monosomics in the critical cross should have more of the dominant phenotype and deviates significantly from the expected ratio.

among and within the different substitution monosomics and reduced fertility of selfed substitution monosomic lines. However, the increased vigour, transmission and fertility of durum substitution monosomics, when compared with durum monosomics, make them the method of choice in durum wheat chromosome analysis (Watanabe et al, 1996).

Procedures on chromosome localization of genes in tetraploid wheat through substitution monosomic analysis

The steps involved in substitution monosomic analysis of durum are summarized in Table 4. The methods described by Sears (1954) on monosomic analysis of hexaploid wheat parallels the substitution monosomic analysis in durum wheat (Table 1).

Developing substitution monosomics

The procedures employed in the production of substitution monosomic in teraploid wheat other than Langdon (LDN) durum is summarized in Table 5. Joppa and Williams (1977) have outlined the procedures of producing the substitution monosomics in the Langdon durum wheat (*T. turgidum* var. *durum*).

Use of Langdon durum D-genome disomic substitutions for chromosome location of major genes

In order to reduce the amount of cytogenetic screening required in maintaining the D-genome substitution monosomics, Joppa and Williams (1988) developed D-genome disomic substitutions among the progenies of D-genome substitution monosomics (Table 4). These segregants were nullisomic for a pair of durum chromosomes and disomic for a pair of homoeologous D-genome chromosomes. In these aneuploids, the D-genome chromosome substituted for homologous A- or B-genome chromosomes. For example the 1D (1A) line was disomic for chromosome 1D from CS and nullisomic for a pair of Langdon durum 1A chromosomes. A complete set of D-genome disomic substitutions aneuploids include; 1D(1A), 1D(1B), 2D(2A), 2D(2B), 3D(3A), 3D(3B), 4D(4A), 4D(4B), 5D(5A), 5D(5B), 6D(6A), 6D(6B), 7D(7A) and 7D(7B). In each of these different homologues of the 14 A- and B-genome chromosomes of durum wheat were replaced by their respective D-genome homoeologues (Joppa and Williams, 1988). The procedure for chromosomal location of genes follows the same steps as described in Table 4.

The use of the LDN D-genome disomic substitutions to determine the chromosomal location of a mutant gene depends on the identification of an F_2 progeny having an aberrant segregation ratio when compared with the segregation in crosses with the other 13 disomic

Table 5. Development of substitution monosomics in tertaploid wheat other than Langdon durum.

Procedure	Action
1	Cross the CS aneuploids (nullisomic for A- or B-genome and tetrasomic for a homologous D-genome chromosome) as females with desired durum parent.
2	Grow the F_1 plants individually.
3	Determine the chromosome number and pairing relationships in PMC of each plant.
4	Bag plants that give 14 bivalents plus seven univalents ($14_{II} + 7_I$) to provide selfed seeds. NB. These F_1 plants are monosomic for one A- or B-genome chromosome, monosomic for six D-genome chromosomes and disomic for one D-genome chromosome.
5	Germinate the F_2 seeds in petri dishes, sample the root tips, and count the chromosome number.
6	Plants with 28 to 32 chromosomes should be grown individually.
7	Determine chromosome pairing in these F_2 plants. Plants with 14 bivalents or 14 bivalents plus one to four univalents are bagged to get selfed seeds. Other plants should be discarded.
8	Germinate F_3 seeds in a petri dish and sample root tips. Plants with 28 chromosomes are grown in pots. Chromosome pairing in PMCs is determined and plants with 14 bivalents are backcrossed to the desired durum parent. Continue selection and backcrosses from the BC_2 to BC_5 generations.

substitutions and a control cross (Joppa and Williams, 1988). If only one gene is segregating, the critical cross should have an excess of the mutant phenotype in the F_2, because the F_1 plant receives only the chromosome with the mutant allele. For example, if the gene was on chromosome 7B, the cross between the disomic substitution 7D (7B) and the plant or line with a mutant phenotype would produce an F_1 plants monosomic for both chromosomes 7B and 7D. The 7B chromosome would come from the mutant plant and the 7D chromosome from the LDN aneuploid. The double monosomics would produce gametes (either male or female) with both monosomic chromosomes, one of them or none.

The Langdon (LDN) D-genome disomic substitution lines have been used to determine the chromosomal location of genes controlling different traits in tetraploid wheat (Konzak and Joppa, 1988; Joppa and Cantrell, 1990; Cantrell and Joppa, 1991; Tsunewaki, 1992; Cai et al., 1999; Shimelis et al., 2005). Konzak and Joppa (1988) have analyzed the chromosomal location of a chocolate-chaff gene (designated *cc*) in durum wheat using this analysis and assigned it to chromosome 7B. Cantrell and Joppa (1991) localized quantitative traits such as grain yield and agronomic traits in wild emmer (*T. turgidum* var. *dicoccoides* L.) using the substitution lines. The authors localized genes controlling grain yield on chromosomes 4A and 4B of *dicoccoides*. Chromosome 6B of this variety was found to have an effect of increasing grain protein content. Cai et al. (1999)

employed both the D-genome chromosome substitution lines of Langdon durum and monosomic lines of common wheat, Abbondanza and localized the recessive cross ability alleles in tetraploid wheat cv. Ailanmai on chromosomes 1A, 6A and 7A. Shimelis et al. (2005) used the Langdon durum D-genome disomic substitution lines and determined the chromosome locations of adult-plant leaf rust resistant genes in tetraploid wheat accessions. Accordingly, the substitution analysis involving accession 104 showed the gene for leaf rust resistance on chromosome 6B. The analysis with accession no. 127 indicated that, chromosome 4A carries a gene for leaf rust resistance.

In summary, the aneuploids of wheat are useful cytogenetic stocks in chromosome locations of major genes in both tetraploid and hexaploid wheat, among other applications. As described, the analyses require proper choice of genetic stocks and careful cytogenetic examinations. The paper highlighted the applications and analytical procedures as well as development of cytogenetic stocks for genetic analysis of candidate genes in both tetraploid and hexaploid wheat. The information may assist wheat researchers to locate and manipulate novel genes in breeding programs.

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