A comparison of monosomic and disomic substitution lines in the chromosomal location of leaf rust resistance genes in tetraploid wheats

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A comparison of monosomic and disomic substitution lines in the chromosomal location of leaf rust resistance genes in tetraploid wheats

BY

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DEDICATION

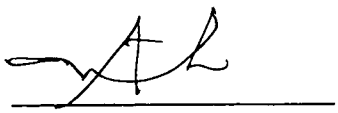
This work is dedicated to:

My son, *Amanuel Shimelis*

DECLARATION

I hereby declare that the dissertation submitted by me for the degree *Philosophiae Doctor* at the University of the Free State is my own independent work and has not previously in its entirety or in part been submitted to any other university. All sources of materials used for the study have been duly acknowledged. I furthermore cede copyright of the dissertation in favor of the University of the Free State.

Signed on the 12th of May 2003 at the University of the Free State, Bloemfontein, South Africa.



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FOREWORD

The study employed and compared two sets of wheat aneuploids (Chinese Spring monosomics and Langdon durum D-genome disomic substitution lines) for the mapping of leaf rust resistance genes of tetraploid wheats. The leaf rust resistance genes have recently been identified in two tetraploid wheat lines that were selected from 353 *Triticum* accessions of different ploidy levels. The substitution lines were further investigated and information collected on genetic variation for important agronomic traits and associations of yield and yield-related traits.

The manuscript is divided into seven separate chapters. The chapters are organized as different investigations, resulting in some inescapable duplication. Chapter 1 introduces the overall study followed by Chapter 2 that reviews and documents literature related to this study. Chapter 3 and 4 are dedicated to chromosomal localization studies of the resistance genes using Chinese Spring A- and B-genome monosomics and Langdon durum D-genome disomic substitutions, respectively. Chapter 5 investigates genetic variation and path coefficient analysis of yield and yield-related traits of Langdon durum D-genome disomic substitution lines. The manuscript discusses and summarizes the major findings of the studies in Chapters 6 and 7, respectively, and terminates with appendices.

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ABBREVIATIONS

%	Percentage
χ^2	Chi-square
°C	Degree Celsius
APR	Adult plant resistance
BC	Back cross
CIMMYT	International Maize and Wheat Improvement Center
CS	Chinese Spring
CSMs	Chinese Spring monosomics
d.p.i.	days post inoculation
e.g.	exempli gratia (for example)
et al.	et alii (and others)
f. sp.	forma specialis
Fig.	Figure
F_1	First-generation hybrid
F_2	Second-generation hybrid
H	hour
g	gram
ha	hectare
h.p.i	hour(s) post inoculation
HR	Hypersensitive resistance
i.e.	id est (that is)
IT	Infection type
l	liter
LDN	Langdon durum
Lr	Leaf rust resistant gene
MI	Meiotic division of the first metaphase
ml	milliliter
MR	Moderately resistant
MS	Moderately susceptible
n	chromosome number in the gametes
PAR	Photosynthetically active radiation
PMC	Pollen mother cell

PR	Partial resistance
R	Resistant
S	Susceptible
subsp.	subspecies
TI	Meiotic division of the first telophase
USDA/ARS	United States Department of Agriculture/ Agricultural Research Service
var.	variety
x	chromosome number in the basic set

Chapter 1

1. Introduction

Wheat is one of the major grain crops of the world. Along with other cereal grains it provides about 63% of the calories and 50% of the protein consumed by humans worldwide (Harlan, 1981). It is projected that by 2020 the demand for wheat will exceed the current production of 552 million tons by 40% (Rosegrant *et al.*, 1997). About 95% of the world wheat production comes from bread wheat (*Triticum aestivum* L., AABBDD, 2n=6x=42). Durum wheat (*T. turgidum* L., AABB, 2n=4x=28) production averages over 30 million tons accounting for less than 5% of the total world wheat production. About 75% of the wheat produced is consumed directly, 15% is consumed indirectly in the form of animal products, and another 10% is for seed and industrial use (Ekboir, 2002).

Wheat frequently suffers from yellow (stripe) rust caused by *Puccinia striiformis* West. f. sp. *tritici*, stem rust (*P. graminis* Pers. f. sp. *tritici* Eriks. and Henn) and leaf rust [*P. triticina* Eriks. [Anikster *et al.*, 1997] {=*P. recondita* Rob. ex Desm. f. sp. *tritici* (Eriks. and Henn) D.M. Henderson}] (Samborski, 1984; Schafer, 1987; Knott, 1989; Das *et al.*, 1992). Yield losses due to rusts are variable because of differences in weather conditions, cultivar susceptibility and availability of inoculum. However, grain losses have been significant and estimated to reach 70% or higher in susceptible varieties (Knott, 1989; Das *et al.*, 1992).

Leaf rust is one of the most serious diseases of wheat worldwide. Because of co-evolution with wheat, various pathotypes are found in different epidemiological zones of the world (Knott, 1989). Yield losses incurred by leaf rust depend on the prevailing environmental conditions and the stage of crop development at the onset of the infection. Susceptible wheat cultivars may show a yield reduction of 5-15% or greater (Kolmer, 1996).

To combat leaf rust, cultural control methods, application of chemicals and use of resistant varieties are employed. The use of resistant varieties developed by resistance breeding programs is the cheapest, most effective and most

environmentally friendly method (Nelson, 1978; Samborski, 1984; Knott, 1989; Messmer *et al.*, 2000; Raupp *et al.*, 2001). Breeding for leaf rust resistance can be achieved via pyramiding major leaf rust resistance (*Lr*) genes that confer complete resistance, accumulating minor *Lr* genes that confer quantitative resistance, or a combination of these approaches. Quantitative resistance, which is often called partial or slow rusting resistance, is more durable. This type of resistance cannot stop the infection completely but delays the spread of the disease. Wheats that show slow rusting have a longer latent period, fewer uredia, and smaller uredinium size than susceptible lines (Kolmer, 1996). *Lr34* (Kolmer, 1996) and *Lr46* (Singh *et al.*, 1998) are examples of slow-rusting genes.

Earlier developed varieties with race-specific *Lr* genes have mostly become susceptible because of the development of new and virulent pathotypes (Samborski, 1982; Statler *et al.*, 1982; Pretorius, 1988; Hussien *et al.*, 1997). Consequently, breeders are constantly developing new lines possessing additional and/or new *Lr* genes to complement the yield potential of their cultivars (Sayre *et al.*, 1998). To date the genetic potential of wheat has been broadened by introgressing useful genes from wild relatives. These include genes that confer different levels of disease resistance (Jiang *et al.*, 1994; McIntosh *et al.*, 1995a). Thus far, 50 *Lr* genes have been catalogued (McIntosh *et al.*, 1998, 1999, 2000, 2002). The search for new sources of resistance is ongoing and breeders in resistance-breeding programs have been constantly selecting for new sources of useful genetic diversity to breed for horizontal resistance that would lead to durability (Johnson, 1981; Knott, 1989; Wolfe, 1993). This is especially important for leaf rust of wheat where durable resistance is based on *Lr* gene combinations and the *Lr34* gene complex (Roelfs, 1988; McIntosh *et al.*, 1995a; Braun *et al.*, 1996; Bender *et al.*, 2000). Accumulating large numbers of resistance genes in a cultivar means more mutations or recombinations are required for the pathogen to overcome resistance (Schafer and Roelfs, 1985). Moreover, accurate identification and utilization of germplasm will aid future conservation of genetic resources as well as exploiting the gene pool to its fullest capability.

Wild relatives of cultivated wheat with which they share homologous chromosome sets, are invaluable sources or reservoirs of genetic attributes including new resistance genes. These materials can be exploited in the improvement of cultivated wheat (Sharma and Gill, 1983; Gill *et al.*, 1986; Knott, 1987, 1989; Cox *et al.*, 1992, 1993; Jiang *et al.*, 1994; Friebe *et al.*, 1996, 1997; Barnard, 1999; Dhaliwal *et al.*, 2002). Successful transfer of genes from these materials, notably from tetraploid to hexaploid wheats, has been described by McIntosh *et al.* (1967), McIntosh and Dyck (1975), Gupta *et al.* (1991) and Dyck (1994). Limitations and altered expression of the genes due to the difference in ploidy level between the two wheat species were also reported by Kerber (1983) and Dyck (1987).

In an effort to select resistant wheat germplasm, the University of the Free State has identified leaf rust resistant lines among 353 *Triticum* accessions (Barnard, 1999). Two accessions, considered excellent sources of adult plant leaf rust resistance, were 104 (*Triticum turgidum* subsp. *dicoccum* var. *arras*) and 127 (*T. turgidum* subsp. *durum* var. *aestivum*).

When a new gene for resistance becomes available, its chromosome location helps to elucidate relationships to other resistance genes. In this regard it is important to determine whether the new gene is allelic to previously reported genes. Besides, chromosomal localization is the first useful step that helps the search of genomic regions responsible for the expression of resistance and hence facilitates the development of molecular markers as a means of marker assisted breeding. To locate genes on chromosomes, different techniques can be employed such as cytogenetic methods using aneuploid stocks and molecular techniques (RFLPs, RAPDs, AFLPs and SSRs). Various cytogenetic stocks are available to localize genes on wheat chromosomes. Among these are the Chinese Spring (CS) monosomics (*Triticum aestivum*, 2n=6x-1=41) and Langdon durum D-genome disomic substitution lines (*T. turgidum*, 2n=4x-2+2=28).

Chinese Spring and other hexaploid wheat monosomics can be used to localize genes in hexaploid (Sears, 1954; McIntosh 1983; Knott, 1989; Marais and du

Toit, 1993; Raupp *et al.*, 1993, 2001; Schroeder *et al.*, 1994; Iwaki *et al.*, 2001; Singh *et al.*, 2001; Zeller *et al.*, 2002) and tetraploid (Allan and Vogel, 1960; Kuspira and Millis, 1967; Bozzini and Giorgi, 1971; Mokhtarzadeh, 1975; Giorgi, 1979; Hanchinal and Goud, 1982) wheat germplasm. The tetraploid, Langdon durum D-genome disomic substitution lines, can be used to localize genes in tetraploid wheats only (Konzak and Joppa, 1988; Joppa and Cantrell, 1990; Cantrell and Joppa, 1991; Tsunewaki, 1992; Cai *et al.*, 1999). Cai *et al.* (1999) employed both the D-genome chromosome substitution lines of Langdon durum and monosomic lines of the common wheat, cultivar Abbondanza. These workers subsequently localized three recessive crossability alleles in tetraploid wheat cultivar Ailanmai on chromosomes 1, 6, and 7 of the A-genome. No comparison of the two methods of locating genes in tetraploid wheats could be found. Salazar and Joppa (1981) reported that considerable morphological variation exists among and within the substitution lines that could be a disadvantage in using them for genetic analysis. However, there is limited information from different environmental situations to validate this conclusion. Therefore, this study was initiated with the following objectives:

- To identify the chromosomal location of genes in two tetraploid wheat lines with adult plant leaf rust resistance, using cytogenetic stocks of CS monosomics and Langdon durum D-genome disomic substitution lines.
- To compare the results and determine which method of analysis works best for localizing genes in tetraploid wheat.
- To study genetic variation for important agronomic traits among the Langdon D-genome disomic substitution lines and the recurrent parent, *T. turgidum* cultivar Langdon.
- To test associations of yield and yield-related traits among Langdon durum D-genome disomic substitution lines through path coefficient analyses.

Chapter 2

2. Literature review

2.1 Wheat

Wheat refers to the cultivated species of the genus *Triticum* (Miller, 1987; Knott, 1989). This genus contains different ploidy levels that include diploids (2n=2x=14), tetraploids (2n=4x=28), and hexaploids (2n=6x=42).

Tetraploid durum wheat (*Triticum turgidum* var. *durum*) and hexaploid common or bread wheat (*T. aestivum* var. *aestium*) are cultivated in various regions of the world (Fig. 2.1). Durum wheat is grown on approximately 8% of the total area devoted to wheat production. It, however, occupies a relatively larger share of the wheat production area in the Middle East, Central India, and the Mediterranean region of West Asia and North Africa. Other production areas include Ethiopia, Argentina, Chile, Russia, Kazakhstan, Mexico, the United States, Italy, Spain, and Canada (Fig. 2.1). Durum wheat is widely used in the production of pasta products such as spaghetti, macaroni, flat or corrugated sheets in lasagna and noodles, and other pasta shapes developed from extrusion of the dough through a die. Moreover, leavened and unleavened bread, couscous and bulgar are made of durum wheat. Durum is unsuitable for producing the light, airy loaves of bread because of its lower gluten strength as compared to common wheat (Joppa and Cantrell, 1990; Bekes *et al.*, 2001; Ekboir, 2002).

Bread wheat is predominantly grown in west, south and central Asia, eastern and southern Africa, north Africa, the southern cone of South America, Mexico/Guatemala, eastern and western Europe and North America. China, India, and Turkey are the most important producers among from developing countries (Fig. 2.1). This crop is grown for products such as leavened breads in loaves or buns, flat breads such as chapattis and tortillas, and many kinds of crackers, cookies, and cakes. Other wheat species are also grown but to a lesser extent (CIMMYT, 1997).

Because of its greater economic importance, most genetic research has concentrated on hexaploid wheat. Joppa and Cantrell (1990) indicated that the

progress and emphasis in genetic research in tetraploid wheat has been limited when compared to the hexaploid wheats. Reasons for this include the lack of suitable cytogenetic stocks, their growth in a small part of the world's total wheat production area, and their limited use in the production of bread products.

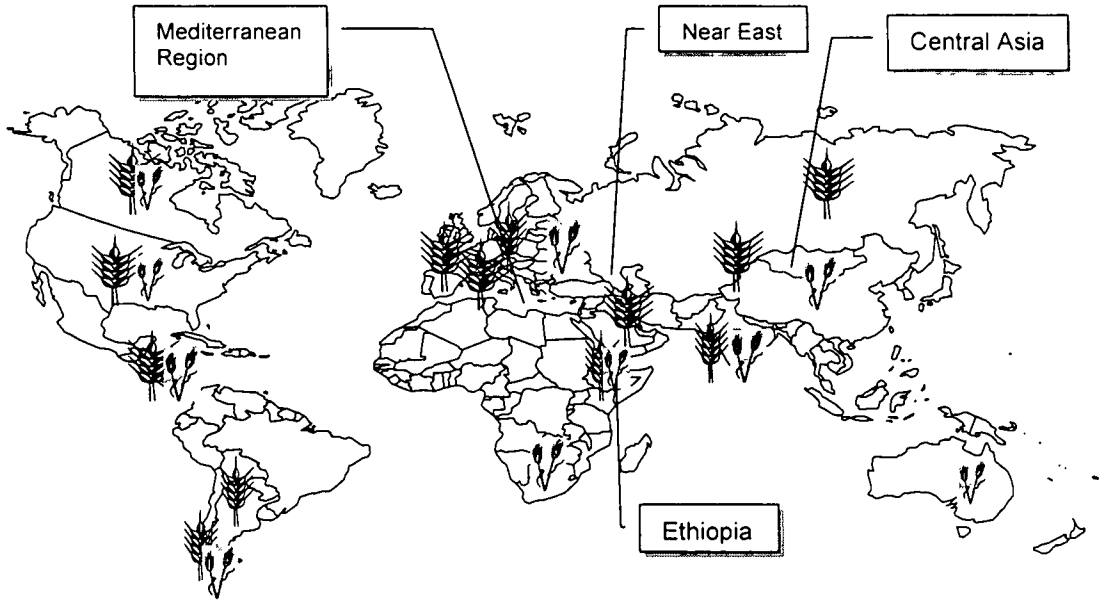


Fig. 2.1 Vavilov's centers of diversity of wheat include Central Asia, Near East, Mediterranean Region and Ethiopia. Prominent durum and bread wheat production areas of the world are shown by single and double tillers, respectively.

The world average wheat yield is 2.6 tons per hectare (t/ha) and in marginal environments yields may not reach 1 t/ha. Low yields are due to different factors, the major being that farmers in marginal areas still grow old, unimproved and disease-susceptible varieties. The major production constraints of wheat include abiotic stresses (drought, heat, waterlogged soils, acidic soils, zinc-deficient soils, and soils with toxic levels of boron) and biotic stresses (diseases, insects, and weeds). Plant diseases alone account for the loss of 9.1% of wheat yield (James, 1981). It is thus crucial for more research on wheat improvement for yield potential, better yield stability and improved disease resistance. To increase yield, breeders are focusing on developing wheats with higher yielding capacity, and improved disease resistance.

2.1.1 Origin and evolution of wheat

Vavilov (1951) described the centers of origins of wheat as Central Asia, Near East, Mediterranean region, and Ethiopia (Fig. 2.1).

As reviewed and cited by Knott (1989) the wheat genome has been extensively studied by different investigators (Sakamura, 1918; Kihara, 1919, 1924; Sax, 1922). Löve (1984), following a broad interpretation of the biological species concept, defined the genus *Triticum* by its unique genome constitution, either as genera of diploids with A-genome or polyploids with BA and BAD-genomes. Thus, the genus *Triticum* was split into three sub genera, each corresponding to one of three ploidy levels in the genus. By studying its genome and the various wild relatives of wheat, geneticists have reconstructed a possible evolutionary history of wheat (Fig. 2.2). An important result of interspecific hybridization was the conclusion that specific chromosomes in different genomes had genes with similar effects.

Allopolyploidization has played a significant role in the evolution of *Triticum* species. The different species are cytogenetically and morphologically distinguished from each other. The D-genome progenitor of common wheat, *Ae. tauschii,* is widely distributed in countries surrounding the Caspian Sea including Turkey, Iran, Pakistan, Afghanistan, Azerbaijan, Armenia, southern Russia (Dagestan) (Kihara, *et al.*, 1965; Gill *et al.*, 1986). *T. monococcum* var. *monococcum,* the only cultivated variety of this species, is grown in the mountainous areas of southern Europe and Turkey (Waines, 1983).

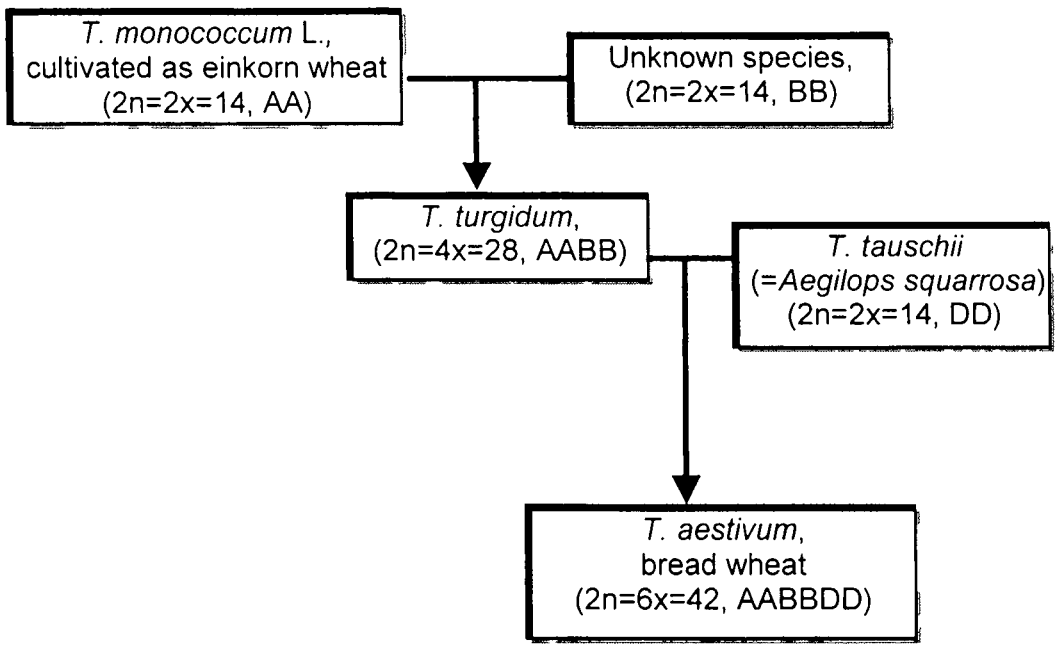


Fig. 2.2 Diagram of the proposed evolution of modern wheats involving amphidiploid production at two points. A, B and D are different genomes (adapted from Griffiths *et al.*, 2000).

2.1.2 Homologous chromosome pairing in wheat

Durum and bread wheats have seven homoeologous groups of chromosomes. In both, each chromosome in one genome should be related and homoeologous to one in each of the one or two genomes as it is reflected in its proposed origin. Homoeologous chromosomes have a similar gene content and can replace each other in nullisomic-tetrasomic combinations (Sears, 1952a, 1966).

During meiosis in durum and bread wheats, 14 and 21 bivalents are formed, respectively. In addition, it has been established that any given chromosome has only one specific pairing partner (homologous pairing). The suppression of homoeologous pairing makes the species more stable and is maintained by numerous genes of which the *Ph* gene on the long arm of chromosome 5B has the strongest effect (Okamoto, 1957; Riley and Chapman, 1958; Sears and Okamoto, 1958; Sears, 1976, 1984; Kimber and Sears, 1987). Thus, the *Ph* gene ensures a diploid-like meiotic behaviour for these polyploid species.

2.1.3 Classification of wheat and proposed genome symbols of the various species of *Triticum*

Wheat belongs to the family Poaceae and genus *Triticum*. Within this family, there are different taxonomic classifications with different genus and species delimitations. The recent classification of *Triticum* and *Aegilops* used by Van Slageren (1994) is presented in Tables 2.1 and 2.2. The classification of Van Slageren (1994) follows that of MacKey (1988) except for minor changes in naming and ranking. Van Slageren's naming of the C-genome species of *Aegilops* (*Ae. caudata* L.) is not accepted by a recent review of the Kansas State University/Wheat Genetics Resource Center (USA) and this species is renamed as *Ae. markgrafii*.

Species of *Triticum* within similar ploidy levels cross readily and give fertile hybrids (Knott, 1989). Durum wheat is the only economically important tetraploid wheat and common/bread wheat the only hexaploid one. Other diploid and polyploid relatives of wheat can serve as germplasm sources to introduce desirable genes into wheat breeding programs (McIntosh *et al.*, 1995a). Most species cross easily with bread and durum wheats but there are exceptions. Wheats also cross to some extent with species of the genera *Agropyron*, *Elymus*, *Hordeum*, and *Secale* (Knott, 1987).

In general, the method of transferring alien genes to wheat largely depends on the evolutionary distance of the species involved (Friebe *et al.*, 1997). Jiang *et al.* (1994) suggested that crosses are possible between wheat and any of the species in the Triticeae and even species from the Panicoideae (Tribe Andropogoneae) such as *Zea mays* and *Sorghum bicolor*. However, such crosses would encounter post-hybridization barriers that would hinder introgression of alien chromosomes or genes. The post-hybridization barriers include chromosome elimination, preferential transmission of certain alien chromosomes, and adverse genetic

Table 2.1 Classification of *Triticum*: ploidy levels, genome formulae and scientific and/or vernacular names (modified from Van Slageren, 1994).

Ploidy level	Genome	Scientific and/or vernacular name
Diploids (2n=2x=14)		*T. monococcum* L.
	A	subsp. *Aegilopoides* (Link) Thell.
	A	subsp. *monococcum* (einkorn wheat)
	A	*T. uratu* Tumanian ex Gandilyan
Tetraploids (2n=4x=28)	AB	*T. turgidum* L.
		subsp. *turgidum* (poulard, rivet or cone wheat)
		subsp. *carthlicum* (Nevski in Kom.) Á. Löve and D. Löve (Persian wheat)
		subsp. *dicoccum* (Schrank ex Schubler) Thell. (emmer wheat)
		subsp. durum (Desf.) Husnot (durum wheat)
		subsp. *paleocolchicum* (Menabde) Á. Löve and D. Löve
		subsp. *polonicum* (L.) Thell (Polish wheat)
		subsp. *turanicum* (Jakubz.) A. Love and D. Love
		subsp. *dicoccoides* (Korn. ex Asch. And Graebner) Thell (wild emmer wheat) .
	AG	*Triticum timopheevii* (Zhuk.) Zhuk.
		subsp. *timopheevii* (Timopheevii wheat)
		subsp. *armeniacum* (Jakubz.) Mackey
		(Armenian wheat)
Hexaploids (2n=6x=42)	ABD	*Trticum aestivum* L.
		subsp. *aestivum* (bread/common wheat)
		subsp. *compactum* (Host) Mackey (club wheat)
		subsp. *macha* (Dekapr. and Menabde) Mackey
		subsp. *spelta* (L.) Thell. (spelt wheat)
		subsp. *sphaerococcum* (Percival) Mackey (shot wheat)
	AAG	*Triticum zhukovskyi* Menabde and Ericzjan

Table 2.2 Classification of *Aegilops*: ploidy levels, genome formulae and scientific/vernacular names (modified from Van Slageren, 1994).

Ploidy level	Genome	Scientific name
Diploids (2n=2x=14)	C	*Ae. caudate* L.
	D	*Ae. tauschii* Cosson
	M	*Ae. comosa* var. *comosa* Sm. in Sibth and Sm.
	M	*Ae. comosa* var. *subventricosa* Boiss
	N	*Ae. uniaristata* Vis.
	S	*Ae. speltoides* var. *speltoides* Jausch
	S	*Ae. speltoides* var. lingustica (Savig.) Fiori
	S	*Ae. bicornis* var. *bicornis* (Forsskal) Jaub and Spach
	S	*Ae. bicornis* var. *anathera* Eig
	S	*Ae. longissima* (Schweinf and Muschl in Muschl.) Eig
	S	*Ae. searsi* Feldman and Kislev *ex.* K. Hammeri
	S	*Ae. sharonensis* Eig
	T	*Amblyopyrum. muticum* var. *muticum* (Boiss) Eig
	T	*Am. muticum* var. *loliacea* (Jaub and Spach) Eig
	U	*Ae. umbellulata* Zhuk
Tetraploids (2n=4x=28)	CD	*Ae. cylindrica* Host
	DM	*Ae. crassa* Boiss
	DN	*Ae. ventricosa* Tausch
	SU	*Ae. peregrina* subsp. *peregrina* (Hackel in J Fraser) Marie and Weiller
	SU	*Ae. peregrina* subsp. *brachyanthera* (Boiss) Marie and Weiller
	UC	*Ae. triuncialis* var. *triuncialis* L.
	UC	*Ae. triuncialis* var. *persica* (Boiss) Eig
	UM	*Ae. biuncialis* Vis.
	UM	*Ae. columnaris* Zhuk.
	UM	*Ae. geniculata* Roth
	UM	*Ae. neglecta* Req. *ex.* Bertol
	US	*Ae. kotschyi* Boiss
Hexaploids (2n=6x=42)	DDM	*Ae. crassa* Boiss
	DMS	*Ae vavilovii (Zhuk)* Chennav.
	DMU	*Ae. juvenalis.* (Thell) Eig
	UMN	*Ae. neglecta* Req. *ex.* Bertol

interactions leading to hybrid dysgenesis (biologically deficient hybrids), chromosome breakage and sterility (Knott, 1989).

To undertake distant hybridization with wheat, selection of diverse wheat and donor genotypes in the initial hybridization is important and would often overcome some of the barriers.

2.1.4 Variation in durum and bread wheats

As with most crop species, modern cultivation techniques have been responsible for rapid genetic erosion in bread wheat (Friebe *et al.*, 1997). Jiang *et al.* (1994) elaborated that wild relatives and related species of wheat can be used to improve the genetic variation of bread wheat. This variability allows for the selection and breeding of different traits such as resistance to wheat leaf rust. Pasquini *et al.* (1979) and Sharma *et al.* (1986) reported that durum wheats carry leaf rust resistance (*Lr*) genes that are different from those in common wheat. The genes can be used to broaden the genetic base of leaf rust resistance in bread wheats. Successful transfer of genes from tetraploid wheats to hexaploid wheats was reported by McIntosh *et al.* (1967), McIntosh and Dyck (1975), Gupta *et al.* (1991) and Dyck (1994). These genes, however, had altered expression due to the difference in ploidy level between the two wheat species (Kerber, 1983; Dyck 1987).

2.1.5 Gene pools and enhancement of genetic variation in bread wheat

Three gene pools were identified to enhance genetic variation in bread wheat (Friebe *et al.*, 1997). These are the primary, secondary and tertiary gene pools. The primary gene pool include landraces of bread wheat, the species of tetraploid wheat such as *T. turgidum* subspp. *turgidum* and *dicoccoides*, the donor species of the A-genome (*T. monococcum* [2n=2x=14, AA]) and the D-genome (*T. tauschii* [2n=2x=14, DD]) of bread wheat. The primary gene pool has homologous genomes in common with bread wheat. The secondary gene pool comprises polyploid *Triticum/Aegilops* species that share at least one homologous genome with bread wheat. In this group are diploid *Aegilops*

species of the section *Sitopsis* which are related to the B-genome of bread wheat, the tetraploid timopheevi wheats (2n=4x=28, A^tA^tGG), and polyploid *Aegilops* species that have the D-genome in common with bread wheat, namely, *Ae. cylindrica* (2n=4x=28, CCDD). Bread wheat has received many *Lr* genes from the genus *Aegilops* including *Lr21*, *Lr22a*, *Lr28*, *Lr32*, *Lr36*, *Lr41*, *Lr42*, and *Lr43* (McIntosh *et al.*, 1998). Mujeeb-Kazi and Hetteel (1995) noted that accessions of *Ae. tauschii* have a wide range of resistance and tolerance to various biotic and abiotic stresses such as karnal bunt, scab, spot blotch, leaf rust, stripe rust, salinity, drought and improved bread making quality. The recent work of Dhaliwal *et al.* (2002) identified and transferred rust resistance genes from *Aegilops ovata* into bread wheat (*Triticum aestivum*).

Gene transfer to bread wheat from the primary and secondary gene pools can be achieved relatively easy through homologous recombination followed by several backcrosses. This gives agronomically well-adapted germplasm containing the target alien gene (Friebe *et al.*, 1997).

Species of the tertiary gene pool are more distantly related to bread wheats. They can be considered as a germplasm source, should a target gene not be available from the primary and secondary gene pools. Members of this gene pool do not share homoeologous genomes with wheat, but rather genetically related individual homoeologous chromosomes. The tertiary gene pool consists of diploid, tetraploid, and hexaploid *Aegilops* species, *Agropyron*, *Secale* and *Hordeum*. Many genes have been transferred from the tertiary gene pool to wheat for disease and pest resistance, but only a few have been exploited in cultivar improvement (Friebe *et al.*, 1997). A number of *Lr* genes derived from the tertiary gene pool are described by McIntosh *et al.* (1998, 1999, 2000, 2002) and summarized in section 2.4.

Tertiary gene pool species are alien chromosome sources to bread wheat. Alien chromosomes can compensate for the loss of homoeologous wheat chromosomes or chromosome segments. Gene transfer from the tertiary gene pool is not possible by homologous recombination. There are other suggested

strategies that take into account the proportion of the alien chromosome to be transferred. These strategies are employed for:

(1) transfer of whole alien chromosome arms to wheat. The approach exploits the centric-breakage-fusion mechanism of univalents at meiosis metaphase I (MI). The procedures are to:
   (a) add the alien target chromosome to the wheat chromosome complement,
   (b) determine the homoeology of this chromosome by either producing compensating chromosome substitutions or by using molecular marker technologies,
   (c) make the alien chromosome and a homoeologous wheat chromosomes monosomic by either crossing the substitution line with wheat or by crossing an addition line with the appropriate monosomics.

In these plants the alien chromosome and a homoeologous wheat chromosome are univalents at MI. Univalents have the tendency to break at the centromere, followed by the fusion of the broken arms (Sears, 1952b). The progenies of such plants, with the desired compensating whole arm translocation, can be recovered at fairly high frequencies (Lukaszewski, 1993; Marais and Marais, 1994).

(2) transfer of segments smaller than the complete arms to wheat. Two strategies are followed to transfer a smaller chromosome arm from tertiary sources to bread wheat including:
   (a) radiation treatment followed by stringent selection for compensating translocations. This has been applied by Sears (1956) for the first time for transferring *Lr9* from *Ae. umbellulata* (2n=2x=14, UU) to bread wheat,
   (b) induced homoeologous recombination. Riley *et al.* (1968) employed this to transfer a yellow rust resistance gene (*Yr8*) from *Ae. comosa* (2n=2x=14, MM) to bread wheat.

2.2 Wheat leaf rust

Wheat leaf rust causes serious economic losses in wheat (Wahl *et al.*, 1984; Kolmer, 1996; Raupp *et al.*, 2001). Transported primarily by wind (Peterson, 1965), leaf rust along with other rust diseases are major restraints to global wheat productivity. After stem rust, leaf rust is the most damaging and widely distributed of the wheat rusts. Yield losses reach 5-15% or more in susceptible wheat varieties (Kolmer, 1996). The fungus attacks the leaf blades and to a lesser extent leaf sheaths and glumes, thus reducing the photosynthetic capacity of the plants and causing related physiological disorders. The disease can cause various degrees of kernel shriveling whereas early and severe attacks may lead to total loss of a crop. Ample moisture and warm weather favour rust development and a crop can be destroyed in a matter of weeks (Peterson, 1965; Knott, 1989).

Like stem and yellow rust, leaf rust belongs to the genus *Puccinia*. The leaf rust fungus differs from the other wheat rusts in terms of morphology, life cycle, and optimal environmental requirements for growth and reproduction (Knott, 1989).

The pustules of leaf rust grow prolifically on the upper leaf surface rather than on the lower surface. The pustules have an orange to brown colour with oval or circular shapes ranging about 1-2 mm in diameter (Schafer, 1987; Knott, 1989).

The spores of leaf rust germinate within 7-10 days at a temperature of 15–25°C. Maximum sporulation will be reached four days after the first sporulation (Roelfs *et al.*, 1992). Goodman and Novacky (1994) demonstrated that symptoms of leaf rust appeared in 2-3.5 days as a hypersensitive reaction, i.e. rapid cell death and subsequent necrosis in the resistant plant tissue, whereas it took 7-12 days in the susceptible tissue.

The sources of inoculum for leaf rust are primary hosts (predominantly bread wheat), alternate hosts (the species of *Thalictrum*, *Anchusa*, *Clematis* and *Isopyron*), and accessory hosts (weedy species of *Triticum*, and *Aegilops* and related species of *Agropyron* and *Secale*). Volunteer wheat serves as a non-crop host (Roelfs *et al.*, 1992).

Leaf rusts specialize on particular host genera to produce so-called *formae speciales* (f. spp.) or *forma specialis* [singular] (f. sp.). Leaf rusts attacking wheat, barley, triticale or relatives of wheat are found under formae specialis *tritici* (Roelfs *et al.*, 1992). This notion, however, has been changed recently when Anikster *et al.* (1997) provided evidence that wheat leaf rust is a separate species, not just a specialized form of rye leaf rust. Subsequent to this, the name *Puccinia triticina* Eriks. has replaced *Puccinia recondita* f. sp. *tritici.*

Ezzahiri *et al.* (1992) from Morocco, North Africa, reported *Anchusa italica* Retz. as an alternate host for *Puccinia recondita* in Morocco. They reported the susceptibility of local durum wheat cultivars to leaf rust in fields infested with *A. italica.* However, few telia or infected *Anchusa* plants were found in bread wheat fields. This pathogen cannot be necessarily considered as *P. triticina.* Thus the leaf rust pathogen populations occurring on common wheat and durum might be a common wheat form both having *Thalictrum* as alternate host or a durum form with *Anchusa* form. Both of the *Thalictrum* and *Anchusa* groups are avirulent when tested on common wheat differentials. It would thus be realized that the current differentials may not be relevant in studying leaf rust of durum wheat.

2.3 Use and development of resistant cultivars to control wheat leaf rust

The use and production of resistant cultivars is the most effective and economical control method for wheat leaf rust. Chemical control has not been completely successful and some compounds must be applied repeatedly, making them unprofitable.

Chester (1946) reported that an attempt to develop rust resistant wheat varieties was made in Kansas in 1911. As cited by Schafer *et al.* (1984), McFadden (1915) crossed emmer wheat, resistant to stem rust, with Marquis as susceptible parent and a cultivar, Hope, was released.

Breeding for resistance has been one of the main objectives in wheat breeding programs. The key strategy in developing durable, effective genetic disease resistance has been to transfer a large number of resistance genes from different

sources into different wheat varieties. This broadens the genetic base of the resistance, which is essential for keeping epidemics from devastating wheat crops over extensive areas. Genes that give resistance are incorporated into new cultivars by crossing, followed by selection. Knowledge of the genetics of resistance and identification and location of specific genes for resistance, are helpful in selecting the appropriate parents for plant breeding programs aimed at producing cultivars with different sources of resistance.

Based on the gene-for-gene concept (Flor, 1942), and the concept of interorganismal genetics of pathogen-host associations (Loegering, 1978, 1985), the presence of specific resistance gene(s) in the host can be demonstrated with suitable combinations of genes for virulence and avirulence in the pathogen. The phenotype of the host : parasite interaction is the infection type (IT). This perception has been used successfully to postulate the genes for resistance to leaf rust and stem rust of wheat (McVey and Long, 1993).

Resistance in wheat can be hypersensitive resistance (HR) or partial resistance (PR). Hypersensitive resistance or race-specific resistance is based on a "major gene" and characterised by a low infection type. Due to the collapse of penetrated host cells, necrotic flecks would appear in the immediate areas of the infection, thus denying the pathogen live tissue as its source of food. HR can be complete or incomplete. This type of resistance is ephemeral, i.e. the pathogen can adapt to produce variants with virulence towards genes conferring HR. Partial resistance, also called race-non-specific or slow rusting resistance, relies on the accumulated effects of numerous minor genes. Partial resistance shows no collapse of cells and allows the rust pathogen to continue feeding on live tissue. However, PR reduces the infection rate to a level that does not seriously damage the plant or reduce yield. During PR the pustules appear normal with high infection type, but temporally slower disease development is observed in the field. Partial resistance is often thought to be durable (Parlevliet, 1981; Messmer *et al.*, 2000).

Resistance can be expressed at the seedling or adult plant growth stages. Adult plant resistance (APR) genes are not effective in seedlings and are the common

sources of durable resistance. Seedling resistance genes are recognised in primary leaves and normally confer resistance at all stages of plant growth (Sawhney *et al.*, 1992).

When compared to susceptible lines, wheat lines with partial resistance are characterized by a reduced infection frequency, longer latent period, and reduced spore production 10 to 14 days after inoculation with leaf rust (Parlevliet, 1979; Lee and Shaner 1985; Pretorius *et al.*, 1987; Kolmer, 1996; Messmer *et al.*, 2000).

According to Knott (1989) most genetic analyses of wheat rust diseases suggested that resistance to the disease is conditioned by a single dominant gene (monogenic), as virulence in the pathogen is conditioned by a matching recessive gene. Some other reports suggested oligogenic resistance. Slow rusting has been attributed to only one to three genes (Geiger and Heun, 1989) and prolonged latent period conditioned by four genes (Shaner *et al.*, 1997) or by at least five genes (Van der Gaag and Jacobs, 1997). According to Braun *et al.* (1996) CIMMYT's strategy to control rusts is through general resistance or slow rusting. Consequently 60% of CIMMYT's materials carry one to four genes for partial resistance, which has been acquired by accumulating several minor genes in different combinations. The latest report by Messmer *et al.* (2000) indicated that durable leaf rust resistance in the Swiss winter wheat variety, 'Forno' was contributed by at least six genes.

The genetic effects of inheritance for partial leaf rust resistance are reported to be predominantly additive (Geiger and Heun, 1989; Das *et al.*, 1992; Messmer *et al.*, 2000). Besides, some crosses were found with epistatic gene action (Geiger and Heun 1989; Shaner *et al.*, 1997). Possible pleiotropic gene action was also reported for *Lr34*, where the gene was suggested to be pleiotropic or closely linked with leaf tip necrosis at anthesis, that was caused by the *Ltn* gene located on the short arm of chromosome 7D (Singh, 1992). The *Ltn* gene was used as an indirect morphological marker of leaf rust resistance, although breeders often select against leaf tip necrosis because varieties with strong leaf tip necrosis are not readily accepted by farmers (Messmer *et al.*, 2000).

2.4 Chromosomal locations and common sources of *Lr* genes

Thus far, 50 leaf rust resistance genes have been reported (McIntosh *et al.*, 1998, 1999, 2000, 2002). Their sources and chromosomal location are presented in Table 2.3. Most of the *Lr* genes have been derived from wild relatives. The distribution of *Lr* genes across the genomes is summarized in Table 2.4. Most *Lr* genes are found on chromosomes 2A, 1B, 4B, 6B, 2D, 3D, and 7D. These chromosomes carry about 58.7% of the hitherto reported genes. Studies revealed that most genotypes in wheat showed durable resistance to leaf rust due to the presence of *Lr12* (Sawhney and Sharma, 1997) and *Lr13* and in combination with *Lr34* (Roelfs, 1988a, Bender *et al.*, 2000; Kolmer and Liu, 2001).

Table 2.3 Genes identified for leaf rust resistance: common sources and chromosomal locations (McIntosh *et al.*, 1998, 2000, 2002).

Gene	Common source(s)[1]	Chromosome location(s)	Source(s) to chromosome location(s)
Lr1	Malakoff, Blueboy, Centenario, Sonora	1B	Soliman *et al.*, 1964
		5D	McIntosh *et al.*, 1965
		5DL	McIntosh and Baker, 1970
Lr2	Webster	1B	Soliman *et al.*, 1964
		2DS	Luig and McIntosh, 1968; McIntosh and Baker, 1968
Lr2a	Webster, Eureka, Waldron, Festiguay	-	
Lr2b	Carina	-	
Lr2c	Brevit, Loros	-	
Lr3	Belocerkovskaja 289, Bennet, Democrat, Fertodi 293, Gage, Hana	6B	Heyne and Livers, 1953
		6BL	McIntosh *et al.*, 1998
Lr3ka	Klein Aniversario	-	
Lr3bg	Bage	-	
Lr4 – Lr8	Purdue 3369-61-1-1-10 (Waban)	-	McIntosh *et al.*, 1998
Lr9	*Triticum umbellulata* (Transfer, Abe, Arthur 71, McNair 701 and 2203, Riley 67, Oasis *Lr11*	6B	McIntosh *et al.*, 1965; Sears, 1961; Sears, 1972
		6BL	Friebe *et al.*, 1996
Lr10	Lee, Exchange, Gabo, Selkirk, Mayo 54, Blueboy	1A	Dyck and Kerber, 1971; McIntosh *et al.*, 1998
		1AS	McIntosh *et al.*, 1998
Lr11	Hussar, Bulgaria 88, Oasis, Hart, Hazen	2A	Soliman *et al.*, 1964
Lr12	Exchange *Lr10 Lr16*, Opal, Sturdy *Lr113*, CS *Lr34*	4B	Dyck and Kerber, 1971
Lr13	Frontana, Chris, Manitou, Neepawa, Era, Polk, Egret, Hustler, Kinsman	2BS	McIntosh *et al.*, 1998
Lr14a	Spica, Hope, Selkirk, Aotea, Glenwari, Hofed	7B	McIntosh *et al.*, 1967
		7BL	Law and Johnson, 1967
Lr14b	Maria Escobar *Lr17*, Bowie *Lr3*, Rafaela *Lr17*	-	
Lr14ab	*Lr14a*/6*Thatcher//*Lr14b*/6*Thatcher	-	
Lr15	Kenya W1483	2DS	Luig and McIntosh, 1968; McIntosh and Baker, 1968
Lr16	Exchange *Lr10 Lr12*, Etoile de Choiosy, Warden *Lr10*, Selkirk *Lr10 Lr14a*, Columbus	4B	Dyck and Kerber, 1971
		2BS	McIntosh *et al.*, 1998

[1] Scientific names of some of the common sources are presented in accordance to the authors.

... Table 2.3 Continued

Gene	Common source(s)	Chromosome location(s)	Reference(s) to chromosome location(s)
Lr17a	EAP 26127, Jupateco, Klein Lucero, Hobbit Sib *Lr13*, Lerma Rojo 64 *Lr13*, Inia 66 *Lr13 Lr14a*, Maria Escobar *Lr14b*, Rafaela *Lr14b*	2A	Dyck and Kerber, 1977
		2AS	Bariana and McIntosh, 1993
Lr17b	Brock, Tarso, Norman	2A	McIntosh *et al.*, 1998
Lr18	Africa 43, Red Egyptian P.I. 170925, Timvera, Sabikei 12	5BL	McIntosh, 1983
Lr19	Derived from *Agropyron elongatum* (Agatha)	7AL	Eizenga, 1987
		7BL	Prins *et al.*, 1997, Marais *et al.*, 2000
		7AgL	McIntosh *et al.*, 1998
		7DL	Sharma and Knott, 1966; Dvorak and Knott, 1977; McIntosh *et al.*, 1977; Kim *et al.*, 1993; Friebe *et al.*, 1994, 1996.
Lr20	Thew, Axminster, Festival, Kenya W744, Normandie	7AL	Watson and Luig, 1963; Sears and Briggle, 1969
Lr21	Tetra Canthatch/ *Triticum tauschii* var. *meyeri*	1D	Kerber and Dyck, 1979
		1DL	Rowland and Kerber, 1974
		1DS	Gill *et al.*, 1991
Lr22	Derived from *Ae. squarrosa*	2DS	Rowland and Kerber, 1974
Lr22a	Tetra Canthatch/ *Triticum tauschii* var. *strangulata*	-	
Lr22b	Thatcher, Cathatch, Marquis	-	
Lr23	Gabo, Lee, Kenya Farmer, Gamenya, Timstein	2BS	McIntosh and Dyck, 1975
Lr24	Derived from *Agropyron elongatum* (Agent, Blueboy II, Fox, Osage, Payne, SST23, SST44, Sears 3D-Ag#1 translocations	3D	Smith *et al.*, 1968; McIntosh *et al.*, 1977
	Amigo, Teewon	1BL	Chen *et al.*, 1994
Lr25	Derived from *Secale cereale* cv. Rosen (Transec, Transfed)	4BS	Driscoll and Anderson, 1967; Driscoll and Bieliy, 1968; Friebe *et al.*, 1996
Lr26	Derivatives of Petkus rye . Iris , Sabina, GR876, Bacanora 88, Amika *Lr3*, Istra *Lr3*, Solaris *Lr3*, Cumpas 88 *Lr13*, Siouxland *Lr24*,	T1BL-1RS	McIntosh *et al.*, 1998
Lr27	Gatcher,.Ocoroni 86, SUN 27A *Lr1 Lr2a*, Timgalen *Lr3 Lr10*,. Anhuac *Lr13 Lr17*, Cocoraque 75 *Lr13 Lr17 Lr34*, Jupateco 73S *Lr17*	3BS	Singh and McIntosh, 1984
Lr28	Derived from *Ae. speltoides*	4AL	McIntosh *et al.*, 1982
Lr29	Derived from *Agropyron elongatum*	7DS	McIntosh *et al.*, 1998
Lr30	Terenzio	4AL	Dyck and Kerber, 1981

Table 2.3 Continued

Gene	Common source(s)	Chromosome location(s)	Reference(s) to chromosome location(s)
Lr31	Chinese Spring, Ocoroni 86	4BL	Sing and McIntosh, 1984
Lr32	Tetra Canthatch/*T. tauschii* RL 5497-1; RL 5713, RL 5713/Marquis-K	3DS	Kerber, 1988
Lr33	PI 268454a, PI 58548, PI 268316 *Lr2c Lr34,*	1BL	Dyck *et al.*, 1987
Lr34	PI 268454, Glenlea *Lr1,* Laura *Lr1 Lr10,* Terenzio *Lr3 Lr30 LrT3,* Chinese Spring *Lr12,* Sturdy *Lr12 Lr13,* Frontana *Lr13,* Parula *Lr13,* PI 58548 *Lr33,* Lageadinho *LrT3*	7D	Dyck, 1987
		7DS	Dyck *et al.*, 1994; Nelson *et al.*, 1997
Lr35	RL 5711	2B	Kerber and Dyck, 1990
Lr36	Derived from *Ae. speltoides.* (line 2-9-2, line E84018)	6BS	Dvorak and Knott, 1990
Lr37	Derived from *T. ventricosum* (Hyka, Madison)	2AS	Bariana and McIntosch, 1993
Lr38	Derived from *Ag. intermedium*	1DL	Friebe *et al.*, 1993, 1996
		2AL	Friebe *et al.*, 1992, 1996
		3DS	Friebe *et al.*, 1993, 1996
		5AS	Friebe *et al.*, 1993, 1996
		6DL	Friebe *et al.*, 1993, 1996
Lr39	Derived from *Ae. tauschii*	2DS	Raupp *et al.*, 2001
Lr40	Derived from *T. tauschii*	-	
Lr41	TAM107*3/*T. tauschii* TA 2460; Thunderbolt	1D	Cox, 1991
Lr42	Century*3/*T. tauschii* TA 2450	1D	Cox *et al.*, 1993
Lr43	Triumph64/3/KS8010-71/TA2470//TAM200, *T. tauschii* TA2470	7D	Hussein *et al.*, 1994
		7DS	Hussein *et al.* 1998
Lr44	Derived from *T. spelta (7B31)*	1B	Dyck and Sykes, 1994
Lr45	Derived from *S. cereale* (ST-1)	2A	McIntosh *et al.*, 1995b; Friebe *et al.*, 1996
Lr46	Pavon F76 *Lr10 Lr13*)	1BL	McIntosh *et al.*, 1998
Lr47	Derived from *Ae. speltoides*	7AS	Dubcovsky *et al.*, 1998
Lr48	CSP44 *Lr34*	-	
Lr49	VL404 *Lr34*	-	
Lr50	WGR36 = TAM107*3/TA870//Wichita, *T. armeniacum* TA870	2BL	McIntosh *et al.* 2002

Table 2.4 The distribution of *Lr* genes across the genome and homoeologous groups of wheat. Chromosomal location and arm positions, i.e. whether on the short (S) or long (L) arm are indicated as summarized from various authors (refer Table 2.3).

		Homoeologous group						
Genome	Arm position	1	2	3	4	5	6	7
A	S	*Lr10*	*Lr17a, Lr17b, Lr37*			*Lr38*		*Lr47*
	L		*Lr38*		*Lr28, Lr30*			*Lr20*
	Not described		*Lr11, Lr45*					
B	S		*Lr13, Lr23, Lr16*		*Lr25, Lr27*		*Lr36*	
	L	*Lr24, Lr26, Lr33*	*Lr50*		*Lr31*	*Lr18*	*Lr3a, Lr3ka, Lr3bg, Lr9*	*Lr14a, Lr14b, Lr14ab*
	Not described	*Lr44, Lr46*	*Lr35*	*Lr27*	*Lr12*	*Lr27*		
D	S	*Lr21*	*Lr2a, Lr2b, Lr2c, Lr15, Lr22a, Lr22b, Lr39*	*Lr32, Lr 38*				*Lr29, Lr34, Lr43*
	L	*Lr38*		*Lr24*		*Lr1*	*Lr38*	*Lr19*
	Not described	*Lr41, Lr42*						

2.5 Cytogenetic analysis of resistance to wheat leaf rust

The use and development of aneuploids

Aneuploids have an important place in genetic research and breeding programs. However, they are generally less vigorous and less fertile than their euploid counterparts (Joppa and Williams, 1977; Knott, 1989).

Aneuploids are employed:

- to localize gene(s) on specific chromosome(s)
- to transfer specific chromosome(s) from one cultivar or line to another
- to determine the crossover frequency between a gene and the centromere
- to study the effect of multiple copies of a gene
- to study the homology of chromosomes and
- to assess phenotypic effects of individual chromosomes and numerous other genetic studies.

Sears (1954) systematically studied and produced the complete sets of aneuploids in the hexaploid common wheat cultivar, Chinese Spring (CS). These aneuploids include: 21 monosomics (2n-1) which are fertile and stable, 21 nullisomics (2n-2) which are low in fertility and lack vigor, 21 trisomics (2n+1) which are reasonably fertile and stable and 21 tetrasomics (2n+2) that are fertile and stable (Knot, 1989). As illustrated (Fig. 2.2) bread and durum wheats are segmental allopolyploids with three and two homoeologous genomes respectively. Pairing of these chromosomes during meiosis is genetically controlled. Deficiencies or excess for one dose of a single chromosome or even multiple chromosomes are tolerated in CS aneuploids.

Some of Sears's aneuploids in CS arose spontaneously as the progeny of either haploid plants or nullisomic 3B plants (Knott, 1989). Currently many other hexaploid monosomic wheat lines are available for genetic analysis (Knott, 1989; Cai *et al.*, 1999; Iwaki *et al.*, 2001; Singh *et al.*, 2001; Tsujimoto, 2001).

The development of the series of 21 aneuploids in CS has furnished a tool for

circumventing, to a certain extent, the difficulties imposed by polyploidy in wheat. These aneuploids have proved immensely useful in elucidating the cytogenetic architecture of bread and durum wheats.

Chinese Spring is generally susceptible to the naturally occurring population of rusts. From crosses of a resistant parent with sets of CS aneuploids, followed by disease testing of segregating lines it is often possible to determine directly whether a given chromosome carries resistance to a given race of rust (Sears, 1956). Nonetheless it has been noted that CS derivatives possesses *Lr28* (McIntosh *et al.*, 1982); *Lr31* (Singh and McIntosh, 1984) and *Lr12* and *Lr34* (Dyck, 1991).

A large number of aneuploids of durum wheat are available for genetic studies (Joppa and Williams, 1977, 1983; Joppa *et al.*, 1987; Joppa and Williams, 1988; Joppa and Cantrell, 1990; Joppa, 1993). These include: monosomics (2n-1=27), D-genome substitution monosomics (2n-1+1=28), monotelodisomics (2n=27+t), ditelomonotelosomics (2n=26+2t+t), double ditelosomics (2n=26+2t+2t) and D-genome disomic substitutions (2n-2+2=28).

2.5.1 Monosomic analysis to identify chromosomes carrying genes for wheat leaf rust resistance

Various aneuploids, particularly monosomics, have been used extensively to identify the chromosomes carrying certain genes in wheat and to map them relative to the centromere (Sears, 1954; Allan and Vogel, 1960; Kuspira and Millis, 1967; Bozzini and Giorgi, 1971; Mokhtarzadeh, 1975; Giorgi, 1979; Hanchinal and Goud, 1982; McIntosh, 1983; Knott, 1989; Marais and du Toit, 1993, Raupp *et al.*, 1993, 2001; Schroeder *et al.*, 1994; Iwaki *et al.*, 2001; Singh *et al.*, 2001, Zeller *et al.*, 2002).

Consequence of selfing monosomics

Theoretically, monosomics produce two kinds of gametes during meiosis: 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 below (Fig. 2.3). From the scheme it can be concluded that there is a 50% chance of recovery of monosomics after selfing.

		Gametes (male parent)	
		n	n-1
Gametes (female parent)	n	2n	2n-1
	n-1	2n-1	2n-2

Fig 2.3 Scheme showing the theoretical progenies of selfed monosomic plants

This scheme, however, describes the normal situation. However, since the monosomic chromosome does not have a homologue with which to pair, it often fails to move normally to a pole during meiosis I or II. As a result, about half the time the monosomic chromosome is not included in a nucleus and appears as a micronucleus in the pollen tetrad. Therefore, only about 25% of the gametes carry all 21 chromosomes and about 75% carry only 20 chromosomes. Besides, when a monosomic plant is selfed the 20-chromosome pollen frequently fails to function due to certation, the frequency of functioning varying from 1 to 19% depending on the particular chromosome (Fig. 2.4) (Sears, 1954; Knott, 1989).

			Pollen-grains	
		Frequency (Range)	n=21 chromosomes 96%(81-99)	n-1=20 chromosomes 4%(1-19)
Eggs	n	25%(14-19)	2n=24%(11-29)	2n-1=1%(0.1-5)
	n-1	75%(61-86)	2n-1=72%(49-85)	2n-2=3%(0.6-16)

Fig. 2.4 The gametic types in monosomic wheat plants, their frequency of functioning, and the progeny from self-pollinating a monosomic plant (Sears, 1954).

The implication is, therefore, that on average about 73% of the progeny of monosomic plants are monosomic (Fig. 2.4). Selfing will consequently maintain monosomic plants and gives disomic (24%) and nullisomic (3%) plants. Nullisomics are recognized by their lack of vigor and narrow leaves. Most nullisomics are almost completely male sterile. However, the Chinese Spring nullisomics 1A, 1D, 3A, 3D, 6A, 6B, and 7D are the most fertile and can be maintained and used in crosses (Law *et al.*, 1987).

Producing monosomic series in other wheat lines

In hexaploid wheat new monosomic series can be produced using the Chinese Spring series as starting material. The procedure is outlined below (see box) following the description of Knott (1989).

- **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.**

- **Select only monosomic plants through chromosome counts and backcross up to five generations using the desired cultivar as a recurrent parent.**

- **Check the presence of genes of the recurrent lines by selfing these monosomic plants and comparing the lines with the recurrent parent.**

Potential problems in producing a new monosomic series include the occurrence of univalent/monosomic shift and reciprocal translocation while backcrossing to the recurrent parent. This would result in a different level of monosomic group (Knott, 1989).

Steps of monosomic analysis in hexaploid wheats:

Chinese Spring monosomic lines can be used to localize genes in both hexaploid and tetraploid wheats. The following is a typical procedure of monosomic analysis in hexaploid wheats (see box). The method was described by Sears (1954).

- **CS monosomic lines are crossed as females with the parent that contains the gene(s) under investigation.**

- **The chromosome number of the F_1 progenies are analyzed from pollen mother cells (PMC) during meiosis or from root tips during mitosis.**

If cytogenetic analysis of PMCs is to be carried out, spikes are collected from F_1 plants when the peduncle lengths are 1 cm. Spikes are fixed in Carnoy's solution (6 parts 95% ethanol: 3 parts chloroform: 1 part acetic acid). After 48 hours at 24°C, heads have to be transferred to 70% ethanol and stored at 2 to 4°C until cytogenetic examination. Squashes are prepared using acetocarmine. Chromosomes can be analyzed by observing under phase contrast microscope. Slides are prepared according to the method described by Belling (1921).

- **The F_1 progenies with monosomic chromosomes (2n=6x-1=41) are advanced to F_2 for further tests and/or segregation analysis.**

In the F_2 the critical and non-critical crosses are decided and the chromosome location of the gene(s) declared from a chi-square goodness of fit test on the proportions of segregants.

The F_2 progenies are analysed and the observed segregation ratios tested for conformity to the expected segregation ratio using chi-square analysis. If the phenotypic ratio in the F_2 is not significantly different from the expected ratio, this cross will be regarded as a non-critical cross. If the observed ratio approximate monosomic inheritance, the cross is a critical one. All F_2 progeny of F_1 plants in the critical cross have the gene under study (see Table 2.5).

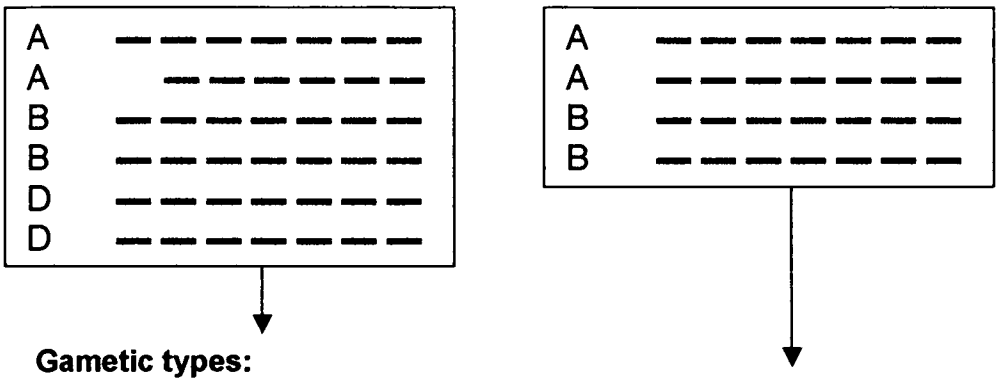
Monosomic analysis in tetraploid wheats (2n=4x=28, AABB)

Two methods might be employed to establish gene-chromosome relationships in tetraploid wheats using common wheat monosomics (Kuspira and Unrau, 1959). One method is to produce a hexaploid by crossing a tetraploid variety with *Aegilops tauschii* (2n=2x=14, DD), polyploidize the hybrid and analyze the F_1 or F_2 generations of crosses between the artificial hexaploid and a series of hexaploid monosomics. An alternative method would be to cross the tetraploid with the A- and B-genome hexaploid monosomics and analyze the F_1 or F_2 generation genetically or cytogenetically.

If tetraploids are crossed with hexaploid monosomics, two types of pentaploid hybrids will be separated (see box next page). One type has 35 chromosomes and represents an euploid pentaploid hybrid (2n=5x=35, AABBD). The other type with 34 chromosomes comprises 14 monopentaploid hybrid (2n=5x-1=34, AABBD). Both hybrids will be monosomic for chromosomes 1D to 7D and either monosomic or disomic for one of the first 14 chromosomes (Kuspira and Millis 1967; Bozzini and Giorgi, 1971).

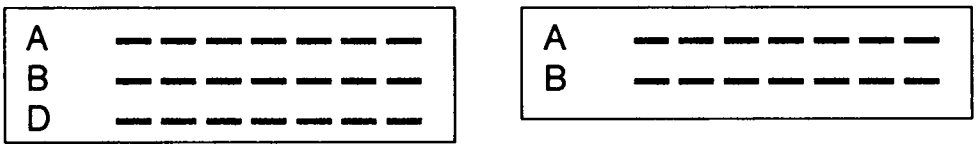
Parents:

P_1: (2n=6x-1=41, AABBDD) (e.g CS monosomic 1A) **X** **P_2: (2n=4x=28, AABB)**

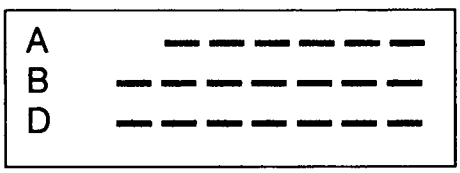


Gametic types:

(i) n=21 **n=14**

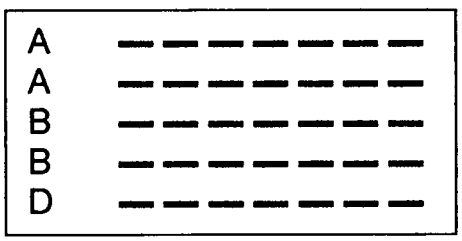


(ii) n-1=20

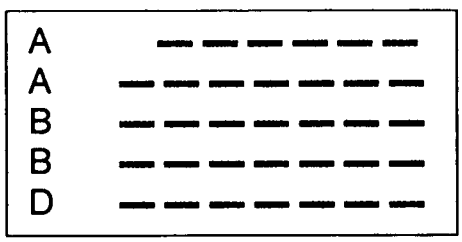


F_1:

(i) Eupentaploid (AABBD; 2n=5x=35)



(ii) Monopentaploid (AABBD, 2n=5x-1=34)



Therefore, in each monopentaploid hybrid one chromosome is represented by a single dose, coming from the donor tetraploid wheat, while the corresponding chromosome of CS wheat is absent. In such a situation the recessive and hemyzigous effective genes carried by the single tetraploid parental type chromosome can express themselves in the F_1 generation (Kuspira and Millis 1967, Bozzini and Giorgi 1971). Consequently, only recessive or partially dominant alleles of the variety to be tested can be identified and attributed to a specific chromosome in the F_1 generation (Kuspira and Millis 1967, Bozzini and Giorgi 1971; Hanchinal and Goud, 1982a). The F_1 monopentaploid hybrids were reported to be considerably sterile (Bozzini and Giorgi 1971; Hanchinal and Goud, 1982a) and seed germination has been a problem (Hanchinal and Goud, 1982b) making F_2 segregation analysis incomplete.

The use of CS monosomics to localize genes in tetraploid wheats has been a difficult task before Joppa and Williams (1977, 1983, 1988) identified the D-genome substitution lines of Langdon durum (see section 2.5.2). Before the substitution lines were made available, few attempts have been made to utilize the monosomics of bread wheats to localize genes influencing various morphological traits of tetraploid wheats (Allan and Vogel, 1960; Kuspira and Millis, 1967; Bozzini and Giorgi, 1971; Mokhtarzadeh, 1975; Giorgi, 1979; Hanchinal and Goud, 1982a). There is no report that attempted to establish gene-locations for leaf rust resistance genes in tetraploid wheats using CS AB-genome monosomics.

Steps of monosomic analysis in tetraploid wheats (2n=4x=28; AABB).

Monosomic analysis in tetraploid wheats can be carried using the following procedure (see box) (Allan and Vogel, 1960; Kuspira and Millis, 1967).

- **Sets of 14 A- and B-genome CS monosomic lines (AABBDD; 2n=6x-1=41) are crossed as maternal parent with the rust resistant accession (AABB; 2n=4x=28)**

- **The chromosome numbers of the F_1 progenies are analyzed from**

pollen mother cells (PMC) during meiosis or from root tips during mitosis.

- **The F_1 monopentaploid plants of the 14 hybrid combinations are selected.**
- **The F_2 individuals of F_1 monopentaploid plants are tested for rust reaction in the F_2.**
- **The F_2 progenies are analyzed and the observed segregation ratios tested for conformity to the expected segregation ratio (see box on page 29 on details of meiotic and chi square analyses).**

There have been limited applications of monopentaploid hybrids derived from crossing CS AB-genome monosomics with 4x wheats for genetic analysis of tetraploid wheats. Allan and Vogel (1960) tried, without success, to analyze smooth awn determination at the F_1 by crossing monosomics of Chinese Spring with durum wheat, which carried this character. It has been described that a factor located on the D-genome of CS may inhibit the expression of the recessive gene responsible for smooth awn in the A- and B-genome of durum wheat. Further, Allan and Vogel (1960) concluded that a recessive gene was incapable of expression in the hemizygous condition at the F_1. Kuspira and Millis (1967), Bozzini and Giorgi (1971), Mokhtarzadeh (1975), and Hanchinal and Goud (1981a) using this technique, attempted to identify the chromosomes controlling different quantitative characters in durum wheat. Bozzini and Giorgi (1971) outlined the chief weakness of an F_1 analysis describing that it is difficult or impossible to determine whether a difference between monosomic and disomic is due to a difference in the genes carried by the two chromosomes concerned, or whether the difference is simply due to a reduced dosage of genes which are the same on the two chromosomes. Another prerequisite for monopentaploid analysis is that attribution of genetic information to specific chromosomes is valid only if homology exists between the A- and B-genomes of CS and the tetraploid parent (Bozzini and Giorgi, 1971).

One of the difficulties of genetic analysis of tetraploids by crossing these with hexaploid monosomics is that the monopentaploid hybrid lines are partially fertile (Hanchinal and Goud, 1981b), thus causing a difficulty in studying further segregating generations. Therefore only recessive or partially dominant alleles of the variety to be tested can be identified and attributed to a specific chromosome in the F_1 generation. It is not possible to locate dominant genes in the F_1 (Mokhtarzadeh, 1975). Besides there are no reports that attempted segregation analysis of these hybrids in the F_2 or later generations.

Seed set was drastically affected in the F_1 monopentaploid hybrids (Hanchinal and Goud, 1982b). Mokhtarzadeh (1975) showed that the chromosomes 1A, 2A, 7A, 1B, 4B and 6B were suggested to carry genes that promote seed set and in the absence of these chromosomes significant reduction in seed set was observed. Disturbance in the seed setting of the interspecific hybrids might be expected as a result of interactions between A- and B- genome chromosomes originating from different sources (Pissarev, 1966). In addition, the absence of chromosomes influencing fertility and crossability may reduce significantly the fertility of the monopentaploid hybrids. Loss of chromosomes carrying genes which promote or suppress fertility can be reflected by very low or very high fertility in the monopentaploid plants when compared with the average of the monosomic lines (Bozzini and Giorgi, 1971). Based on the results of Hanchinal and Goud (1982b), chromosomes 2A, 3A, 1B, 4B, 5B and 6B in the donor durum wheat could be considered as the carriers of promoter genes for seed fertility.

With regard to seed germination, the F_1 progenies of crosses involving 1A, and 2A of CS AB-genome monosomics with tetraploid wheat had reduced germination (42.6%) (Hanchinal and Goud, 1982b). Failure to obtain viable seeds could largely be due to adverse chromosome interactions between embryo and endosperm (Stebbins, 1958) and the dosage of genes and genomes in the endosperm (Sasakuma and Maan, 1978).

When CS D-genome monosomic lines were crossed to durum wheat, Hanchinal and Goud (1982a) observed that the transmissions of monosomic condition in

the monopentaploid plants were only 52.51 percent. They attributed this to a reduced viability or inviability of n-1 spores or reduced viability of 2n-1 zygotes. This may be due to a differential transmission rate of monosomic condition to its progeny. Bozzini and Giorgi (1971) also observed such variability in transmission rates of monosomic condition in different A- and B-genome monosomic lines.

Monosomic analysis works best when the pattern of inheritance is known and one or two genes are involved. The number of genes involved and the type of gene action dictate the interpretation of monosomic analysis results (see Table 2.5). Depending on the number of genes conferring leaf rust resistance and the nature of inheritance, the expected F_2 phenotypic segregation of critical and non-critical crosses during monosomic analysis of common wheat is described in Table 2.5 (McIntosh, 1987; Knott, 1989; Kosner and Bartos,1995). Description of each model is presented following Table 2.5.

Table 2.5 Types of gene action, number of genes conditioning leaf rust resistance and the expected F_2 segregation ratios of non-critical and critical crosses.

Type of gene action and number of genes conditioning resistance	F_2 Segregation ratio	
	non-critical crosses	critical crosses
One dominant gene	3R : 1S	Proportion of resistant plants greater than the expected ratio
Two independent dominant genes	15R:1S	Proportion of resistant plants greater than the expected ratio
Two dominant complementary genes	9R:7S	3R:1S
One dominant and one recessive gene	13R:3S	Proportion of resistant plants greater than the expected ratio or 13R:3S

One dominant gene

In the case of dominant monogenic inheritance, the expected F_2 ratio of the non-critical cross yields 3R:1S. The deviation from 3:1 may occur in different

conditions. If a cross has an excess of susceptible plants, the deviation will be attributed to chance. If two crosses show an excess of resistance plants, one often will have a much larger excess than the other and is probably the critical cross. This can be confirmed by growing more plants from the two crosses. In the subsequent generation the critical cross will continue to have an excess of resistant plants and the non-critical cross will fit to a 3:1 ratio. Further confirmation is possible through meiotic chromosome counts on the susceptible plants. In the critical crosses these plants will be nullisomic or carry a product of misdivison of the univalent, while in the non-critical cross they will be dismoic, monosomic or even nullisomic.

Two independent dominant genes

When two independent dominant genes govern inheritance, the expected F_2 ratio in the 19 non-critical crosses will fit to a 15R:1S ratio. In the two critical crosses, the ratio will be >15R:1S. However, it is usually difficult to distinguish the two critical crosses from 15:1. As a result one can carry out meiotic analysis on F_2 susceptible plants. These plants from the 19 non-critical crosses could be identifiable as disomics, monosomics, or nullisomics. In the two critical crosses the susceptible plants are distinguishable as only nullisomics or misdivision products such as monotelosomics.

Two completely dominant genes giving distinctively different infection types

If two completely dominant genes that give distinct infection types such as IT 0 and IT 2 are involved, the segregation within F_2 families in the 19 non-critical cross will show 12 IT 0:3 IT 2:1 IT 4. In one critical cross all plants should be IT 0 and only a few plants will be IT 2 or IT 4. Nevertheless, the second critical cross is thought to give segregation similar to the non-critical cross.

Two dominant complementary genes

If two dominant complementary genes condition resistance the expected phenotypic ratio in the F_2 of the non-critical crosses will be 9R:7S. The two critical crosses should give about 3R:1S and can be distinguishable if F_2 families of at least 100 plants are tested. The F_3 families can be grown from

resistant F_2 plants in the apparently critical crosses. In a critical cross, about 2/3 of the families should segregate approximately to 3R:1S and 1/3 should be all or mostly resistant. No families will segregate 9R:7S. In a non-critical cross, about 4/9 of the F_3 families from resistant F_2 plants should segregate 9R:7S, 4/9 3R:1S and 1/9 all R.

One dominant and one recessive gene

If one dominant and one recessive gene are involved, the expected ratio in the F_2 of the non-critical cross should be in the order of 13R:3S. In the critical cross of a chromosome harboring the dominant gene, most of the plants should be resistant. However, in the critical cross of a chromosome carrying the recessive gene the ratio will be about 13R:3S and the cross will not easily be detectable. Further chromosome counts on the susceptible F_2 plants of a chromosome carrying the recessive gene is thought to be either monosomic or nullisomic. In the non-critical cross, about 24% of the susceptible plants will be disomic.

If the inheritance of the resistance genes involved is more complicated the individual genes should firstly be separated in different lines before carrying out monosomic analysis.

2.5.2 Langdon durum D-genome disomic substitution analysis to identify chromosomes carrying genes for wheat leaf rust resistance

The complete set of CS monosomics developed by Sears (1954) has been used to determine the chromosomal location of genes for many traits of hexaploid wheat. The same cytogenetic stocks can also be used to locate genes in tetraploid wheat but the use of a set of tetraploid wheat aneuploids would be more efficient and eliminate the confounding effect caused by the D-genome chromosomes (Joppa and Williams, 1988).

The first attempts to develop a set of monosomics in durum wheat (*Triticum turgidum* var. *durum*) were made by Mochizuki (1968, 1970). The monosomics lacked vigor, had low seed set, and averaged only 27% transmission of the monosomic condition as compared to the 73% transmission in CS.

Tetraploid wheat aneuploids, such as nullisomics, monosomics, telosomics, and other aneuploids have rarely been used in the genetic analysis of *T. turgidum*. This is because of the inability of the species to tolerate the loss of one or more chromosomes or part of a chromosome compared to hexaploid wheat. To circumvent this, Joppa and Williams (1977, 1983, 1988) have developed, characterized and discussed the uses of different aneuploid and other stocks of the durum cultivar Langdon in genetic analysis of durum wheat. These stocks include: double-ditelosomics, dimonotelosomics, D-genome substitution-monosomics, D-genome disomic substitutions, intercultivar chromosome substitution lines, and homozygous recombinant lines. The D-genome substitutions 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). It was also found that univalent shift was less of a problem in these lines than in the hexaploid monosomics, but they were still inferior to the CS monosomics in vigor, fertility, and transmission frequency.

The Langdon substitution monosomics have been used to determine the chromosomal location of genes including stem rust resistance in Langdon durum (Salazar and Joppa, 1981). The disadvantages of the substitution monosomics in genetic analysis include: a lower rate of transmission (28%) than hexaploid common wheat monosomics, the necessity for careful cytogenetic analysis to preclude translocations between the A and D or between B and D homoeologous chromosomes, the existence of considerable morphological variation among and within the different substitution monosomics, and reduced fertility of selfed substitution monosomic lines. However, the increased vigor, transmission, and fertility of durum substitution monosomics, as compared to durum monosomics, make them the method of choice in durum wheat chromosome analysis (Salazar and Joppa, 1981).

Steps in producing substitution monosomics

Joppa and Williams (1977) have outlined the procedures of producing the substitution monosomics in durum wheat Langdon (*Triticum turgidum* var. *durum*) (see box for summary).

- Cross the CS aneuploids (nullisomic for A- or B-genome and tetrasomic for a homoeologous D-genome chromosome) as females with Langdon durum.
- Grow the F_1 plants in individual pots.
- Determine the chromosome number and pairing relationships in PMC of each plant.
- 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.

- Germinate the F_2 seeds in petri dishes, sample the root tips, and count the chromosome number.
- Plants with 28 to 32 chromosomes are transferred to individual pots to grow.
- Study 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 are discarded.
- Germinate F_3 seeds in a petri dish and sample root tips. Plants with 28 chromosomes are grown in pots. Chromosome pairing in PMCs are determined and plants with 14 bivalents are backcrossed to Langdon.

Continue selection of plants with 14 bivalents and backcrossing procedure from the BC_2 to BC_5 generations.

Steps of substitution monosomic analysis

The methods described by Sears (1953) of monosomic analysis are applicable to substitution monosomic analysis in durum. The steps of the analysis are as follows:

- **Cross a durum line (carrying a dominant homozygous gene) with each of the substitution monosomics.**
- **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} by cytogenetic identification of PMCs.**
- **Analyze the F_2 progenies. The F_2 progenies of F_1 plants with chromosomal configurations of either $13_{II} + 2_I$ or 14_{II} are tested for susceptibility (ITs of 3 and 4) or resistance (ITs of 0, ;, 1, and 2) according to their reactions to the races. Progenies of F_1 substitution monosomic plants of non-critical crosses should segregate 3: 1. All F_2 progeny of F_2 substitution-monosomics in the critical cross should have the dominant phenotype.**

Langdon durum D-genome disomic substitutions

In order to reduce the cytogenetic screening required in maintaining the D-genome substitution monosomics, Joppa and Williams (1983) observed D-genome disomic substitutions among the progenies of D-genome substitution monosomics. These segregates were nullisomic for a pair of durum chromosomes and disomic for a pair of homoeologous D-genome chromosomes. In these plants, the D-genome chromosome substituted for homoeologous 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 (LDN) 1A chromosomes. A complete set of D-genome disomic substitutions includes: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. These sets are available for use in cytogenetic studies in tetraploid wheat. Their fertility and agronomic characteristics, transmission frequency, methods for use in chromosomal allocation of genes, and chromosome substitutions from one cultivar or line into another, were described by Joppa and Williams (1988).

Growing conditions

D-genome disomic substitutions aneuploids are best grown in a soil or peat mixture maintained at a temperature of 20 to 25°C. Light must be supplemented to maintain a day length of 16 h.

Determining the chromosome location of genes

Compared to CS or other hexaploid monosomics, D-genome disomic substitutions have rarely been applied to the chromosome location of genes in tetraploid wheats. The procedure for chromosomal location of genes follows the same steps described above for substitution monosomics.

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 as compared to the segregation in crosses with the 13 other disomic 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 gene under study would produce an F_1 plant monosomic for both chromosomes 7B and 7D. The 7B chromosome would come from the line under study and the 7D chromosome from the LDN substitution. The double monosomics would produce gametes (either male or female) with both monosomic chromosomes, one of them, or none.

The 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). Konzak and Joppa (1988) have analyzed a chocolate-chaff gene (designated *cc*) in durum wheat using this analysis and unambiguously assigned it to chromosome 7B. Cantrell and Joppa (1991) who localized quantitative traits such as grain yield and agronomic traits in wild emmer (*Triticum turgidum* var. *dicoccoides* L.) also used the substitution lines. They have identified genes controlling grain yield on chromosomes 4A and 4B of *T.*

dicoccoides. Chromosome 6B of this species was found to increase 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 crossability alleles in tetraploid wheat cv. Ailanmai on chromosomes 1A, 6A, and 7A.

Konzak and Joppa (1988) have found that the D-genome chromosomes often have genes that are dominant to the mutant gene under investigation.

Considerable morphological variation exists among and within the different D-genome disomic substitution lines (Salazar and Joppa, 1981). These variations may hamper the use of substitution lines in genetic analyses.

2.6 Genetic variation and analysis

Within all crop species a wide range of variation is the normal pattern. Frankel *et al.* (1995) outlined driving forces of variation within a plant population. These forces embrace inter-relationships among biotic factors, physical environment, artificial selection and plant characters, mating system, mutation, migration and dispersal.

Genetic variation is the basis of plant breeding programs. It buffers vulnerability of a crop species against biotic and abiotic stress and guarantees long-term selection gains (Messmer *et al.*, 1993; Barrett and Kidwell, 1998). An analysis of genetic relatedness among the existing germplasm of a crop species is important for designing a selection scheme and for effective management of the diversity that exists in a germ pool (Manjarrez-Sandoval *et al.*, 1997).

Different methods of analysis have been employed for genetic diversity studies in crop species. These include morphological characterization (Souza and Sorrells, 1991a; Van Beuningen and Busch, 1997b; Grzesik, 2000), pedigree analysis (Van Beuningen and Busch, 1997a), biochemical markers (Souza and Sorrells, 1991b; Tsegaye *et al.*, 1994; Labuschagne *et al.*, 2000; Metakovsky *et al.*, 2000), DNA based markers (Siedler *et al.*, 1994; Barrett and Kidwell, 1998;

Bohn *et al.*, 1999) and seed storage proteins (Souza and Sorrells, 1991b, Gregova *et al.*, 1997; Labuschagne *et al.*, 2000).

The use of morphological traits for genetic analysis depends on the magnitude of differences in the characters. These traits have been widely used to discern genetic distances in agricultural crop species (Schut *et al.*, 1997). It is often assumed that phenotypic similarities for morphological characters are accurate reflections of genotypic similarities of individuals in a crop species (Van Beuningen and Bush, 1997b).

Agronomic traits provide a true picture of the performance of an ideotype in a given environment. There are statistical packages and procedures for data analysis and interpretation of these characters. For this and other reasons these traits still continue to serve as first useful steps in genetic variation studies (Van Beuningen and Busch, 1997b). Nevertheless there are arguments against morphological traits analysis describing them as lengthy and costly processes (Cooke, 1984). Further, Smith and Smith (1989) described that genetic control of some of the traits are complex and often with epistatic genetic effect. The sensitivity of such traits to genotype x environment interaction and the subsequent requirement of replicated trials have been indicated by Yee *et al.* (1999).

Qualitative and quantitative variation

Variation within populations may be qualitative or quantitative (Griffiths *et al.*, 2000). With qualitative variation it is possible to group the individuals in clearly recognizable classes. In crosses between contrasting types, clear segregation ratios may be observed in the F_2, for instance 1 : 2 : 1 or 3 : 1 (monogenic inheritance) and 15 : 1, 12 : 3 : 1, 13 : 3, 9 : 7, 9 : 6 : 1, 9 : 3 : 3 : 1 (digenic inheritance). Many important agricultural traits, however, show continuous variation (Bos and Caligary, 1995; Asíns, 2002) and it is not possible to classify the phenotypes of individuals into distinct categories. Neither is it possible to assess all individuals of the group. Consequently, for meaningful comparisons of variation of quantitative characters, it is advisable to use the coefficient of variation (CV). The CV is the ratio of the standard deviation and the mean and

is expressed as a percentage [$CV=100(\frac{Sx}{\bar{X}})$]. To calculate the CV, the mean ($\bar{X}$) and standard deviation (S_x) can be determined from a representative sample (Falconer and Mackey, 1996; Griffiths *et al.*, 2000).

Components of quantitative variation

Knowledge of components of quantitative variation is a prerequisite for improving a particular trait through selection, estimating heritabilities and determining genetic correlations (Falconer and Mackey, 1996).

Phenotypic variation (Vp) is the result of the interaction between the genetic information (Vg) of the individual and its environment (Ve). This may be represented as Vp = Vg + Ve (Dudley and Moll, 1969; Falconer and Mackay, 1996; Griffiths *et al.*, 2000). The environmental variance (Ve) reduces selection responses by obscuring the true relationship of genotypes and phenotypes (Lynch and Walsh, 1998).

The genetic variance is of special interest to the breeder since selection response of a character depends on Vg. High Vg among genotypes is required in transgressive breeding (Kisha *et al.*, 1997). According to Dudley and Moll (1969) the total genetic variance can be portioned into additive genetic variance (V_A), dominance genetic variance (V_D) and epistatic genetic variance (V_I). The Va is the additive genetic variance contributed by individual loci with additive effect. Additive genetic variance causes resemblance between relatives and therefore determines the observable genetic properties of the population and the response to selection (Falconer and Mackay, 1996). The V_D consists of the variance due to intra-locus interaction and the V_I represents the inter-locus interaction.

Estimation of heritability

Heritability may be defined as the genetic portion of the total variability (Allard *et al.*, 1960) or a measure of the correspondence between breeding values and phenotypic values (Falconer and Mackey, 1996). Heritability estimation can be separated into two categories, depending on whether that estimation refers to

genotypic values or to breeding values. These are heritability in the wider sense or wide sense heritability [h^2_w=Vg/(Vg+Ve)] and heritability in the narrow sense or narrow sense heritability [h^2_n=Va/(Va+Vd+Vi+ Ve)].

Estimation of heritability values depends on the method of reproduction or mating system of a crop. In general, heritability estimates provide an indication of the expected response to selection for a given character. Heritability estimates vary from 0 to 1. Theoretically, traits with high heritability values will respond best to selection and can be improved easier than those with low values. The heritability estimates depend strongly on the Ve. If Ve is reduced, the h^2 increases. Many screening methods were improved by reducing the environmental variance. Resistance to pathogens forms an important part of screening aims. Field screening has often been inaccurate due to the fluctuating presence of the pathogen, other diseases, irregular distribution of the pathogen over the field, climatic variation, etc. The screening for resistance has often been made independent of these variations resulting in a strongly reduced Ve and greatly increased h^2, often close to one. Through multi-location tests over a few years one can detect genotypes that have a too large genotype by environment interaction (Parlevliet and Niks, 1992).

There are different ways of partitioning variance components and hence calculating heritability values. In identically reproducing crops such as clonally multiplied and self-fertilizing crops analysis of variance (ANOVA) has been described (Comstock and Robinson, 1948, 1952; Bos and Caligari, 1995). A very simple situation is explained below using ANOVA to partition variance components and calculate the heritability value of a character (Table 2.7). This model assumes a random sample containing I genotypes with identical reproduction, evaluated by growing in J plots each containing K plants (Bos and Caligary, 1995).

Table 2.6. A model of ANOVA when evaluating I genotypes at J plots (see Bos and Caligary, 1995)

Source of variation (SV)	Degrees of freedom (df)	Sums of square (SS)	Mean square (MS)	Expected mean square (E(MS))
Lines	I-1	SSg	MSg	$\sigma_r^2 + J\sigma_g^2$
residual	I(J-1)	SSr	MSr	σ_r^2

Where σ_r^2 is the error variance, its unbiased estimator is the mean square of residual (MSr). The quantity, σ_r^2, is regarded as an environmental variance (Ve). The component, σ_g^2, is the genotypic variance (Vg) among the tested lines. The unbiased estimator of this component is given by $\sigma_g^2 = \frac{MSg - MSr}{J}$. In this equation MSg represents the mean square of genotypes (lines). The heritability in broad sense can thus be calculated as $h_b^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$.

In cross-fertilizing crops the regression of offspring on parents has been applied to estimate heritability values (Frey and Horner, 1957; Smith and Kinman, 1965, Falconer and Mackay, 1996).

Heritability estimates do not have extrapolative power; estimates are specific to the population and the environment from which the estimate was taken (Griffiths *et al.*, 2000).

Correlation analysis

Usually the number of traits a breeder has to select for, is fairly large. Simultaneous selection for several traits has a significant effect on the selection intensity per trait and so for the response to selection for that trait (Bos and Caligary, 1995).

Two quantitative traits may vary independently of one another or they vary in association with each other. The degree of association can be expressed by the correlation coefficient (r). High correlation coefficients indicate that the variance

for one trait is largely explained by the variance of the other trait. Two types of variance can be distinguished in these associations: the explained variance (r^2), which is attributable by an independent/casual variable on the dependent/response variable and the remainder ($1-r^2$) or unexplained variance. Part of this unexplained variation is caused by the experimental error (residual variance) and part is due to differences in the response variable independent of the casual variable (Falconer and Mackay, 1996; Lynch and Walsh, 1998).

In quantitative genetics three types of correlations are of importance: phenotypic (r_P), genotypic (r_g) and environmental correlations (r_E). Phenotypic correlation measures the extent to which any two observed characters are phenotypically but linearly related. It is determined from measurements of the two characters in a number of individuals of the population. Phenotypic correlations can normally be estimated with a high degree of accuracy. Genetic correlation measures to what degree the same genes or closely linked genes cause co-variation in any given two different characters. Estimates of genetic correlations, however, usually have high standard errors because of difficulties to avoid the directional effects of confounding factors (i.e., dominance and epistatic genetic effects) on additive genetic correlation estimates (Lynch and Walsh, 1998). Furthermore, genetic correlations are strongly influenced by gene frequencies and therefore may differ markedly in different populations (Falconer and Mackay, 1996). The correlation of environmental deviations together with non-additive genetic deviations (i.e. dominance and epistatic genetic deviations) is referred to as environmental correlation (Falconer and Mackay, 1996). Correlation studies on various characters of crop plants are useful to set selection criteria in crop improvement programs. Correlation coefficients may range in value from –1 to +1. High values of genetic correlations may indicate considerable genetic association between the characters tested.

In wheat breeding programs, increased grain yield is a desired trait. Yield is a quantitative trait and is the product of inter-related variables such as number of spikes per unit area, average kernel weight and the number of kernels per spike. The direct and indirect influences of a character on yield could not be discerned from simple correlation coefficients. Simple correlation measures

mutual associations without regard to cause (Sidwell *et al.*, 1976; Alexander *et al.*, 1984; Yildirim *et al.*, 1995). Subsequently it is required to indirectly select yield via other character(s). Genetic correlations are useful if indirect selection gives greater response to selection for a character than direct selection for the same character (Falconer and Mackay, 1996).

Path coefficient analysis of characters facilitate indirect selection. The analysis was developed by Wright (1921) and later described by Wright (1923, 1934) and Li (1948, 1956). Numerous studies reported on the use of path coefficient analysis in plant breeding (Dewey and Lu, 1959; Duarte and Adams, 1972; Sidewell *et al.*, 1976; Puri *et al.*, 1982; Kang *et al.*, 1983; Miligan *et al.*, 1990; Gravois and Helms, 1992; Samonte *et al.*, 1998).

Path coefficient is a standardized partial regression coefficient that helps to measure the direct influence of one variable upon another and permits the separation of the correlation coefficient into components of direct and indirect effects. Subsequently it will ease the viewing of important relationships and discerning patterns among subsets of predictor variables. The use of this method requires a cause and effect relation among the variables, and the researcher must assign the causal system based on *a priori* grounds or experimental evidence (Dewey and Lu, 1959; Samonte *et al.*, 1998). The direct and indirect influences of a character on the response trait would not be discernible from simple correlation coefficients. Simple correlation measures mutual associations without regard to causation, the path coefficient analysis specifies the causes and measures their relative importance enabling indirect selection of a character.

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Chapter 3

3. Monosomic analysis of chromosome locations of leaf rust resistance genes in two tetraploid wheats

Abstract

A study was conducted to identify the chromosomel locations of adult-plant leaf rust resistance genes in tetraploid (2n=4x=28, AABB) wheat accessions 104 (*Triticum turgidum* subsp. *dicoccum* var. *arras*) and 127 (*T. turgidum* subsp. *durum* var. *aestivum*). Fourteen A- and B-genome Chinese Spring (CS) monosomic lines (2n=6x-1=41) were crossed as females with the resistant lines. The F_1 of each cross was subjected to meiotic chromosome analysis and monopentaploid (2n=5x-1=34, AABBD) plants were selected. Selected plants were selfed to test for rust reaction in the F_2. The F_2 segregants were inoculated during the flag leaf stage of growth with pathotype UVPrt2 of *Puccinia triticina*. The F_2 of the cross involving accession 104 and monosomic line 1A gave an excess of resistant plants, suggesting that the gene for leaf rust resistance is located on this chromosome. The analysis of accession 127 showed that F_2 plants descended from selfed monopentaploids of chromosome 4A, gave an excess number of resistant plants, indicating the occurrence of a gene for leaf rust resistance on this chromosome. However, the monopentaploid hybrids of both crosses showed a high degree of sterility and poor seed germination that made F_2 segregation analysis incomplete.

3.1 Introduction

Wheat is one of the major grain crops of the world. It is estimated that the demand for wheat will increase by 40% in 2020 from the current production of 552 million tons. About 95% of the world's wheat production comes from bread/common wheat whereas durum wheat production averages more than 30 million tons, accounting for less than 5% of the total world wheat production (Rosegrant *et al.*, 1997; Ekboir, 2002).

Wheat leaf rust caused by *Puccinica triticina* Eriks. is regarded as one of the greatest impediments to increased yield (Samborski, 1984; Schafer, 1987; Knott, 1989; Das *et al.*, 1992). Yield losses incurred by leaf rust depend on the prevailing environmental conditions and the stage of crop development at the onset of rust infection. Susceptible wheat cultivars may show a yield reduction of 5-15% or even greater (Kolmer, 1996). To counteract losses, cultural control methods, application of chemicals and use of resistant cultivars are employed by wheat growers. The use of resistant cultivars is the best option (Nelson, 1978; Knott, 1989; Raupp *et al.*, 2001). Breeding for leaf rust resistance can be achieved via pyramiding major *Lr* genes that confer complete resistance or accumulating minor *Lr* genes that confer quantitative resistance. Quantitative resistance, which is often called partial or slow rusting resistance, is more durable. This type of resistance cannot stop the infection completely but delays the spread of the disease. Wheats that show slow rusting have a longer latent period, fewer uredina, and smaller uredina size 10 to 14 days after inoculation with leaf rust compared to susceptible wheat lines (Kolmer, 1996). *Lr34* (Kolmer, 1996) and *Lr46* (Singh *et al.*, 1998) have been described as slow-rusting genes.

Earlier developed leaf rust-resistant cultivars, containing single *Lr* genes, became ineffective because of the development of new and virulent races of the pathogen (Samborski, 1982; Statler *et al.*, 1982; Pretorius, 1988; Hussien *et al.*, 1997). Consequently breeding programs are focused on developing new lines that possess additional and/or new leaf rust resistance genes (Browder, 1980; McIntosh *et al.*, 1995; Sayre *et al.*, 1998). Thus far, 50 *Lr* genes have been

reported (McIntosh *et al.*, 1998, 1999, 2000, 2002) and the search for new sources of resistance remains important.

The genetic effects of inheritance for partial leaf rust resistance are reported to be predominantly additive (Geiger and Heun, 1989; Das *et al.*, 1992; Messmer *et al.*, 2000). Besides, some crosses were found with epistatic gene action (Geiger and Heun 1989; Shaner *et al.*, 1997). Possible pleiotropic gene action was also reported for *Lr34*, where the gene was suggested to be pleiotropic or closely linked with leaf tip necrosis at anthesis, that was caused by the *Ltn* gene located on the short arm of chromosome 7D (Singh, 1992). The *Ltn* gene has been used as an indirect morphological marker of leaf rust resistance, although breeders often select against leaf tip necrosis because varieties with strong leaf tip necrosis are not readily accepted by farmers (Messmer *et al.*, 2000).

Inheritance to leaf rust resistance is often monogenic (Knott, 1989; Peusha *et al.*, 1996; Peusha and Enno, 1998; Singh *et al.*, 1998). There are, however, various reports that described oligogenic inheritance. Slow rusting was attributed to one to three genes (Geiger and Heun, 1989), prolonged latent period conditioned by four genes (Shaner *et al.*, 1997) or by at least five genes (Van der Gaag and Jacobs, 1997) and partial resistance by three genes (Kolmer and Liu, 2001). A recent report by Messmer *et al.* (2000) suggested that the Swiss winter wheat variety, 'Forno', exhibited oligogenic resistance. This variety was found to have at least six genes that contributed to the high level of durable leaf rust resistance.

Wild and close relatives of wheat are the sources of new genes for leaf rust resistance that can be exploited in wheat breeding (Sharma and Gill, 1983; Gill *et al.*, 1986; Knott, 1987, 1989; Cox *et al.*, 1992, 1993; Jiang *et al.*, 1994; Friebe *et al.*, 1996, 1997; Dubcovsky *et al.*, 1998). In general, the method of transferring alien genes to wheat largely depends on the evolutionary distance of the species involved (Friebe *et al.*, 1997). Successful transfer of genes from tetraploid wheats to hexaploid wheats have been reported by McIntosh *et al.* (1967), McIntosh and Dyck (1975), Gupta *et al.* (1991) and Dyck (1994). However, the recombined genes, may sometimes have altered expression due to the

difference in ploidy level (Kerber, 1983; Dyck, 1987). It is important to identify new sources of resistance from wild as well as close relatives of wheat to breed for durable resistance (Johnson, 1981; Knott, 1989; Wolfe, 1993). Moreover, accurate identification and characterization of germplasm will aid genetic conservation as well as exploiting the gene pool towards resistance breeding.

In an effort to select leaf rust resistant germplasm, researchers at the former Department of Plant Pathology (University of the Free State) identified two tetraploid wheat lines among 353 *Triticum* accessions that had been screened for resistance (Barnard, 1999). The selected accessions 104 (*Triticum turgidum* subsp. *dicoccum* var. *arras*) and 127 (*T. turgidum* subsp. *durum* var. *aestivum*) were considered sources of adult plant leaf rust resistance.

When a new gene for leaf rust resistance is available, its chromosome localization is useful for several reasons. Firstly, it helps to elucidate possible relationships to previously reported resistance genes. Secondly, information on the chromosomal location of the resistance genes is a first step towards finding suitable markers for marker-assisted breeding. Various cytogenetic stocks and techniques are available to assign genes to wheat chromosomes. Chinese Spring (CS) and CS-derived monosomics (*Triticum aestivum* L., 2n=6x-1=41) are among the cytogenetic stocks used to localize genes in both hexaploid and tetraploid wheats (Sears, 1954; Allan and Vogel, 1960; Kuspira and Millis, 1967; Bozzini and Giorgi, 1971; Mokhtarzadeh, 1975; Giorgi, 1979; Hanchinal and Goud, 1982a; McIntosh 1983; Knott, 1989; Marais and du Toit, 1993; Raupp *et al.*, 1993; 2001; Schroeder *et al.*, 1994; Iwaki *et al.*, 2001; Singh *et al.*, 2001, Zeller *et al.*, 2002). This study was aimed at identifying the chromosomes harboring resistance genes in two recently identified tetraploid wheat lines.

3.2 Materials and methods

3.2.1 Plant materials

Leaf rust-resistant tetraploid wheat (2n=4x=28, AABB) viz. *Triticum turgidum* subsp. *dicoccum* var. *arras* (accession 104) and *T. turgidum* subsp. *durum* var. *aestivum* (accession 127) and 14 CS A- and B-genome monosomic lines (2n=6x-

1=41, AABBDD) were used for this experiment. Accession 104 shows a trace resistance (tR) leaf rust severity and reaction type in the adult plant. It takes 89 days to flag leaf formation, has an average plant height of 140 cm and intermediate growth habit. Accession 127 has a resistant (R) adult plant leaf rust reaction type, takes 69 days to flag leaf formation, has an average plant height of 110 cm and the same growth habit as Line 104 (Barnard, 1999). Chinese Spring monosomics (CSM) (CSM1A, CSM1B, CSM2A, CSM2B, CSM3A, CSM3B, CSM4A, CSM4B, CSM5A, CSM5B, CSM6A, CSM6B, CSM7A, and CSM7B) lines were kindly made available by the Department of Genetics, University of Stellenbosch.

3.2.2 Growing conditions

Parental stocks, as well as their F_1 progenies, were grown in a temperature-controlled glasshouse. The day and night temperature of the glasshouse were maintained at 20 ± 5 °C and 14 ± 5 °C, respectively. The F_2 segregates were raised at the same temperature conditions and in a leaf rust free, air-conditioned glasshouse cubicle. Daylight was supplemented with 14 h of 120 $\mu molm^{-2}s^{-1}$ photosynthetically active radiation (PAR) that was emitted from cool white fluorescent tubes arranged directly above plants. Two weeks after planting and every fortnight after that till maturity, 35 ml of 2 g/l Chemicult hydroponic nutrient solution was applied as soil drench to each pot. Chemicult® contains macro elements (N, P, K, Ca, Mg, at respective percentages of 6.5, 2.7, 13.0, 7.7, 2.2) and microelements (Fe, Mn, B, Zn, Cu, Mo at percentages of 0.15, 0.024, 0.024, 0.005, 0.002, 0.001, respectively). For the control of aphids Metasystox® 2.5 ml/l was sprayed once at late tillering stage. Other recommended cultural practices and procedures were followed to establish and grow strong and vigorous plants.

3.2.3 Rust pathotype

Pathotype UVPrt2 of *P. triticina* was used for inoculating the F_2 individuals that descended from all the crosses. Based on the infection types on the South African differential set UVPrt2 was avirulent to *Lr1, Lr2a, Lr2b, Lr3ka, Lr11, Lr15, Lr17,*

Lr20, Lr24, Lr26, and Lr30 and virulent to *Lr2c, Lr3a, Lr3bg, Lr10, Lr14a,* and *Lr16.*

3.2.4 Preparation of fresh inoculum

For producing fresh and sufficient inoculum of UVPrt2, seedlings of the leaf rust-susceptible variety Zaragoza were grown in plastic pots in the greenhouse. When seedlings were 2 to 3 cm long a solution of maleic hydrazide (MH) was prepared at a rate of 0.3 g/l and 50 ml/pot was added at the base of seedlings of each pot. Seedlings were fertilized two days after applying MH with a solution that contained 12.5%N, 8.3%P, 4.2%K and 0.5%Zn (Omnia Fertilizer Limited) at a rate of 10 g/l and a solution of 50 ml was applied to every pot. One week-old seedlings were infected by spraying with them leaf rust urediospores of pathotype UVPrt2 that were suspended in light mineral oil. Inoculated seedlings were allowed to dry for about 30 minutes before they were incubated for 16 h by placing them in a moist chamber (100% RH). Seedlings were taken from the moist chamber and allowed to dry slowly and moved to greenhouse benches until sufficient spores were harvested for the infection of adult plants.

3.2.5 Crosses and chromosome analysis

Sets of 14 A- and B-genome CS monosomic lines (2n=6x-1=41; Fig. 3.2 A) were crossed as maternal parents with the rust resistant accessions (2n=4x=28; Fig. 3.2 B). Three sets of parental lines were planted at two weeks intervals to synchronize flowering.

Cytogenetic analyses of pollen mother cells (PMCs) were made from the F_1 progenies that were grown in a greenhouse. From each cross five to ten plants were sampled 58 days after planting and at two days intervals (Table 3.1). Tillers of each sampled plant were marked and spikes sampled when the peduncles lengths were 1 cm. Spikes were fixed in Carnoy's fluid (6 parts 95% ethanol: 3 parts chloroform: 1 part acetic acid). After 48 h at 24°C, spikes were transferred to 70% ethanol and stored at 2 to 4°C until cytogenetic examination of PMCs. Slides were prepared according to the method described by Belling

(1921). Chromosomes were squashed in 2% aceto-carmine solution. Cover slips were removed by freezing using CO_2 and permanent slides were made after soaking in ethanol and mounted in Euparol (Bown, 1956). The chromosome numbers were confirmed by counts from at least five cells of each plant and by observing under 100x magnification using phase contrast on a Nikon Microphot-FXA (Nikon Corporation, Tokyo, Japan) microscope.

3.2.6 Inoculation and incubation

The seeds of selfed monopentaploid plants from all crosses were used for F_2 tests. In the text, crosses are designated by codes, e.g. 1A4 represents a cross between CS monosomic series 1A and accession 104, or 1A7 a cross of the same monosomic line with accession 127. F_2 seeds were produced by seven (1A4, 1B4, 2B4, 4A4, 6A4, 7A4, 7B4) and eight (1A7, 1B7, 2B7, 4A7, 5A7, 6A7, 7A7, 7B7) F_1 hybrids with 34 chromosomes, respectively. For each cross 16 to 70 seeds were sown in 2-liter capacity plastic pots with an appropriate soil mix. Six to ten seeds were planted per pot. The F_1 seeds of crosses 6A4, 2B7, 5A7, and 6A7 failed to germinate. Ten seeds/pot of accessions 104 and 127 and the susceptible variety Zaragoza were grown for comparative assessment. Freshly harvested spores of pathotype UVPrt2, at standard spore concentration of 40 x 10^4 urediospores/ml oil was suspended in distilled water containing a drop of Tween20® for inoculation. The fully expanded leaves of adult plants were inoculated uniformly by applying urediniospores by means of a compressed air sprayer. Inoculated plants were allowed to dry for about 2 h before they were incubated in a moist chamber (100% RH) for 16 h. Upon completion of the dew period plants were allowed to dry slowly and moved to a 6.5 m^2 air-conditioned glasshouse cubicle.

3.2.7 Assessment

Infection types (ITs) were taken from flag leaves of the F_2 individuals 10 - 12 days post inoculation (d.p.i). The Stakman *et al.* (1962) 0 to 4 scale as modified by Roelfs *et al.* (1988) was used as a guide (Appendix I) (Table 3.2). The IT readings of 3 (medium-size uredia with/without chlorosis) and 4 (large uredia

without chlorosis or necrosis) were regarded as compatible reactions. Other readings, i.e. 0 (immune), ; (fleck), 1 (small uredia with necrosis), 2 (small to medium uredia with chlorosis or necrosis), X (mesothetic, heterogeneous infection types), Y (variable size ITs with large uredia towards the leaf tip), and Z (variable size ITs with large uredia towards the leaf base) were incompatible. Pustules that were accompanied by chlorosis or necrosis were indicated by "C" and "N"; respectively (Tables 3.2). The variations above the established pustule sizes were indicated by a plus or minus sign (McIntosh *et al.*, 1995).

3.2.8 Segregation analysis

The chromosomal locations of the resistance genes were proposed after calculation of F_2 segregation ratios according to the chi-square goodness of fit (Snedecor and Cochran, 1989): $\chi^2 = \sum_{i=1}^{k} \frac{(Oi - Ei)^2}{Ei}$, where *Oi* and *Ei* are the observed and expected frequencies of resistance and susceptible plants and K is the number of classes whose contributions are summed in finding the χ^2. The statistic, χ^2, was calculated using Agronomix Software INC. (Agrobase, 2000). The observed numbers of resistant and susceptible plants were derived from Table 3.2. The expected phenotypic segregation ratio was 13:3 for the respective resistant and susceptible plants in the crosses between accession 104 and CS monosomics. A ratio of 3R:1S was used to obtain the expected numbers of F_2 plants for the crosses between accession 127 and CSMs. The two segregation ratios were established from genetic analysis of pentaploid hybrids . The inheritance of the resistance genes in 104 and 127 were studied using pentaploid hybrids (2n=5x=35; AABBD) derived from crosses of CS A- and B-genome monosomic lines with the two accessions (H.A. Shimelis, unpublished data). In accession 104 the analysis at the F_2 showed that nine of the pentaploid hybrids (1B4, 2A4, 2B4, 3A4, 4A4, 4B4, 5B4, 6A4, and 7A4) segregated into a ratio of 13 resistant and 3 susceptible (13:3). At the F_3 the same crosses and cross 7B4 gave the 13:3 ratio. In accession 127, F_2 and F_3 segregates of 12 pentaploid hybrids were found to fit the monogenic segregation ratio of 3:1. Cross 4A7 (F_2 and F_3), 7A7 (F_3), and 7B7 (F_2) did not segregate in the order of 3:1. In both crosses the critical cross will show more resistant plants in the F_2 than

the expected genetic ratios (McIntosh, 1987). The non-critical crosses, however, will give rise to the two expected segregation ratios or a proportion of expected resistant plants different than these ratios. Besides, a contingency chi-square analysis was done and Pearson chi-square estimated to test whether the F_2 segregates of pentaploid and monopentaploid crosses could come from the same population.

3.3 Results

3.3.1 Preliminary tests

Preliminary tests with pathotype UVPrt2 indicated that it was virulent on A- and B-genome CS monosomic lines. Typical leaf rust reactions of the CS A- and B-genome monosomics and the resistant lines are depicted in Fig. 3.1. Disease reactions of the monosomic lines when tested with pathotype UVPrt2 and three other pathotypes (UVPrt3, UVPrt9 and UVPrt13) are included in Appendix III.

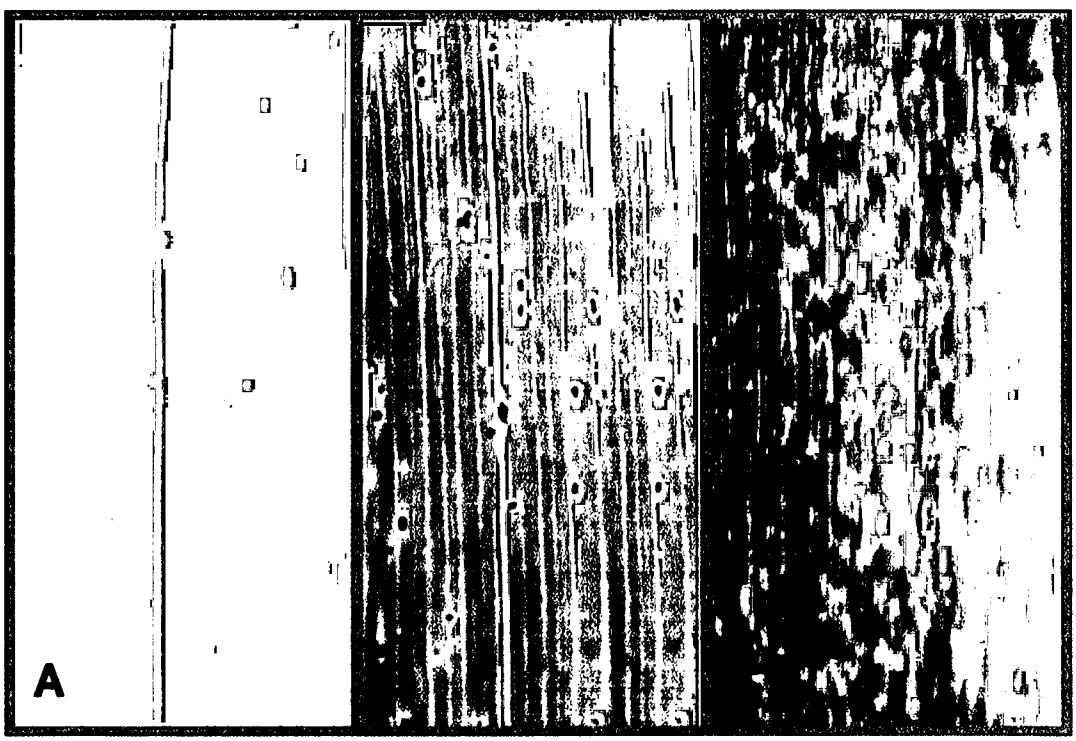

Fig. 3.1. Responses of accessions 104, IT=1N (A) and 127, IT=2C (B) and CS monosomic 4A, IT=3 (C) 10-days after inoculation with pathotype UVPrt2 of *Puccinia triticina.*

3.3.2 Selection of pentaploid hybrids

Crosses of the two tetraploids with the hexaploid monosomics produced two types of pentaploid hybrids (Table 3.1 and Fig. 3.2). One type had 34 chromosomes similar to the monopentaploid hybrid (2n=5x-1=34, AABBD). The other type with 35 chromosomes comprises an euploid pentaploid hybrid (2n=5x=35, AABBD). Both hybrids are monosomic for chromosomes 1D to 7D and either monosomic or disomic for one of the first 14 chromosomes. In each monopentaploid hybrid the monosome comes from the donor tetraploid wheat, whereas the corresponding chromosome of CS wheat is absent.

The F_1 monopentaploid plants of the 14 hybrid combinations that gave 34 chromosomes (denoted with number 1 in Table 3.1 and depicted in Fig. 3.2 C) were selected to test for rust reaction in the F_2. Selfed monosomics and F_1 plants with 35 and other chromosome numbers were discarded (Table 3.1 and Fig. 3.2 A and D).

The outcome of the chromosome analysis of F_1 plants is presented in Table 3.2. In the crosses of CSMs with accession 104, combinations 7A4 and 5B4 gave the lowest proportions of monopentaploid (14%) and pentaploid hybrids (17%), respectively. Crosses 4A4 and 7A4 gave the highest percentage of monopentaploid (67%) and pentaploid (86%), respectively. In the crosses of accession 127 with CSMs the chance of encountering monopentaploid hybrids ranged from 25% (5B7) to 57% (4B7 and 6B7) and the proportion of pentaploid plants with 2n=35 varied from 22% (1A7) to 67% (3B7). On average, for both crosses, there was about a 50% and 40% chance of selecting F_1 monopentaploid and normal or eupentaploid hybrids, respectively. It is therefore, necessary to sample an adequate number of F_1 individuals to select monopentaploid hybrids for F_2 segregation analysis.

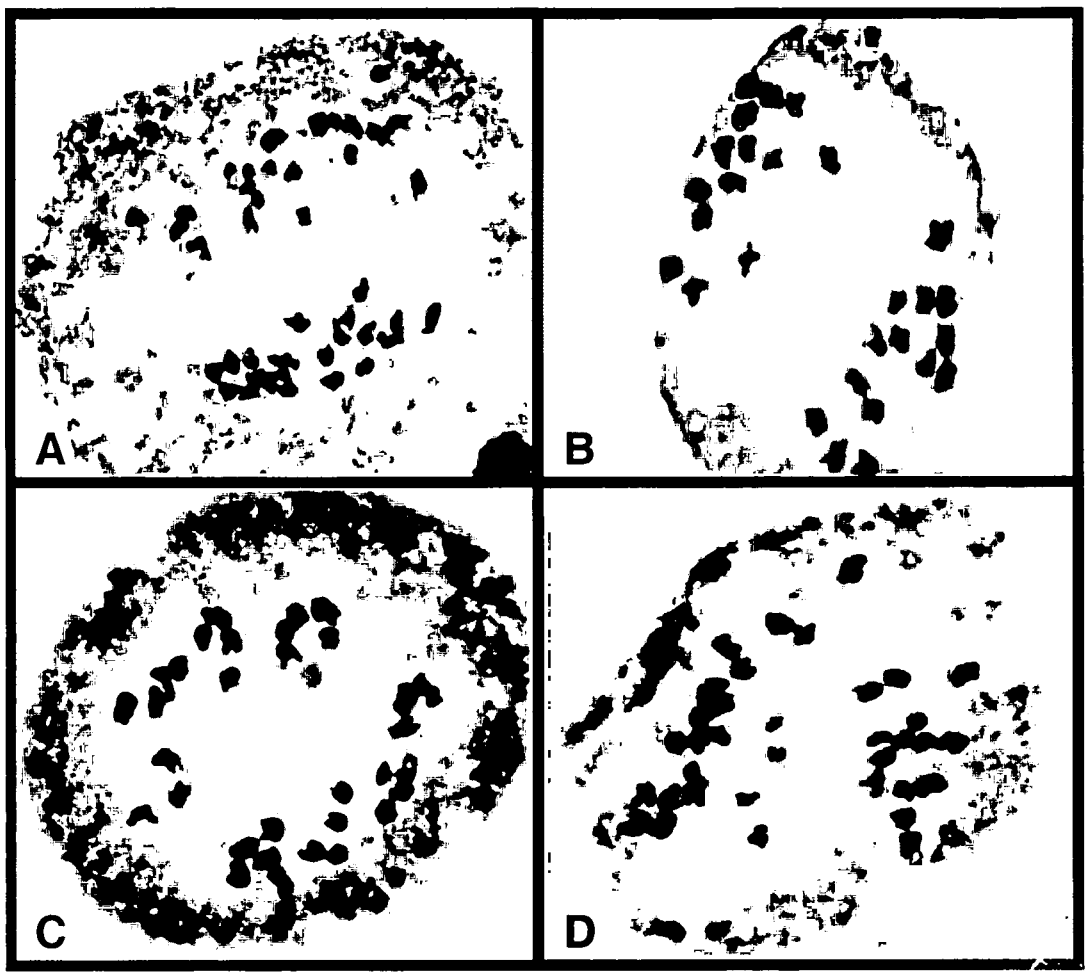

Fig. 3.2. Anaphase I chromosomes of wheat plants: (A) Chinese Spring monosomic 1A, (2n=6x-1=41), (B) Tetraploid accession 104, (2n=4x=28), (C) F_1 plant (2n=5x-1=34) that resulted from a cross of (A) and (B), (D) F_1, 2n=5x=35, arisen as indicated in "C".

Table 3.1. Summary of sampled and cytogenetically examined F_1 plants obtained after crossing Chinese Spring A- and B-genome monosomics with accessions 104 and 127. Plants with chromosome numbers of 34, 35, 41, and others are denoted as 1, 2, 3, and 4, respectively

Cross[a]	Examined F_1 plants										Cross[b]	Examined F_1 plants								
	1	2	3	4	5	6	7	8	9	10		1	2	3	4	5	6	7	8	9
C1A4	2	1	2	1	1	1	1	2	2	3	C1A7	1	2	2	1	3	1	1	4	
C1B4	1	1	1	2	1	2	2	3			C1B7	1	2	2	2	2	1	2	1	4
C2A4	1	1	1	2	2	4					C2A7	2	1	2	1	1	1	2	1	
C2B4	1	1	1	2	1	2	1	2			C2B7	2	3	2	1	2	1	1	1	2
C3A4	1	1	1	3	2	2					C3A7	1	1	2	1	1	3	2	2	1
C3B4	2	1	3	1	1	1	2	2	2		C3B7	1	2	2	2	2	1	2	1	
C4A4	1	1	1	1	2	2					C4A7	2	2	1	1	1	2	2	1	2
C4B4	1	1	1	2	3						C4B7	1	1	2	1	1	2	4		
C5A4	2	2	2	2	2	2	1	1	1	1	C5A7	1	2	2	2	1	2	1	1	
C5B4	1	1	2	1	4	4					C5B7	1	1	2	2	3	2	4	2	
C6A4	2	2	2	1	1	1	1	1			C6A7	1	1	2	1	3	2			
C6B4	1	1	1	1	2	2	2				C6B7	2	2	1	1	1	2	1		
C7A4	2	2	1	2	2	2	2				C7A7	2	1	2	1	1	1	2	2	
C7B4	1	2	1	1	2	1	2				C7B7	1	1	2	1	2	2	1	2	

a. crosses in a column are between CS monosomics and accession 104
b crosses in a column are between CS monosomics and accession 127

Table 3.2 Numbers of F_1 plants examined (crosses of CS A- and B-genome monosomics with accessions 104 and 127) and percentages with 2n=34 and 2n=35.

Cross[a]	F_1 plants			Cross[b]	F_1 plants		
	Examined	2n=34 (%)	2n=35 (%)		Examined	2n=34 (%)	2n=35 (%)
1A4	10	50	40	1A7	9	44	22
1B4	8	50	38	1B7	8	38	63
2A4	6	50	33	2A7	9	56	44
2B4	8	62	38	2B7	9	56	33
3A4	6	50	33	3A7	8	50	38
3B4	9	44	44	3B7	9	33	67
4A4	6	67	33	4A7	8	50	50
4B4	5	60	20	4B7	7	57	29
5A4	10	40	60	5A7	8	50	50
5B4	6	50	17	5B7	8	25	50
6A4	8	62	38	6A7	6	55	33
6B4	7	57	43	6B7	7	57	43
7A4	7	14	86	7A7	8	50	50
7B4	7	57	43	7B7	9	56	44

[a] crosses in a column are between CS A- and B-genome monosomics and accession 104.

[b] crosses in a column are between CS A- and B-genome monosomics and accession 127.

Since F_1 plants had favorable growing conditions, seed set was not a problem in the pentaploid F_1 plants, except that two, three and one plant(s), respectively, were sterile for the cross between accession 104 and CSM 5A, 7A and 3B. However, significant proportions of the monopentaploid hybrids were sterile. In the crosses of accession 104 with the hexaploid monosomic stocks seven monopentaploid hybrids (2A4, 3A4, 3B4, 4B4, 5A4, 5B4 and 6B4) were completely sterile. Two of the hybrids (1B4 and 6A4) were partially fertile. Similarly, in crosses of accession 127 with the monosomic stocks, six monopentaploid hybrids (2A7, 3A7, 3B7, 4B7, 5B7, and 6B7) were sterile. In

this cross monopentaploid hybrids 2B7, 5A7 and 6A7 showed reduced seed set.

3.3.3 Infection types of F_2 segregates

Data on infection types (ITs) from F_2 individuals are presented in Table 3.3. As indicated in the table the ITs of 65 plants were recorded for the crosses of monosomic 7B with 127 while ITs of 12 plants were scored for cross 1B4.

3.3.4 F_2 segregation analysis

During metaphase I the F_1 monopentaploid hybrids will be disomic for 13 chromosome pairs of the A- and B-genomes and monosomic for eight chromosomes i.e. 1D to 7D plus one chromosome of the A- or B-genome. The monosomic chromosome of the A- or B-genome will derive from the tetraploid parent with the resistance gene. For the critical A- or B- genome chromosome, these plants will produce zygotes with one or two or none of the chromosomes carrying the *Lr* allele of the tetraploid wheat. The F_2 segregation (presence/absence) of this chromosome then allows for the assignment of the gene to the specific chromosome.

The result of the F_2 segregation analyses of monopentaploid hybrids (2n=34) in line 104 is summarized in Table 3.4. Due to failure of seed set (crosses 2A4, 3A4, 3B4, 4B4, 5A4, 5B4 and 6B4) and germination failure (cross 6A4) only six crosses were available for the F_2 segregation analysis (Table 3.4). The result of the analysis suggested that the chromosome groups 2B4, 4A4, and 7B4 segregated according to the expected segregation ratio of 13R:3S. Cross 7A4 had a larger proportion of susceptible plants than expected. However, cross 1A4 was found to be the critical group that displayed all resistant plants with typical infection types of 0 (immune) to 2 (small to medium uredia with chlorosis or necrosis, Table 3.3). This critical cross, therefore, indicates that one of the *Lr* gene(s) of 104 is located on chromosome 1A. The chi square test was not possible for cross 1B4 since few F_2 plants were available for disease testing (Table 3.4).

Table 3.3 Infection types produced by F_2 segregates of selfed monopentaploid plants of crosses of CS A- and B-genome monosomics with tetraploid wheat lines 104 and 127. Inoculation was done with the pathotype UVPrt2 of *Puccinia triticina*.

No.	Cross[a]						Cross[b]				
	1A4	1B4	2B4	4A4	7A4	7B4	1A7	1B7	4A7	7A7	7B7
1	;N	1N	;N	0	3	2N	1	3C	0	4	;
2	1	3C	1	1	3	y	2N	1	;	3	;N
3	0	3	2N	;N	1N	;	;	3	;N	3	1
4	;	;N	1N	1N	3	3	1N	3C	1N	3C	2
5	;	4	;	4	3C	2	1N	3	2N	3C	2N
6	;N	2N	;N	3	4	1N	2	3	3	3	3C
7	X	1	1	0	3	2N	3C	;	1	3	1
8	0	2++	2	;	3C	;	;N	3	1N	4	1N
9	2N	1	1	2N	3	3C	3	3C	;	3	2
10	1	3C	0	1	3	2	1	3	0	0	2N
11	;	1N	;N	;	3	1	2N	3C	;	3	3
12	;N	3	1	1	3	;N	0	1N	1	3C	;
13	;		3C	4	4	2	1	4	1	3C	;N
14	1N		3	;	1	3C	2	3	3C	3	1
15	;		1	0	3C	2N	2	3C	2	3	2
16	;N		;	3C	3C	;N	4	3	1	4	3C
17	0		0	;N	3	y	3	1N	1N	3C	1
18	X		1N	1		1N	1N	3C	1N	3C	3C
19	;		2	1N		4	0	3	1	3C	3
20	2		3C	;		2	;	3	;	3C	1N
21	1		1++	1N		;N	1N	;N	;N	2N	2
22	;N		1N	2		1N	3C	4	;	3	1
23	1		;N	;N		3	3	3	0	3	;
24	;		1	3		2	1	z	;N	3	1N
25	1N		3	1		;	2N		2N	3	3
26	;N		1	2		3	1N		1N	3C	2N
27	;		0	;		2N	4		1	3C	3
28	0		1N	1		1N	4		1N	3C	4
29	0		3C			3	;		;	3C	1
30	;N		;			2N	3		1	4	2
31	;					1	2		2	3C	1N
32	1					;N	2N		1	3C	3C
33	0					1	1		2N	3	1N
34	1					;	4		1	;N	1
35	;N					;	4		;N	4	;
36						3C	3C		2	3	1
37						2	1N		2N	3	2
38						1N	2		0	3	1
39						2N	1			3	2N
40						3C	;N			3	1N
41						4				3C	3C
42						1N				3C	3
43										1N	1N

[a, b] crosses in a column are between CS monosomics and accessions 104 and 127, respectively.

... Table 3.3. Continued.

No.	Cross[a]						Cross[b]				
	1A4	1B4	2B4	4A4	7A4	7B4	1A7	1B7	4A7	7A7	7B7
44										3C	1
45										4	2
46										3	2N
47										3	1N
48										1	1
49											
50											2N
51											1N
52											3C
53											1
54											1N
55											3
56											2N
57											1N
58											1
59											2
60											3
61											1N
62											2N
63											N
64											1
65											1N

Results of the monopentaploid analysis of accession 127 is given in Table 3.4. The F_1 of crosses of the accession with the CS monosomics 2A7, 3A7, 3B7, 4B7, 5B7, and 6B7 were sterile. The monopentaploid hybrids 2B7, 5A7 and 6A7 had reduced seed set and the seeds failed to germinate. Consequently, the segregation analysis was carried out on the remaining crosses (see Table 3.4). The analysis suggested that 4A7 was the critical cross that showed the highest transmission of the resistance compared with other groups. Thus, the resistance gene in the accession may be located on chromosome 4A. Other remaining crosses segregated according to the expected segregation ratio or the proportions of resistant plants were considerably less than the expected.

Table 3.4 The F_2 segregation of F_1 selfed monopentaploid hybrids after inoculation with leaf rust pathotype UVPrt2 of *Puccinia triticina*.

Cross[a]	F_2 plants			Cross[b]	F_2 plants		
	Resistant	Susceptible	χ^2 (13:3)		Resistant	Susceptible	χ^2 (3:1)
1A4	35	0	8.077**	1A7	28	12	0.533
1B4	6	6	-	1B7	6	18	32.00
2B4	25	5	0.085	4A7	36	2	7.895***
4A4	23	5	0.015	7A7	5	43	106.8
7A4	2	15	53.878	7B7	51	14	0.415
7B4	32	10	0.706				

a, and b crosses in a column are between CSMs and accessions 104 and 127, respectively .

** and *** denote significant differences at 0.01 and 0.001 levels of probability, respectively.

A contingency chi-square analysis showed that the F_2 segregates of pentaploid hybrids of the critical cross 4A7 did not show significant differences suggesting that both segregates could come from the same population (Table 3.5). There were no susceptible plants in pentaploid hybrid 1A4 subsequently it was not possible to conduct contingency chi-square and test the associations of the F_2 data. However, the two data sets are unlikely to show any significant differences. The F_2 segregates from crosses 7A4, 1B7 and 7B7 showed significant differences suggesting that the pentaploid and monopentaploid population couldn't derive from the same population. These differences in the first two crosses could be attributed to a low number of F_2 individuals available for segregation analysis from monopentaploid hybrids.

Table 3.5 A contingency chi-square comparing the F_2 segregation of pentaploid and monopentaploid hybrids after inoculation with leaf rust pathotype UVPrt2 of *Puccinia triticina*. Hybrids derived from crosses of accessions 104 and 127 with CS A- and B-genome monosomics.

Cross[a]	F_2 plants			Cross[b]	F_2 plants		
	Resistant	Susceptible	χ^2		Resistant	Susceptible	χ^2
1A4[c]	**35**	**0**	-[d]	**1A7**	**52 (48.63)**	**10 (13.37)**	2.765
1A4	57	5		1A7	28 (31.37)	12 (8.63)	
1B4	**49 (46.4)**[e]	**16 (18.57)**	3.198	**1B7**	**58 (47.48)**	**11 (21.52)**	28.942***
1B4	6 (8.57)	6 (3.43)		1B7	6 (16.52)	18 (7.48)	
2B4	**39 (41.41)**	**16 (13.59)**	1.611	**4A7**	**63(62.83)**	**3 (3.17)**	0.027
2B4	25 (22.59)	5 (7.41)		4A7	36 (36.17)	2 (1.83)	
4A4	**45 (46.36)**	**15 (13.64)**	0.555	**7A7**	**49 (30.65)**	**14 (32.35)**	49.483***
4A4	23 (21.64)	5 (6.36)		7A7	5 (23.35)	43 (24.65)	
7A4	**45 (36.76)**	**16 (24.24)**	21.344***	**7B7**	**52 (53.41)**	**18 (16.59)**	0.325
7A4	2 (10.24)	15 (6.76)		7B7	51(49.59)	14(15.41)	
7B4	**44 (46.17)**	**21 (18.83)**	0.895				
7B4	32 (29.83)	10 (12.17)					

a and b crosses in a column are between CSMs and accessions 104 and 127, respectively.

c Bold faced scripts show segregation from pentaploid hybrid.

d Chi-square could not be calculated since there were no susceptible plants as segregates of pentaploid hybrid 1A4 giving unequi-probable cells.

e Expected frequencies are shown in brackets

*** denotes significant differences at 0.001 level of probability.

3.4 Discussion

Two methods might be employed to establish gene chromosome location in tetraploid wheats using common wheat aneuploids (Kuspira and Unrau, 1959). One method is to produce a hexaploid by crossing a tetraploid variety with *Aegilops squarrosa* (2n=2x=14, DD), polyploidize the hybrid and analyze the F_1 and F_2 generations of crosses between the artificial hexaploid and a series of hexaploid monosomics. An alternative method would be to cross the tetraploid

with the A- and B-genome hexaploid monosomics and analyze the F_1 or F_2 generation genetically or cytogenetically.

The present study attempted to localize adult-plant leaf rust resistance genes in two tetraploid wheat accessions using CS A- and B-genome monosomics (2n=6x-1=41, AABBDD). The 14 monosomic stocks were crossed with the accessions and F_1 hybrids were selected with 34 (2n=5x-1; AABBD = monopentaploid) and 35 chromosomes (2n=5x; AABBD = normal or eupentaploid). The F_2 of monopentaploid hybrids were analyzed for the segregation of the monosomic chromosome with the resistance allele from the tetraploid wheat (Bozzini and Giorgi, 1971; Hanchinal and Goud, 1982a).

The F_2 segregation analysis involving accession 104 indicated that the gene for leaf rust resistance may be located on chromosome 1A. The analysis of line 127 showed that 4A7 may be the critical cross that gave an excess of resistant plants, indicating the occurrence of a gene for leaf rust resistance on the chromosome. Only two susceptible plants were identified in cross 4A7 that were weak and with narrow leaves, characteristic of nulllisomiy (Knott, 1989). Most of the monopentaploid F_1 hybrids had considerable sterility. Consequently the F_2 segregation analysis with accession 104 failed to locate the supposed second resistance gene. Genetic analysis using pentaploid hybrids showed that accession 104 possessed dominant and recessive resistance genes (H.A. Shimelis, unpublished data).

In the present study seven F_1 hybrids of accession 104 with the 14 monosomic lines were sterile whereas six F_1 hybrids of the crosses of accession 127 with the monosomics were sterile. A relatively good F_1 seed set was found in crosses of both accessions with CS monosomics 1A, 2B, 4A, 7A, and 7B. The seeds of these crosses germinated well in the F_2. Hybrid sterility as a chief weakness of monopentaploids, derived from crossing CS AB-genome monosomics with tetraploid wheats, was discussed by Bozzini and Giorgi (1971) and Hanchinal and Goud (1982a). According to Mokhtarzadeh (1975), chromosomes 1A, 2A, 7A, 1B, 4B and 6B carried genes that promote seed and in the absence of these chromosomes significant reduction in seed was

observed. Another report from Hanchinal and Goud (1982b) indicated that chromosomes 2A, 3A, 1B, 4B, 5B and 6B of durum wheat carry promoter genes for seed fertility. Disturbance in the seed set of interspecific hybrids may be expected as a result of interactions between A- and B-genomes originating from different sources (Pissarev, 1966). Loss of chromosomes carrying genes which promote or suppress fertility can be revealed by very low or very high fertility in the monopentaploid plants when compared with the average of the monosomic lines (Bozzini and Giorgi, 1971). Kihara (1968) and Suemoto (1968) have ruled out a cytoplasmic effect of the hexaploid parent in reducing hybrid fertility. Unlike the monopentaploids, the normal pentaploid hybrids of both crosses had no sterility problem. Sasakuma and Maan (1978) also reported that the pentaploid (AABBD) produced from crossing bread and durum wheats was highly fertile (96.5%) and set seeds in 83.2% of florets.

With regard to seed germination of monopentaploids it was found that 10 seeds sown from an F_1 hybrid that resulted from crossing 104 with CS monosomic 6A failed to germinate. In addition, three F_1 monopentaploid hybrids from crosses of 127 with CS monosomics 2B, 5A and 6A failed to germinate when 12, 18, and 16 F_1 seeds were sown, respectively for F_2 analysis. The seeds of these hybrids were weak and shriveled. Such seeds could have lacked proper embryo and endosperm development. Hanchinal and Goud (1982a) reported drastic germination failures when F_1 monopentaploid seeds were planted. Hanchinal and Goud (1982b) described that seeds of F_1 progenies of crosses involving 1A, and 2A of CS AB-genome monosomics with tetraploid wheat had reduced germination (42.6%). Failure to obtain viable seeds could largely be due to abnormal chromosome interactions between embryo and endosperm (Stebbins, 1958) and the dosage unbalance between the endosperm and embryo genome constitutions (Sasakuma and Maan, 1978). However, the recent report of Aung *et al.* (1998) stated that embryo survival, germination and vigor of the pentaploid seeds were not affected by the chromosomal differences of the endosperm or outer layers.

The present study showed that sterility of the F_1 and seed germination of selfed monopentaploids were potential hindrances that would make F_2 segregation

analysis inconclusive. An additional problem of such hybrids would be the differential transmission rate of a monosomic condition to its progeny. For instance Hanchinal and Goud (1982a) reported that monopentaploid plants might show low transmission (52.51%) of the monosomic condition in the F_2. They attributed this to reduced viability or inviability of n-1 spores or reduced viability of 2n-1 zygotes. They further indicated a maximum transmission of monopentaploid condition in the F_2 (81.82%) only in the individuals of hybrids derived from crosses of CSM 5D with the tetraploid wheat. Bozzini and Giorgi (1971) also observed such variability in the transmission rate of the monosomic condition in different A- and B- genome lines.

There is no report on studies that attempted to establish gene-locations for leaf rust resistance genes in tetraploid wheats using CS AB-genome monosomics. However, few attempts have been made to utilize the monosomics of bread wheat to localize genes influencing various morphological traits of tetraploid wheats (Allan and Vogel, 1960; Kuspira and Millis, 1967; Bozzini and Giorgi, 1971; Mokhtarzadeh, 1975; Giorgi, 1979; Hanchinal and Goud, 1982a). These studies focused on analyzing monopentaploid hybrids in the F_1 generation since the tetraploid parents carried recessive and hemyzigous effective genes (Kuspira and Millis 1967, Bozzini and Giorgi 1971; Hanchinal and Goud, 1982a). Allan and Vogel (1960) for example, tried, without success, to analyze smooth awn determination in the F_1 by crossing monosomics of Chinese Spring with durum wheat, which carried this character. They illustrated that a factor located on the D-genome of CS may inhibit the expression of the recessive gene responsible for smooth awn in the A- and B-genome of durum wheat. Further, Allan and Vogel (1960) concluded that a recessive gene was incapable of expression in the hemizygous condition in the F_1. Kuspira and Millis (1967), Bozzini and Giorgi (1971), Mokhtarzadeh (1975), and Hanchinal and Goud (1981a) using this technique, attempted to identify the chromosomes controlling different quantitative characters in durum wheat. Bozzini and Giorgi (1971) outlined the primary weakness of an F_1 analysis describing that it is difficult or impossible to determine whether a difference between a monosomic and a disomic is due to a difference in the genes carried by the two chromosomes concerned, or whether the difference is simply due to a reduced dosage of

genes which are the same on the two chromosomes. An additional prerequisite for monopentaploid analysis is that attribution of genetic information to specific chromosomes is valid only if homology exists between the A- and B-genomes of CS and the tetraploid parent (Bozzini and Giorgi, 1971). Mokhtarzadeh (1975) indicated the unfeasibility of analyzing a dominant gene carried by the tetraploid parent at the F_1. Since the gene is not effective when hemizygous (Knott, 1989).

Employing the CS monosomic analysis of monopentaploid hybrids it was possible to assign a leaf rust resistance gene on chromosome 1A in accession 104 and on chromosome 4A of accession 127. To designate these genes as new it is required to carry out linkage studies with earlier reported *Lr* genes that are located on these chromosomes. Previous work indicated that wheat chromosome 1A carries *Lr10* (McIntosh *et al.*, 1998) and 4A carries *Lr28* (McIntosh *et al.*, 1982) and *Lr30* (Dyck and Kerber, 1981). Knowledge of the linkage relationship of the genes will ascertain whether they are new or similar to those reported (McIntosh *et al.*, 1998). If the linkage analysis suggested that the genes are different from earlier identified ones, mapping of the genes will be undertaken with respect to known molecular markers. Following mapping of the genes new gene symbols will be assigned for the genes in the accessions 104 and 127. Furthermore, it is equally important to identify molecular markers flanking these genes in order to screen their presence in future breeding material.

An example of inheritance governed by two complementary interacting dominant and recessive genes similar to accession 104 was reported by Davoyan *et al.* (1994, 1996). It was displayed by a genome addition synthetic hexaploid, *Triticum miguschovae,* obtained from a cross of two wild species: *T. militinae* and *Aegilops squarrosa*. The synthetic has been used for transfer of leaf rust resistance to common wheat with the two genes located on chromosomes 7B and 1D, as revealed by monosomic analysis. Many workers have reported monogenic inheritance of leaf rust resistance similar to accession 127, see for instance, Knott (1989), Peusha *et al.* (1996), Peusha and Enno (1998) and Singh *et al.* (1998). According to Friebe *et al.* (1997) the two subspecies of tetraploid wheat, *T. turgidum* subsp. *turgidum* and *T. dicoccoides,* alongside landraces of bread wheat and the donor species of the A-genome (*T.*

monococcum [2n=2x=14, AA]) and D-genome (*T. tauschii* [2n=2x=14, DD]) of bread wheat are primary gene pools sources for bread wheat. Dyck (1994) reported one accession of *Triticum turgidum* subsp. *dicoccoides* that gave excellent resistance to leaf rust. This accession had three *Lr* genes when crossed to a leaf rust susceptible durum and tested in the F_3. Two of these genes were transferred to hexaploid wheat (*T. aestivum* cv. Thatcher) by repeated backcrosses. One of the transferred genes, however, was reportedly the same as *Lr33*. In the same report the second gene gave a fleck reaction to a leaf rust race and appeared to be fully incorporated into hexaploid wheat where it segregated to a one-gene ratio. Backcross lines with this gene gave excellent resistance to leaf rust, although one race was virulent to the gene. There is no report that identified *Triticum turgidum* subsp. *dicoccum* var. *arras* and *T. turgidum* subsp. *durum* var. *aestivum* as sources of resistance genes.

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Chapter 4

4. Langdon durum D-genome disomic substitution analysis for chromosomal locations of leaf rust resistance genes in two tetraploid wheats

Abstract

Langdon durum D-genome disomic substitution lines were employed to verify the chromosomal locations of adult-plant leaf rust resistance genes in tetraploid wheat. The genes derived from two accessions viz. 104 (*Triticum turgidum* subsp. *dicoccum* var. *arras*) and 127 (*T. turgidum* subsp. *durum* var. *aestivum*). The complete sets of 14 Langdon durum D-genome disomic substitution lines were crossed as female parents with the two accessions. F_1 hybrids from each cross were used for meiotic chromosome analysis and to select F_1 individuals with 13 bivalents and two univalent chromosomes at metaphase I. Segregating F_2 plants were inoculated during the flag leaf stage with pathotype UVPrt2 of *Puccinia triticina*. The substitution analysis involving accession 104 showed that the gene for leaf rust resistance is located on chromosome 6B. The substitution analysis with accession 127 indicated that chromosome 4A carries a gene for leaf rust resistance.

4.1 Introduction

Wheat is a major food crop (Ekboir, 2002) that belongs to the genus *Triticum* and family Poaceae (Miller, 1987; Knott, 1989). Durum wheat (*Triticum turgidum* L., 2n=4x=28, AABB) and common or bread wheat (*T. aestivum* L., 2n=6x=42, AABBDD) are the two widely cultivated species.

Genetic research has been limited in durum wheat as compared to common wheat. This is the result of the limited production of durum in smaller sections of the world's total wheat production area, their limited use in the production of bread products and the unavailability of suitable cytogenetic stocks (Joppa and Cantrell, 1990). However, additional research is required on tetraploid wheats since durum wheat (*T. turgidum* var. *turgidum*) occupies a relatively larger share of the wheat production area in some areas of the world such as parts of Italy, the Middle East, Central India, Ethiopia, Argentina, Chile, Russia, Kazakhstan, the USA, Spain and Canada (Joppa and Cantrell, 1990; Ekboir, 2002). The wild forms of the species are important sources of resistance to diseases including leaf rust of wheat (Gill *et al.*, 1986; Knott, 1987, 1989; Cox *et al.*, 1992, 1993; Jiang, 1994; Friebe *et al.*, 1996, 1997; Dubcovsky *et al.*, 1998; Dhaliwal *et al.*, 2002; McIntosh *et al.*, 2002).

Wheat leaf rust caused by the fungus *Puccinia triticina* Eriks. *(=P. recondita* Rob. ex Desm. f. sp. *tritici)* causes significant yield loss in wheat (Samborski, 1984; Schafer, 1987; Knott, 1989; Das *et al.*, 1992, Messmer *et al.*, 2000, Raupp *et al.*, 2001). Susceptible cultivars show 5-15% or greater yield losses (Kolmer, 1996). A new and virulent form of the pathogen often develops after a resistant cultivar is released. Consequently the search for new sources of leaf rust resistance (*Lr*) genes and their incorporation into a susceptible cultivar is a key strategy in resistance breeding programs (Browder, 1980; McIntosh *et al.*, 1995; Sayre *et al.*, 1998).

Wheat rust workers at the University of the Free State recently selected leaf rust resistant germplasm after screening wild *Triticum* accessions that consisted of diploid, tetraploid and hexaploid species (Barnard, 1999). The selected

accessions 104 (*Triticum turgidum* subsp. *dicoccum* var. *arras*) and 127 (*T. turgidum* subsp. *durum* var. *aestivum*) carry adult plant resistance genes. In an earlier attempt to allocate the resistance genes using Chinese Spring (CS) monosomics, the gene in accession 104 was assigned to chromosome 1A (Chapter 3). The gene in accession 127 was localized on chromosome 4A, using a similar approach. It was concluded that the use of a set of tetraploid wheat aneuploids might provide more meaningful information on the chromosomal location of the genes. This was cognizant of the fact that tetraploid aneuploids have been described to avoid the confounding effects of the D-genome chromosomes of CS (Joppa and Williams, 1988).

The Langdon durum D-genome disomic substitution lines are useful tetraploid stocks to localize genes in tetraploid wheat chromosomes. Joppa and Williams (1983) selected the lines as segregates from the progenies of Langdon durum D-genome substitution monosomics. The substitution monosomics were produced from crosses of CS A- and B-genome nullisomics that were also tetrasomic for a homoeologus D-genome chromosome with Langdon durum (*T. turgidum* var. *durum*, 2n=4x=28, AABB).

The Langdon durum D-genome disomic substitution lines are nullisomic for a pair of Langdon durum A- or B- genome chromosomes and disomic for a pair of homoeologous D-genome chromosomes from CS. For example, the substitution line 1D(1A) is disomic for chromosome 1D from CS and nullisomic for the Langdon durum 1A pair of chromosomes. In the set each of the 14 A- and B-genome chromosomes of Langdon durum wheat was substituted by their respective D-genome homoeologues from CS. These materials were more vigorous and fertile than the tetraploid monosomics described by Mochizuki (1968) because of the compensation of the D-genome chromosomes (Joppa and Williams, 1977; Salazar and Joppa, 1981). The Langdon durum D-genome disomic substitution lines have been applied 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).

The major objective of the present study was to employ the Langdon durum D-genome disomic substitution lines to verify that adult-plant leaf rust resistance genes in two tetraploid wheats are located on chromosome 1A (accession 104) and 4A (accession 127) as revealed by a previous study using appropriate CS monosomics.

4.2 Materials and methods

4.2.1 Plant materials

Two tetraploid wheat accessions (2n=4x=28, AABB) viz. accessions 104 and 127 and 14 Langdon durum D-genome disomic substitution lines (2n=4x-2+2=28) were used for this study. The USDA/ARS (Northern Crop Science Lab, State University Station, Fargo, North Dakota, U.S.A) kindly supplied the substitution lines. Details of the substitution lines are presented in Table 4.1.

Table 4.1 List, code and generation of Langdon durum D-genome disomic substitution lines used in the study.

Line[a]	Code	Generation[b]
LDN1D(1A)/*12LDN	J99S 1884	F_7 O.P.
LDN1D(1B)/*12LDN	J99S 1913	F_9 O.P.
LDN2D(2A)/*12LDN	J99S 1889	F_{10} O.P.
LDN2D(2B)/*12LDN	J99S 1917	F_{10} O.P.
LDN3D(3A)/*12LDN	J99S 1891	F_9 O.P.
LDN3D(3B)/3D addition line	J99S 1919	F_3 O.P.
LDN4D(4A)/4D(4A)	J99S 1898	F_7 Self
LDN4D(4B)/*12LDN	J99S 1922	F_9 O.P.
LDN5D(5A)/*12LDN	J99S 1900	F_{10} O.P.
LDN5D(5B)/*12LDN	J99S 1927	F_9 O.P.
LDN6D(6A)/*12LDN	J99S 1905	F_9 O.P.
LDN6D(6B)/6D(6A)//6D(6B)	J99S 1932	F_7 O.P.
LDN7D(7A)/*12LDN	J99S 1908	F_9 O.P.
LDN7D(7B)/*12LDN	J99S 1936	F_{10} O.P.

[a] = pedigrees of lines is presented in accordance to the suppliers.
[b] = lines have been maintained as open pollinated (O.P.) or self for the specified generation.

4.2.2 Growing conditions

For detailed descriptions about the growing conditions see chapter 3 section 3.2.2.

4.2.3 Rust pathotype

Pathotype UVPrt2 of *P. triticina* was used for testing F_2 individuals derived from the crosses of substitution lines with resistant accessions. Relevant checks were included in the test for relative assessment. The avirulence/virulence formula of the pathotype is presented in chapter 3 section 3.2.3 and Appendix II.

4.2.4 Crosses and chromosomal analysis

The method of analysis described by Konzak and Joppa (1988) and Joppa and Cantrell (1990) was applied.

- The complete set of the substitution lines were crossed as female parents with the two accessions with resistance genes (Fig. 4.2 A and B).

- The F_1 plants were grown in a greenhouse. Each plant was sampled at early boot stage. Chromosome pairing was determined from pollen mother cells (PMCs) at metaphase I (MI) using the acetocarmine squashing technique (chapter 3, section 3.2.5) to select F_1 double monosomics ($13_{II} + 2_I$). Selection was done after confirminf from at least eight cells per plant. One of the univalents in the F_1 double monosomics would be a D-genome chromosome from the LDN disomic substitution parent and the other an A or B- genome chromosome from the resistant parent. For example, if the gene for resistance was on chromosome 2A, the cross between the D-genome substitution line 2D(2A) and the resistant accession would produce an F_1 monosomic for both chromosomes 2D and 2A. The 2A chromosome would come from the resistant line and the 2D chromosome from the Langdon aneuploid.

- The F_1 plants were selfed by covering the spikes with a glassine bag prior to flowering.

- The F_1 plants whose spikes could not be analyzed cytogenetically and plants with other chromosome configurations such as $11_{II} + 2_I$, $11_{II} + 3_I$ were discarded.

- The F_2 plants from all of the crosses with 13_{II} and 2_I were grown in a glasshouse and inoculated with the pathotype UVPrt2 to classify them as resistant or susceptible.

- Chromosomal locations were proposed after the χ^2 test for goodness of fit.

4.2.5 Inoculation and Incubation

The seeds of double monosomics from all crosses were used for F_2 tests. For each cross 60 to 70 seeds were sown in 2-liter capacity plastic pots. Ten seeds were planted per pot. The recurrent parent of the substitution lines, cultivar Langdon and the susceptible control variety Zaragoza were included. Testing procedures were as outlined in chapter 3 section 3.2.6.

4.2.6 Assessment

Assessment procedures are explained in chapter 3 section 3.2.7. 52- 70 plants were scored at F_2.

4.2.7 Segregation analysis

The procedure of the segregation analyses described in chapter 3 section 3.2.8 was followed. A contingency chi-square analysis was carried out to test whether the F_2 segregates of pentaploid and double monosomic plants could come from the same population.

4.3 Results

4.3.1 Substitution analysis

4.3.1.1 Preliminary test

Preliminary tests were carried out to test leaf rust responses of the set of substitution lines using four pathotypes (UVPrt2, UVPrt3, UVPrt9 and UVPrt13) that were used in the initial screening of the two leaf rust resistant accessions. The result indicated that pathotype UVPrt2 was the most virulent on the substitution lines except line 1D1A (see Fig. 4.1 A and B for typical leaf rust reaction and Appendix III for infection types of the substitution lines and checks). Consequently this pathotype was included for the genetic study of the resistance genes. 1D1A had a resistance reaction by showing minute uredia surrounded by some necrotic tissue after inoculation by this and three other pathotypes during preliminary tests (see Appendix III). This may suggest that the substituted chromosome 1D may harbor resistance gene.

Fig. 4.1 Leaf rust reactions of Langdon durum substitution line 2D2B, IT=3 (A) and 1D1A, IT=1N (B) ten days after inoculation by pathotype UVPrt2 of *Puccinia triticina*.

4.3.1.2 Selection of double monosomics

The proportions of sampled F_1 plants of each cross and the results from cytogenetic analysis are presented in Table 4.2. From each cross five to 12 plants were sampled 58 days after planting. The F_1 plants whose spikes were difficult for chromosomal analysis and plants with other chromosome configurations such as $11_{II} + 2_I$, $11_{II} + 3_I$ (Figure 4.2) were considered as others (Table 4.2) and discarded. The F_1 plants with 13_{II} and 2_I chromosomes were often associated with cells having two micronuclei at meiosis telophase I (Fig. 4.2 G).

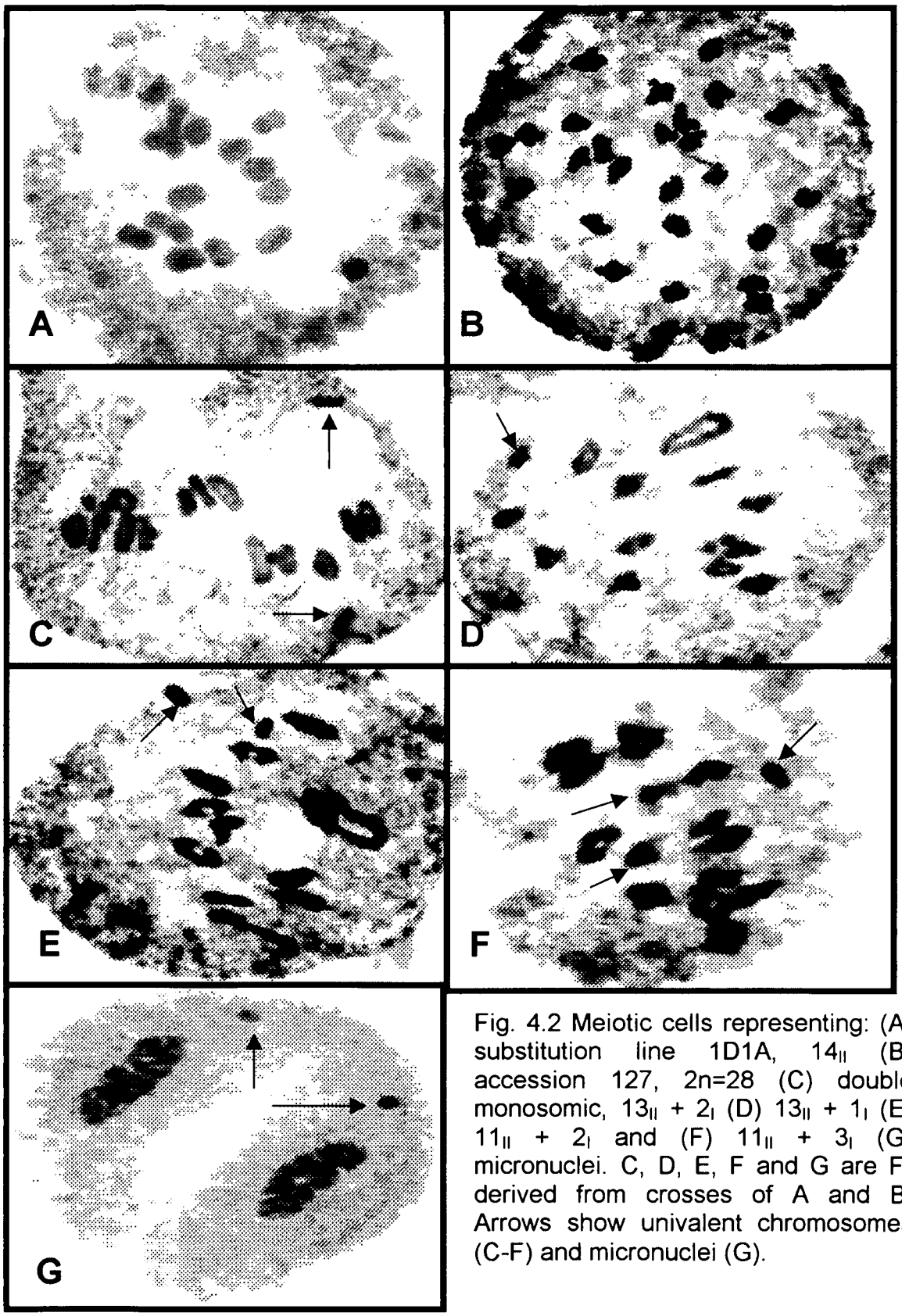

Fig. 4.2 Meiotic cells representing: (A) substitution line 1D1A, 14_{II} (B) accession 127, 2n=28 (C) double monosomic, 13_{II} + 2_{I} (D) 13_{II} + 1_{I} (E) 11_{II} + 2_{I} and (F) 11_{II} + 3_{I} (G) micronuclei. C, D, E, F and G are F_1 derived from crosses of A and B. Arrows show univalent chromosomes (C-F) and micronuclei (G).

Table 4.2 Summary of cytogenetic examinations of F_1 plants obtained after crossing Langdon durum D-genome disomic substitution lines with accessions 104 and 127. Plants with chromosome configuration of $13_{II} + 2_I$, 14_{II}, $13_{II} + 1_I$ and others are denoted as 1, 2, 3, and 4, respectively

Cross[a]	Examined F_1 plants												Cross[b]	Examined F_1 plants											
	1	2	3	4	5	6	7	8	9	10	11	12		1	2	3	4	5	6	7	8	9	10	11	12
1D1A4	1	1	1	2	1	1	1	1	3	2			1D1A7	1	1	2	2	3	3	1	2	2	2	1	1
1D1B4	1	1	1	2	3	1	2	4	4				1D1B7	2	1	2	3	1	2	1	1	2			
2D2A4	1	1	1	2	1	1	2	1	1	2	1	1	2D2A7	1	1	2	2	2	2	1	1				
2D2B4	2	3	1	1	1	1	3	2	3	2			2D2B7	1	2	2	1	1	1	1	1	2	2		
3D3A4	2	2	1	2	2	1	3	3	2	1			3D3A7	2	2	1	2	1	1	1	3	4	4	4	
3D3B4	1	1	1	1	2	2	2	2	4				3D3B7	1	1	1	1	1	2	1	2	1	2		
4D4A4	2	1	1	2	2	1	2	3					4D4A7	2	1	1	1	1	1	1	3	2			
4D4B4	1	1	1	1	2	2	2						4D4B7	1	1	3	2	1	3	2					
5D5A4	1	1	1	1	1	2	2	3	3				5D5A7	2	1	2	1	1	1	1	3				
5D5B4	2	2	1	3	1	3							5D5B7	1	1	1	1	1	1	3	3	2	2		
6D6A4	2	1	1	1	1	2	4	4					6D6A7	1	2	1	1	1	1	1	2	3	4		
6D6B4	1	1	2	2	1	3	1	1					6D6B7	2	2	1	1	2	3	3	1	3	1	1	
7D7A4	1	3	3	1	4								7D7A7	3	2	2	2	1	1						
7D7B4	1	2	1	2	1								7D7B7	1	1	2	3	3							

a. crosses in a column are between Langdon durum D-genome substitution lines and accession 104.
b. crosses in a column are between Langdon durum D-genome substitution lines and accession 127.

Table 4.3 shows the summary of the chromosome analysis of F_1 plants of the crosses of Langdon durum D-genome substitution lines with *Triticum turgidum* subsp. *dicoccum* var. *arras* and *T. turgidum* subsp. *durum* var. *aestivum*. The results indicated that in the crosses involving accession 104, group 3D3A gave the lowest (30%) and 2D2A the highest (75%) number of F_1 double monosomics. In crosses of accession 127 with the substitution lines, the chance of selecting for F_1 plants with 13 bivalents and two univalents ranged from 33% (7D7A) to 70% (3D3B). On average, and for both crosses the frequency of F_1 plants with 13_{II} and 2_I chromosomes was about 50%. F_1 plants with 14_{II} were the second most frequent group (see Table 4.2) suggesting homoeologus pairing between the D-genome and A- or B-genome chromosomes of the tetraploid resistant accessions.

Table 4.3 Numbers of F_1 plants examined and percentages of F_1 plants with 13_{II} and 2_I chromosomes obtained from the crosses of Langdon durum D-genome disomic substitution lines with accession 104 (*Triticum turgidum* subsp. *dicoccum* var. *arras)* and accession 127 (*T. turgidum* subsp. *durum* var. *aestivum*).

	F_1 plants			F_1 plants	
Cross[a]	Examined	13_{II} and 2_I (%)	Cross[b]	Examined	13_{II} and 2_I (%)
1D1A4	10	70	1D1A7	12	42
1D1B4	9	44	1D1B7	9	44
2D2A4	12	75	2D2A7	8	50
2D2B4	10	40	2D2B7	10	60
3D3A4	10	30	3D3A7	11	36
3D3B4	9	44	3D3B7	10	70
4D4A4	8	37	4D4A7	9	67
4D4B4	7	57	4D4B7	7	43
5D5A4	9	56	5D5A7	8	63
5D5B4	6	33	5D5B7	10	60
6D6A4	8	50	6D6A7	10	60
6D6B4	8	63	6D6B7	11	45
7D7A4	5	40	7D7A7	6	33
7D7B4	5	60	7D7B7	5	40

[a, b] see footnote in Table 4.2.

4.3.1.3 Infection types of F_2 segregates

The scores of infection types of F_2 individuals are presented in Tables 4.4 and 4.5. As indicated the ITs of 70 plants were scored for the crosses of 6D(6B) and 7D(7A) with 104 and 2D(2A) with 127. For crosses of 3D(3B) X 104 and 6D(6B) X 127 the ITs of 54 and 52 plants were scored , respectively.

4.3.1.4 Segregation analysis

Table 4.6 summarizes the results of the F_2 substitution analysis. The substitution analysis for accession 104 showed that chromosome groups 2D2A, 3D3A, 4D4A, 5D5A, 6D6A, 7D7A, 1D1B, 2D2B, 3D3B, 4D4B, 5D5B, and 7D7B segregated according to expected phenotypic ratios (13R:3S). In these groups the proportions of resistant plants were considerably less than expected (Table 4.6). However, the segregation of F_2 individuals in the cross 6D6B (accession 104) showed an excess of resistant plants. The observed ratio differed significantly from the expected ratio ($P<0.001$). This cross suggests that the gene for leaf rust resistance could reside on chromosome 6B of accession 104. Earlier analysis using Chinese Spring monosomics showed that accession 104 carried a resistance gene on chromosome 1A.

Table 4.4 Infection types produced by F_2 segregates when tested with pathotype UVPrt2 of *Puccinia triticina*. Crosses were between Langdon durum D-genome substitution lines and tetraploid wheat line 104.

No	Cross													
	1D1A	1D1B	2D2A	2D2B	3D3A	3D3B	4D4A	4D4B	5D5A	5D5B	6D6A	6D6B	7D7A	7D7B
1	3	2	;	1	2C	1	3	0	3	1N	1	1	3C	2C
2	1N	1	3	;	1	;	3C	2	3	1	;	1N	2	2
3	1	1	1	1	3	0	1	;	2C	1	1	;	2	3
4	3	1N	2C	2C	3	;	;	3	0	2	2C	;	1N	;
5	3	2C	3	2	;	2C	;	1	3	;	3	1	1	;
6	;	2	1	;	1	0	1N	2	;	1	;	;	2	2
7	3C	1	;	1	1N	1	2C	2	1	1	2	1	3	1
8	1N	;	1	1N	2	1	1	1N	1	3	1	2	2	1
9	1N	1	1	3	1N	2	3	2	;	1	1N	;	;	1N
10	1	3	2C	1	1	0	1	1N	0	1	1	;	2C	2C
11	2	1N	3	3	2	;	3	1	2	;	3C	1N	1N	3C
12	2	1	;	1	2C	3	1	1	1	1	3	;	1	2C
13	3	2	1	3	1	2	2	4	1	2	;	;	2	1
14	3	1	3C	3C	3	1	2C	3C	3C	1N	3	1N	2C	1
15	3C	3C	;	3	1	1	1N	3	3	1	3C	1	2	2
16	3	1	2C	;	4	2C	3	3C	1	3	1	2C	3	1N
17	3	3C	3	;	3	;	1	1N	1	1	2	;	2	3
18	1N	;	1	2	3C	0	0	0	3	1	1N	1	2	3
19	1	1N	1	3C	1N	;	;	1	;	1	2	1N	2	2C
20	1	3	3C	1	1	3	1	2	1N	3	1	1	1	1
21	2	;	3	1	1	1	1N	1	3	1	1	0	1	3
22	;	1N	2C	3	3	1	2	2	1N	;	3	;	1N	1N
23	2C	2	1N	1N	0	3	1	1	;	1N	3	;	2	2C
24	2	1	;	2	3	3	3	0	3	3	1N	;	2C	1
25	3	;	1	1N	;	2	3C	;	1N	1N	3	1	2	1
26	1N	1	2	2	4	2C	2C	2	3	;	2	1	1	3C
27	4	2C	1	1	2C	3C	1	2	1	3	1	;	1	1N
28	3	3	3C	3	2	3	;	1	2	3	1	1	;	2C
29	2	1	;	;	1	;	0	3	2	1	3	0	1	1N
30	1	3C	2	2C	;	1	1	3	1	1N	;	2	4	2
31	3	1	1N	2	1N	;	2C	3C	1N	1	1	;	3	2C
32	2	2	3	1	2	1N	1	2	;	3	;	;	2	3
33	1N	1	;	1	1	3	3	1N	3	;	2	;	3	3
34	;	3	;	1N	1	;	3C	1	1N	;	1	1N	2C	2C
35	;	1	3	3	3	1	1N	2	3	1N	1N	1	2	1
36	1	1N	1	3C	;	0	3	3C	;	1	3	;	1N	1
37	1	;	1	1	2	3C	2C	4	2	1	3	;	2	1N
38	3C	1	2	2	1	3	1N	1	3	1	1N	1N	2	2
39	2C	1N	3C	;	1	;	1N	1	1	1N	1	;	1	2
40	1	2	1N	1	1N	;	3	2	3	2	1	1	;	2C
41	1N	1N	3C	1N	2	3	;	2	;	;	2	1	1	1N
42	3C	;	1	3C	1N	1	0	;	2	1N	2C	;	1N	1
43	3	1	3	1	3C	2C	;	1N	1	1	3C	;	3	1

... Table 4.4. Continued

No.	Cross													
	1D1A	1D1B	2D2A	2D2B	3D3A	3D3B	4D4A	4D4B	5D5A	5D5B	6D6A	6D6B	7D7A	7D7B
44	1	1	1	1	1N	;	1	1N	1	1	4	1N	4	3
45	2	1	3C	2C	2	2C	2	3	2C	1	3	1	2	2C
46	1N	2C	2C	1	1	2	;	2C	1N	1N	1	1	;	2
47	1	1N	3	2	2	;	2C	3	1	1	3	2	2C	1
48	3C	2	;	;	1N	3C	2	1N	1	3C	1	;	3C	1N
49	3	1	4	2	1N	1	;	;	3C	2	1	2C	1	3C
50	2C	3	1	1N	3C	;	1	3C	3	1	3	1	;	2
51	3C	1N	1N	3	3	1N	1N	2C	1	1	3C	1N	1	3
52	1	2	2C	1N	1N	;	;	3	3C	1N	1N	1	2	3
53	2	1	1	3C	1		1	3	1	;	;	2	2	3C
54	2	0	1N	;	1		3C	2	2C	;	1	;	2	;
55	1	1	1N		;		2C	2C	;	1	;	1	2	2
56	;	;	1N		2		1N	1N	1	1N	1	;	1	3
57		3	1		1		2C	;	3	1	2C	;	1N	1N
58		1	;		3		1	1	;		0	;	3	1
59		;	1		;		1	2			3	3C	1	2
60		1N	2C		3		;	1N			2C	1	2C	1
61			;		1		;	;			1	1	4	3
62			1		0		1	0			1N	1N	1	2
63			1		3		2				1	3	1	;
64			1N		2		2C					1N	3C	1N
65			1		1N							1	3	
66			;		1							1	1N	
67					1							1	4	
68					2							;	1	
69												;	1	
70												2C	;	

Table 4.5 Infection types produced by F_2 segregates when tested with pathotype UVPrt2 of *Puccinia triticina*. Crosses were between Langdon durum D-genome substitution lines and tetraploid wheat line 127.

No	Cross													
	1D1A	1D1B	2D2A	2D2B	3D3A	3D3B	4D4A	4D4B	5D5A	5D5B	6D6A	6D6B	7D7A	7D7B
1	;	1N	1N	;	1	0	;	;	;	1	1N	2	2C	3
2	;	;	1	1	;	1N	;	1	1	2	3	1N	1	1
3	1N	;	1	1	;	;	1	2	1N	2C	1	1	1	1N
4	1N	1	2	1	1N	1	1	1	2	;	2	4	1	3C
5	2C	0	;	2	1N	1	;	1	;	1N	2	3	4	1
6	1	1	3	1N	2	2	1N	1	1	3	;	1N	;	3
7	0	2	1	2	2C	;	2C	3C	1	;	1N	;	;	;
8	0	3C	4	3	1	2C	;	1N	2C	2	0	1	1	3
9	1N	1N	;	3	3	1N	1N	1	;	1	0	3	3C	2C
10	3C	1N	;	1N	3C	2	2C	3C	2	1	1	;	3	1N
11	1	1	2C	1	1	1N	;	4	1	2	;	2	;	2
12	1	2C	1N	3C	2	2	2	1N	1N	1	1N	1N	3	3C
13	1	3	3C	3C	2	3C	1N	3	3	1	3C	3	1	1
14	;	3C	1	1	2C	1	;	3	1	2	1	3	2C	1
15	;	3	1	3	3	3	1	3C	3	1	1N	3C	3C	3
16	3	3C	2	1	;	2	;	1	1	1N	1	3	;	1
17	3	3	4	3	3	1N	1N	3	1	2	2	1N	1	3
18	4	2	4	3C	3C	2C	2	1N	1N	1	1	1	3C	1
19	1	3	2C	;	;	;	;	2	3C	3C	0	3	1	1
20	0	3	3	3	2	1N	1	1	1	0	1N	;	3	;
21	;	1	3	3	1N	2	;	2	;	;	;	1	1N	1N
22	1N	0	;	;	1	2	;	1	1N	;	2C	2	3C	1
23	1N	;	1N	1	;	;	1	1	;	1	0	;	1	2
24	4	1N	4	1	2	3C	0	2	3C	1	2	3	;	1
25	2C	1N	;	1	1	1N	1	3C	1	3C	;	1N	1	3
26	2	3	2	2C	1	1	2	1	2C	3	3	2C	3	2
27	4	;	3C	2	1	2	;	3	;	1	1N	;	1	;
28	3	2	1N	1N	3	1	1	1	1N	3C	;	4	;	1
29	2	3	3	3	1N	3	1	1	2C	1	1	3	;	3
30	3C	3C	1	;	2	1	;	3	;	3	2	1N	1	1N
31	4	3	2C	3	1	3	1	1	3	2C	2C*	1	2	3
32	;	4	1N	1N	1	1N	2C	3C	1N	1N	1N	1	;	3C
33	1	1N	1	3	2	2C	1	3	1	3C	3C	3	3C	2
34	1N	3C	1	1	2	3C	;	1N	1	2	;	;	1	1N
35	0	2C	3C	1	4	;	2	3	1N	1N	2	;	1N	1
36	1N	1N	3	3	1N	1	;	1	2	1	0	2C	3	2
37	4	0	1N	1	2C	2	1	1	1N	1	;	1	1	1
38	;	1	3C	2	;	0	;	1	3C	3C	;	3C	;	3
39	3C	1	;	3C	2	2	1	1	1N	2	2	1N	;	1N
40	;	2C	2	1	2	;	;	1N	2	2	1N	1	1	;
41	2	2	;	1N	1	1N	3	1	;	1	2	;	1	1
42	1N	0	2	3	1	3	;	2C	1N	3C	;	1N	2	3
43	1	1N	1N	;	1	;	;	1	3	1	1N	3	;	1N

... Table 4.5. Continued.

No.	Cross													
	1D1A	1D1B	2D2A	2D2B	3D3A	3D3B	4D4A	4D4B	5D5A	5D5B	6D6A	6D6B	7D7A	7D7B
44	1	3	1N	1	1N	1	;	1	1N	3	3	;	1N	1
45	1N	3C	2C	3C	2C	2C	;	1	1	1N	1N	1N	3C	2C
46	4	4	2	1	1	3C	;	3C	1	1	;	;	;	1
47	;	3C	1	3	1	3C	1	3	3	3C	3	2C	3	2
48	1	3	3	1N	2C	1N	1	3	1N	1	1	1	;	1N
49	3	;	1	2C	3	1	2C	3	3C	3	;	3	3	3C
50	0	1N	1N	1	1N	1	3	1N	;	3	2C	2	1N	1N
51	;	1	2	3	2C	3	;	1	1	3	3	3C	3	3C
52	3C	2C	2	1N	1	1N	1	1	1	3C	1	;	1	3C
53	1	;	1N	3	1N	2C	;	1	3	1	2C	1N	3	1
54	1	1N	3C	3	1	;	0	1	;	2	;	2C	1	3
55	2C	2	1N	0	;	2C	1	2	1	2	0		3	2
56	1	;	1	0	2	3	;	1N	1N	1N	3C		;	1
57	3	2C	1	;	1	1	1N	1	3C	;	0		3	2
58	0	;	2	1	2C	1	2C	1	2	3	1N		;	1N
59	;	1N	2	1	3	2C	;	3		3	1		1	;
60	3C		1N	2	1N	1	1N	1		1	1N		2C	;
61	1N		1		1	3	0	3C		3C	;		1	1N
62	;		3C		3C	1N	1	;		1	;		3	
63	2		1N		1		;	3		4	3		3	
64	2		1		2C		;	3		1	3		1	
65	1N		1				1	;					3	
66	0		2C					4					4	
67	0		1N										;	
68			2										1N	
69			1N										3	
70			1											

The substitution analysis of accession 127 showed that F_2 progenies of chromosome group 4D4A included an excess number of resistant plants (Table 4.6). The segregation ratio showed highly significant deviation from 3:1. The gene for leaf rust resistance in line 127 is, therefore, probably located on chromosome 4A. This result is in agreement with the Chinese Spring (CS) monosomic analysis that revealed that accession 127 carries the resistance gene on chromosome 4 of the A-genome. Other chromosome groups 1D1A, 2D2A, 3D3A, 5D5A, 6D6A, 7D7A, 1D1B, 2D2B, 3D3B, 4D4B, 5D5B, 6D6B, and 7D7B gave the expected segregation ratio, i.e. 3R:1S and the proportion of resistant plants were considerably different than the expected.

Table 4.6 The F_2 segregation of F_1 double monosomic plants after inoculation with leaf rust pathotype UVPrt2 of *Puccinia triticina*.

Cross[a]	F_2 plants		χ^2 (13:3)	Cross[b]	F_2 plants		χ^2 (3:1)
	Resistant	Susceptible			Resistant	Susceptible	
1D1A4	37	19	8.469**	1D1A7	51	16	0.045
1D1B4	51	9	0.554	1D1B7	40	* 19	1.633
2D2A4	50	16	1.307	2D2A7	54	16	0.171
2D2B4	41	13	1.005	2D2B7	40	20	2.222
3D3A4	52	16	1.019	3D3A7	54	10	3.000
3D3B4	41	11	0.197	3D3B7	50	12	1.054
4D4A4	52	12	0.000	4D4A7	63	2	16.662
4D4B4	47	15	1.206	4D4B7	44	22	2.444
5D5A4	41	17	4.246*	5D5A7	47	11	1.126
5D5B4	49	8	0.832	5D5B7	45	19	0.750
6D6A4	45	18	3.989	6D6A7	54	10	3.000
6D6B4	68	2	11.606***	6D6B7	38	16	0.617
7D7A4	56	14	0.072	7D7A7	46	23	2.556
7D7B4	49	15	0.923	7D7B7	44	17	0.268

[a, b] see footnote in Table 4.2 .

*, **, and *** significant differences at 0.05, 0.01 and 0.001 levels of probability, respectively .

Table 4.7 summarizes contingency chi-square tests on the association of data from F_2 segregates derived from pentaploid and double monosomic individuals. The result shows that except two crosses (1A4 and 1B7) the two data sets show non-significant differences suggesting that both segregates of the respective crosses could come from the same population. The contingency chi-square test confirmed that the F_2 data set of the two critical crosses, 6D6B4 and 4D4A7, did not show any significant differences suggesting that both segregations came from same population.

Table 4.7 A contingency chi-square comparing the F_2 segregation of pentaploid and double monosomic individuals after inoculation with leaf rust pathotype UVPrt2 of *Puccinia triticina*. F_1 pentaploids and double monosomics were derived from crosses of accessions 104 and 127 with CS A- and B-genome monosomics and D-genome substitution lines, respectively.

Cross[a]	F_2 plants		χ^2	Cross[b]	F_2 plants		χ^2
	Resistant	Susceptible			Resistant	Susceptible	
1A4[c]	**57 (49.4)**[d]	**5 (12.61)**	12.148***	**1A7**	**52 (49.50)**	**10 (12.50)**	1.202
1D1A4	37 (44.61)	19 (11.39)		1D1A7	51(53.50)	16 (13.50)	
1B4	**49 (52)**	**16 (13)**	1.803	**1B7**	**58 (52.83)**	**11 (16.17)**	4.686*
1D1B4	51 (48)	9 (12)		1D1B7	40 (45.17)	19 (13.83)	
2A4	**45 (45.24)**	**15 (14.76)**	0.009	**2A7**	**49 (49.99)**	**17 (16.01)**	0.156
2D2A4	50 (49.76)	16 (16.24)		2D2A7	54 (53.01)	16 (16.99)	
2B4	**39 (40.37)**	**16 (14.63)**	0.351	**2B7**	**45 (42.85)**	**16 (18.15)**	0.730
2D2B4	41(39.63)	13 (14.37)		2D2B7	40 (42.15)	20 (17.85)	
3A4	**47 (45.57)**	**11 (12.43)**	0.387	**3A7**	**42 (45.22)**	**15 (11.78)**	2.102
3D3A4	52 (53.43)	16 (14.57)		3D3A7	54 (50.78)	10 (13.22)	
3B4	**42 (44.46)**	**18 (15.54)**	1.136	**3B7**	**46 (48.38)**	**17 (14.62)**	1.021
3D3B4	41(38.54)	11(13.46)		3D3B7	50 (47.62)	12 (14.38)	
4A4	**45 (46.94)**	**15 (13.06)**	0.710	**4A7**	**63 (63.48)**	**3 (2.52)**	0.192
4D4A4	52 (50.06)	12 (13.94)		4D4A7	63 (62.52)	2 (2.48)	
4B4	**43 (43.88)**	**16 (15.12)**	0.136	**4B7**	**46 (42.86)**	**14 (17.14)**	1.540
4D4B4	47 (46.12)	15 (15.88)		4D4B7	44 (47.14)	22 (18.86)	
5A4	**39 (41.65)**	**24 (21.35)**	1.040	**5A7**	**45 (46.78)**	**15 (13.22)**	0.625
5D5A4	41(38.35)	17 (19.65)		5D5A7	47 (45.22)	11 (12.78)	
5B4	**45 (49.35)**	**18 (13.65)**	3.726	**5B7**	**44 (44.50)**	**20 (19.50)**	0.037
5D5B4	49 (44.65)	8 (12.35)		5D5B7	45 (44.50)	19 (19.50)	
6A4	**47 (46)**	**16 (17)**	0.161	**6A7**	**46 (50.00)**	**18 (14.00)**	2.926
6D6A4	45 (46)	18 (17)		6D6A7	54 (50)	10 (14)	
6B4	**67 (67.01)**	**2 (1.99)**	0.000	**6B7**	**46 (44.56)**	**15 (16.44)**	0.369
6D6B4	68 (67.99)	2 (2.01)		6D6B7	38 (39.44)	16 (14.56)	
7A4	**45 (47.03)**	**16 (13.97)**	0.716	**7A7**	**49 (45.34)**	**14 (17.66)**	2.015
7D7A4	56 (53.97)	14 (16.03)		7D7A7	46 (49.66)	23 (19.34)	
7B4	**44 (46.86)**	**21 (18.14)**	1.261	**7B7**	**52 (51.30)**	**18 (18.70)**	0.077
7D7B4	49 (46.14	15 (17.86)		7D7B7	44 (44.70)	17 (16.3)	

a and b crosses in a column are between CSMs or substiution lines with accessions 104 and 127, respectively.

c Bold faced scripts show segregation from pentaploid hybrid.

d Expected frequencies are shown in brackets.

* and *** denote significant differences at 0.05 and 0.001 levels of probability, respectively.

4.3.2 Comparisons of CS monosomics and substitution analyses

4.3.2.1 Selection of F_1 individuals

The outcome of the chromosome analyses of F_1 plants of the crosses of CS A and B-genome monosomics and Langdon durum D-genome substitution lines with accessions 104 and 127 are presented in Table 4.8 and Fig. 4.3.

To select monopentaploid plants with 2n=5x-1=34 for F_2 segregation analysis a total of 103 F_1 plants were cytogenetically examined in the crosses of CS monosomics with accession 104. The sample size was 113 for the other set of crosses between CS monosomics and accession 127. In the F_1 analyses of the crosses of CS monosomics with the resistant accession 104, group 7A4 (= monosomic 7A crossed with accession 104) gave the lowest (14%) of monopentaploid plants and group 4A4 the highest number (67%). In crosses of CS monosomics with accession 127, 5B7 gave the lowest number (25%) of monopentaploid plants (Table 4.8). The crosses of monosomics 4B and 6B with the same accession gave the highest number of monopentaploid plants (57%).

On average and regardless of the monosomic groups, there was about 50% success in selecting F_1 monopentaploid plants from crosses of CS A- and B-genome monosomics with accessions104 and 127 (Table 4.8 and Fig. 4.3).

The average proportion of F_1 pentaploid plants with 2n=35 were 10% less than plants with 2n=34. Other unselected plants such as selfed monosomics with 2n=41 and F_1 plants with 32, 33, etc chromosomes were discarded. The proportions of these plants were low (10%) (see Fig. 4.3).

For chromosome analyses of F_1 plants derived from crossing the substitution lines with accession 104, 116 plants and for the crosses with accession 127, 126 F_1 plants were examined. The analyses indicated that from both sets there was about a 50% frequency of F_1 plants with 13 bivalents and 2 univalent chromosomes (Fig. 4.3B). Table 4.8 indicates that the lowest proportions of double monosomic plants were 30% in the crosses of 3D3A with accession 104 (=3D3A4) and 7D7A with accession 127 (=7D7A7). The highest frequencies

were 75% obtained from crossing 2D2A with accession 104. The average proportion of F_1 double monosomic plants maintained for F_2 segregation analysis from both crosses almost equals that of F_1 monopentaploid hybrids (Fig. 4.3B). Furthermore the different proportions of unselected F_1 plants with 14_{II} (≈30%), $13_{II}+1_I$ (≈10%), and others such as $12_{II}+1_I$ and 13_{II} (≈10%) at MI were significantly less than the F_1 selects.

Table 4.8 Summary of cytogenetic examinations of F_1 plants obtained after crossing Chinese Spring A- and B-genome monosomics and Langdon durum D-genome disomic substitution lines with accession 104 (*Triticum turgidum* subsp. *dicoccum* var. *arras*) and accession 127 (*T. turgidum* subsp. *durum* var. *aestivum*).

Cross[a]	F_1 plants					Cross[b]	F_1 plants				
	Examined	2n=34	2n=35	2n=41	Others		Examined	2n=34	2n=35	2n=41	Others
	CS monosomics										
1A4	10	0.50	0.40	0.10	0.00	1A7	9	0.44	0.22	0.11	0.22
1B4	8	0.50	0.38	0.13	0.00	1B7	8	0.38	0.63	0.00	0.00
2A4	6	0.50	0.33	0.00	0.17	2A7	9	0.56	0.44	0.00	0.00
2B4	8	0.62	0.38	0.00	0.00	2B7	9	0.56	0.33	0.11	0.00
3A4	6	0.50	0.33	0.17	0.00	3A7	8	0.50	0.38	0.13	0.00
3B4	9	0.44	0.44	0.11	0.00	3B7	9	0.33	0.67	0.00	0.00
4A4	6	0.67	0.33	0.00	0.00	4A7	8	0.50	0.50	0.00	0.00
4B4	5	0.60	0.20	0.20	0.00	4B7	7	0.57	0.29	0.00	0.14
5A4	10	0.40	0.60	0.00	0.00	5A7	8	0.50	0.50	0.00	0.00
5B4	6	0.50	0.17	0.00	0.33	5B7	8	0.25	0.50	0.13	0.13
6A4	8	0.62	0.38	0.00	0.00	6A7	6	0.55	0.33	0.17	0.00
6B4	7	0.57	0.43	0.00	0.00	6B7	7	0.57	0.43	0.00	0.00
7A4	7	0.14	0.86	0.00	0.00	7A7	8	0.50	0.50	0.00	0.00
7B4	7	0.57	0.43	0.00	0.00	7B7	9	0.56	0.44	0.00	0.00
Total/mean	**103**	**0.51**	**0.40**	**0.05**	**0.04**		**113**	**0.48**	**0.41**	**0.05**	**0.04**
	D-genome substitutions										
		$13_{II} + 2_I$	14_{II}	$13_{II} + 1_I$	Others			$13_{II} + 2_I$	14_{II}	$13_{II} + 1_I$	Others
1D1A4	10	0.70	0.20	0.10	0.00	1D1A7	12	0.42	0.42	0.17	0.00
1D1B4	9	0.44	0.22	0.11	0.22	1D1B7	9	0.44	0.44	0.11	0.00
2D2A4	12	0.75	0.25	0.00	0.00	2D2A7	8	0.50	0.50	0.00	0.00
2D2B4	10	0.40	0.30	0.30	0.00	2D2B7	10	0.60	0.40	0.00	0.00
3D3A4	10	0.30	0.50	0.20	0.00	3D3A7	11	0.36	0.33	0.11	0.33
3D3B4	9	0.44	0.44	0.00	0.11	3D3B7	10	0.70	0.30	0.00	0.00
4D4A4	8	0.38	0.50	0.13	0.00	4D4A7	9	0.67	0.22	0.11	0.00
4D4B4	7	0.57	0.43	0.00	0.00	4D4B7	7	0.43	0.29	0.29	0.00
5D5A4	9	0.56	0.22	0.22	0.00	5D5A7	8	0.63	0.25	0.13	0.00
5D5B4	6	0.33	0.33	0.33	0.00	5D5B7	10	0.60	0.20	0.20	0.00
6D6A4	8	0.50	0.25	0.00	0.25	6D6A7	10	0.60	0.20	0.10	0.10
6D6B4	8	0.63	0.25	0.13	0.00	6D6B7	11	0.45	0.27	0.27	0.00
7D7A4	5	0.40	0.00	0.40	0.20	7D7A7	6	0.30	0.50	0.17	0.00
7D7B4	5	0.60	0.40	0.00	0.00	7D7B7	5	0.40	0.20	0.40	0.00
Total/mean	116	0.50	0.31	0.14	0.06		126	0.51	0.32	0.09	0.03

[a,b] See footnote in Table 4.2

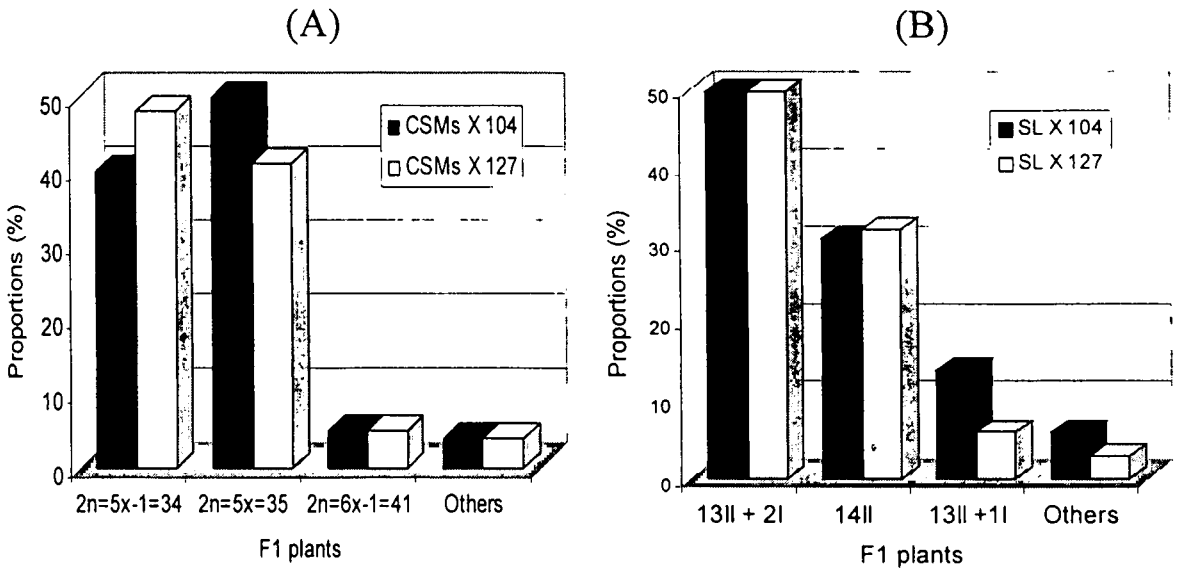

Fig. 4.3 Average proportions (%) of examined F_1 plants with different chromosome constitutions. Figure A represents crosses of CS monosomics with accessions 104 (CSMsX104) and 127 (CSMsX127) and B indicates the crosses of Langdon substitution lines with 104 (SLx104) and 127 (SLx127).

Compared to the substitution lines the CS monosomics were less vigorous and a wire support was needed to prevent lodging during crossing. The major characteristic of the D-genome substitution lines was their morphological heterogeneity. A detailed analysis of the morphological variation of these aneuploids has been given and presented in the subsequent chapter. Under greenhouse conditions the substitution lines were moderately vigorous and fertility was not a problem in the selfed F_1 double monosomic plants. However, the selfed F_1 monopentaploid hybrids showed considerable sterility. It was found that seven monopentaploid hybrids that derived from the crosses of accession 104 with the hexaploid monosomics were completely sterile. Additionally, in the same cross, two hybrids (1B4 and 6A4) were also partially fertile giving unproductive tillers. Similarly, in crosses of accession 127 with the monosomic stocks, six monopentaploid hybrids were sterile while three showed reduced seed set. Unlike the monopentaploid hybrids, seed set was less of a problem in the normal pentaploids excepting a few sterile plants in the crosses between accession 104 and CSM 5A (2 plants), 7A (3), and 3B (1).

4.3.2.2 Segregation analysis

In crosses of the resistant tetraploid with the hexaploid aneuploids, the F_2 segregation analysis for identifying the critical cross and hence the chromosome location depends on differential segregation of resistance from selfed monopentaploid plants. At metaphase I F_1 monopentaploid hybrids are disomic for 13 chromosome pairs of the A- and B-genomes and monosomic for eight chromosomes i.e. 1D to 7D plus one chromosome of the A- or B-genome. The monosomic chromosome of the A- or B-genome will derive from the tetraploid parent with the resistance gene. For the critical A- or B- genome chromosome, these plants will produce zygotes with one or two or none of the chromosomes carrying the *Lr* allele of the tetraploid wheat. The F_2 segregation (presence/absence) of this chromosome then allows for the assignment of the gene to the specific chromosome.

The results of F_2 segregation analysis (CS monosomics) involving line 104 show that the chromosome groups 2B, 4A, and 7B segregated according to expected ratios (13R:3S). In the chromosome group 7A4, the proportions of resistant plants were less than expected. F_2 progenies of the cross between 104 and monosomic line 1A gave excess numbers of resistant plants, suggesting that the gene for leaf rust resistance is located on this chromosome. Chi square test was not possible for cross 1B4 since too few F_2 plants were available for disease testing (Table 3.4, Chapter 3).

In the CS monosomic analysis of accession 127, F_2 progenies of chromosome groups 1A, and 7B gave the expected segregation ratio, i.e. 3R:1S, while in groups 1B and 7A the proportions of resistant plants were considerably less than the expected. Therefore, the gene in this accession could not be located on those chromosomes. Since chromosome 4A gave an excess of resistant plants the gene for resistance in line 127 is located on this chromosome.

The major problem of the monosomic analyses was the lack of F_2 data for many of the combinations due to sterility and poor seed germination.

The results of F_2 segregation analyses from the substitution lines are summarized in Table 4.6. In the crosses with the substitution lines, F_1 plants with 13 bivalent and two univalent chromosomes were selected. Similar to the monopentaploid hybrids the substitution analysis exploits the segregation of selfed F_1 individuals with the univalent chromosome of the resistant parent. Selfing would produce F_2 individuals with one or two or none of the chromosomes carrying the *Lr* allele of the resistant tetraploid wheat. A subsequent testing with an appropriate leaf rust pathotype yields the F_2 segregation.

The substitution analysis of the resistance gene in accession 104 indicated that the gene is located on chromosome 6B (Table 4.6). This accession, nevertheless, carried another gene on chromosome 1A as revealed from crossing CS 1A.

The substitution analysis for the resistance gene in accession 127 confirms the result obtained using CS monosomic analysis. It was observed that the substitution line with chromosome group 4D4A was the only critical cross from the F_2 segregants. In this group the 4D chromosome came from the Langdon durum substitution line and the 4A from the resistant accession that was selected carrying $13_{II} + 2_{I}$ chromosomes at the F_1. Therefore, the gene in this accession was localized on chromosome 4A.

4.4 Discussion

Various cytogenetic techniques and stocks are available to localize genes on wheat chromosomes. Among others are the hexaploid Chinese Spring (CS) or CS-derived hexaploid monosomics (AABBDD, 2n=6x-1=41) and the tetraploid Langdon durum D-genome disomic substitution cytogenetic stocks (2n=4x-2+2=28).

The tetraploid substitution lines are useful to localize genes in tetraploid wheats (Konzak and Joppa, 1988; Joppa and Cantrell, 1990; Cantrell and Joppa, 1991; Tsunewaki, 1992; Cai *et al.*, 1999) whereas CS monosomics have been utilized to localize genes in both hexaploid and tetraploid wheats (Sears, 1954; Allan

and Vogel, 1960; Kuspira and Millis, 1967; Bozzini and Giorgi, 1971; Mokhtarzadeh, 1975; Giorgi, 1979; Hanchinal and Goud, 1982a; McIntosh 1983; Knott, 1989; Marais and du Toit, 1993; Raupp *et al.*, 1993; 2001; Schroeder *et al.*, 1994; Iwaki *et al.*, 2001; Singh *et al.*, 2001; Zeller *et al.*, 2002). The rationale behind both analyses is the identification of an F_2 progeny having an aberrant segregation ratio, often referred to as the critical cross as compared to other crosses with a normal pattern of segregation (Bozzini and Giorgi, 1971; Joppa and Williams 1988; Knott, 1989). Analysis of a dominantly inherited resistance gene in tetraploid wheats using CS aneuploids utilizes an F_2 progeny of selfed F_1 monopentaploid plants (AABBD; 2n=5x-1=34) (Bozzini and Giorgi, 1971; Giorgi, 1979; Hanchinal and Goud, 1982a) while the D-genome substitution analysis relies on the progeny of selfed F_1 double monosomics (Konzak and Joppa, 1988; Joppa and Cantrell, 1990). Compared to CS or other hexaploid monosomics, D-genome disomic substitutions have seldomly been used for determining the chromosomal location of genes in tetraploid wheats.

Joppa and Williams (1988) noted that the use of tetraploid aneuploids in genetic analysis of tetraploid wheats would avoid the confounding effect of the D-genome chromosomes of the CS aneuploids. Cai *et al.* (1999) employed both the D-genome chromosome substitution lines of Langdon durum and monosomic lines of the common wheat, cultivar Abbondanza, and localized the recessive cross-ability alleles in tetraploid wheat cultivar Ailanmai on chromosomes 1, 6, and 7 of the A-genome. There has been no report that compared the two methods of analysis in locating genes in tetraploid wheats.

Resistance in accession 104 is conditioned by one dominant and one recessive gene (see Chapter 3 section 3.2.8). The 13R:3S ratio is characteristic of recessive or dominant suppressor (Griffiths *et al.*, 2002). Suppression occurs when an allele of one gene reverses the effect of another gene, resulting in the normal phenotype. For example resistance may occur in the presence of one dominant and one recessive gene say $R_1\_r_2r_2$ or $r_1r_1R_2\_$ gene combinations. If resistance was conditioned by $R_1R_1r_2r_2$ genes, these plants when crossed to a leaf rust susceptible parent e.g. $r_1r_1R_2R_2$, the phenotypes of the resistant plants at the F_2 will be $R_1\_$ $R_2\_$ (9/16), $R_1\_r_2r_2$ (3/16), and $r_1r_1r_2r_2$ (1/16) constituting

13/16. The recessive gene r_1r_1 is suppressor of the R_2 gene consequently the $r_1r_1R_2\_$ plants (3/16) would have the susceptible phenotype. Evidence on CS monosomic analysis of wheat suggested that when one dominant and one recessive gene are involved in conferring resistance, the expected ratio in the F_2 of the non-critical cross should be in order of 13R:3S. In the critical cross of a chromosome harboring the dominant gene, most of the plants should be resistant. However, in the critical cross of a chromosome carrying the recessive gene the ratio will be about 13R:3S and the cross will not be distinguishable. Further chromosome counts on the susceptible F_2 plants of a chromosome carrying the recessive gene is thought to be either monosomic or nullisomic. In the non-critical cross, about 24% of the susceptible plants will be disomic (Knott, 1989).

Based on this proposition the present study employing the D-genome substitution analysis located the resistance gene in accession 104 on chromosome 6B. The resistance gene in accession 104 presumably occurring on chromosome 6B could not be studied using the CS monosomic analyses since the appropriate F_1 monopentaploid was sterile. In accession 127 the gene was localized on chromosome 4A using the substitution lines. The chromosomal position of the gene in accession 127 was consistent with that of the earlier analysis using CS monosomics. Also, the gene in accession 104 that had been assigned to chromosome 1A by CS monosomic analysis could not be localized from the substitution analysis. From preliminary tests it was noted that line 1D1A had a resistant reaction to pathotype UVPrt2 of *P. triticina*. Line 1D1A might have possessed a suppressor gene that inhibited the expression of resistance in accession 104. The segregation of F_2 individuals that resulted from the cross of substitution line 1D1A and accession 104 may indicate that the two lines carried different resistance genes. The distortion of the F_2 segregation in cross 1D1A4 that should have given a 13R:3S ratio could be assumed due to gene interaction from the D-genome chromosome of 1D1A.

There is evidence that the D-genome of wheat may affect expression of leaf rust resistance genes present on the A- or B-genomes (The and Baker, 1975; Kerber, 1983; Dyck 1987; Bai and Knott, 1992). It has further been pointed out

by Konzak and Joppa (1988) that the D-genome chromosomes in the substitution lines often have genes that are dominant to the gene under study. Bai and Knott (1992) found that chromosomes 1D and 3D of the substitution lines carried suppressor genes for resistance to leaf rust present on the A- or B-genome of hexaploid wheats. Bai and Knott (1992) did several tests to demonstrate the occurrence of genes on D-genome chromosomes that may suppress resistance to leaf rust in bread wheat. Following crosses of 10 rust-resistant wild tetraploid wheats (*T. dicoccoides*) with both durum and bread wheats, it was found that in all cases, resistance to leaf rust was expressed in the hybrids with durum wheats but suppressed in the hybrids with bread wheats. In another set of crosses made between five durum and four bread wheats, seedlings from the pentaploid hybrid of 12 crosses were tested with leaf rust race 15 and in all cases the resistance of the durum parents was suppressed. Testing of the 14 D-genome disomic chromosome substitution lines of the durum wheat Langdon with leaf rust race 15, Bâi and Knott (1992) illustrated that chromosomes 2B and 4B carried genes for resistance to leaf rust, and 1D and 3D carried suppressors. Other crosses between 7 D-genome monosomics of Chinese Spring and three *T. dicoccoides* accessions showed that Chinese Spring possesses genes on 3D that suppresses the leaf rust resistance of all three *T. dicoccoides* accessions, plus a gene or genes on 1D that suppresses the leaf rust resistance of only one of them. They concluded that the high frequency of suppressors in the bread wheat population suggests that they must have a selective advantage. In wheat, chromosome 1D carries *Lr21* (Gill *et al.*, 1991), *Lr38* (Friebe *et al.*, 1993), *Lr41* (Cox, 1991) and *Lr42* (Cox *et al.*, 1993).

This study confirmed the application of the substitution lines in chromosomal location of genes in tetraploid wheats. It is, however, worthwhile to carry out preliminary tests of the substitution lines with the known pathotype of the rust to avoid gene interactions emerging from the lines and its subsequent camouflaging on the phenotype of the desired parent under study.

The comparative analyses of two sets of aneuploids suggested that the resistance gene in accession 127 is located on chromosome 4A. In accession 104 two genes

were localized on chromosomes 1A using CS monosomic and 6B by Langdon durum D-genome substitution analyses.

The F_1 monopentaploid plants derived from the crosses of the hexaploid monosomic series with the resistant accessions showed by considerable sterility. Consequently, the resistance gene of accession 104 that was localized on chromosome 6B by the substitution lines could not be localized by CS analysis due to its exclusion from F_2 segregation. On average, 47% of the F_1 monopentaploid hybrids from the two crosses were found to be sterile. In both crosses of the accessions with the hexaploid monosomics a relatively good seed set was found in monosomics 1A, 2B, 4A, 7A, and 7B. The seeds of these crosses germinated well in the F_2. Hybrid sterility of monopentaploids derived from crossing CS AB-genome monosomics with tetraploid wheats were reported by Bozzini and Giorgi (1971) and Hanchinal and Goud (1982a). Mokhtarzadeh (1975) suggested that chromosomes 1A, 2A, 7A, 1B, 4B and 6B carry genes that promote seed set and in the absence of these chromosomes significant reduction in seed set was observed. Based on the results of Hanchinal and Goud (1982b), chromosomes 2A, 3A, 1B, 4B, 5B and 6B in the donor durum wheat could be considered as the carriers of promoter genes for seed fertility. Disturbance in the seed set of interspecific hybrids may be expected as a result of interactions between A- and B- genomes originating from different sources (Pissarev, 1966). Loss of chromosomes carrying genes which promote or suppress fertility can be reflected by very low or very high fertility in the monopentaploid plants when compared with the average of the monosomic lines (Bozzini and Giorgi, 1971). A cytoplasmic effect of the hexaploid parent in reducing the hybrids fertility have already been ruled out by Kihara (1968) and Suemoto (1968).

Another problem of the CS monosomic analysis through F_1 monopentaploids is germination failure. It was observed that even if there was seed set in some of the hybrids about 10–18% of them showed poor germination. The seeds of the hybrids involving monosomics 2B, 5A, and 6A were shriveled. Such seeds could have lacked the proper development of embryo and endosperm. When the seeds were planted, germination was drastically reduced. To improve seed

germination it could be valuable to rescue embryos and raise them in artificial medium before planting to the soil. Hanchinal and Goud (1982b) described that seeds of the F_1 progenies of crosses involving 1A, and 2A of CS AB-genome monosomics with tetraploid wheat had reduced germination (42.6%). Failure to obtain viable seeds could largely be due to adverse chromosome interactions between embryo and endosperm (Stebbins, 1958) and the dosage unbalance between the endosperm and embryo chromosomes. The 5D chromosome of common wheat is reported to carry genes that restore seed viability (Sasakuma and Maan, 1978). However, Aung *et al.* (1998) suggested that embryo survival, germination and vigor of the pentaploid seeds were not affected by the chromosomal differences of the endosperm or outer layers.

Unlike crosses of the accessions with the monosmics there were complete seed set in the F_1 hybrids of double monosomics resulted from crosses to the substitution lines. Raised in a greenhouse the substitution lines grew vigorously and fertility of hybrids as well as germination of F_2 seeds were not a problem in F_1 hybrids. Besides seed germination was not a problem in selfed double monosomics for F_2 segregation analysis. However, the second gene in accession 104 localized on chromosome 1A by the CS analysis could not be confounded using the substitution analysis. Therefore, the gene on chromosome 1A might be recessive and therefore could not be expressed in the substitution analysis. This gene might have been suppressed by another matching gene that was on the chromosome 1D of the substitution line 1D1A. It is thus possible that there could be interference with the expression of this gene from the 1D chromosome of the 1D1A substitution lines.

Studies indicated that normal CS monosomics show a relatively high transmission frequency (73%) of the monosomic condition compared to the substitution monosomics of tetraploid wheat (Joppa and Williams, 1988; Knott, 1989) and monopentaploid hybrids (Hanchinal and Goud, 1982b). However, monosomic shift and reciprocal translocations were reported to be more of a problem in CS monosomics than with the tetraploid aneuploids. The Langdon durum D-genome substitution lines were reportedly inferior in vigor and fertility compared to CS monosomics (Joppa and Williams, 1988).

The tetraploid aneuploids were found more commendable than hexaploid monosomics for genetic analysis in the tetraploid accessions. Firstly, the F_1 monopentaploid hybrids resulting from crossing CS monosomics with the tetraploid wheats had a high degree of sterility and in some of the fertile hybrids seed germination was a problem. These made a complete F_2 segregation analysis impossible. Secondly, the smaller numbers of chromosomes in the F_1 hybrids from crosses between the tetraploid aneuploids and tetraploid disomic wheats make meiotic chromosome analysis easier than in hybrids with CS monosomics. A major problem noticed with regard to the D-genome substitution lines was their morphological heterogeneity.

It was found that accession 104 carries resistance genes on chromosomes 1A and 6B and accession 127 on chromosome 4A. Earlier work suggested that wheat chromosomes 1A carries *Lr10* (McIntosh *et al.*, 1998), 4A carries *Lr28* (McIntosh *et al.*, 1982) and *Lr30* (Dyck and Kerber, 1981), and 6B carries *Lr36* (Dvorak and Knott, 1990), *Lr3a*, *Lr3ka*, *Lr3bg* (McIntosh *et al.*, 1998) and *Lr9* (Friebe *et al.*, 1996). However, there is no report that described genes derived from *Triticum turgidum* subsp. *dicoccum* var. *arras* and *T. turgidum* subsp. *durum* var. *aestivum* as sources of leaf rust resistance. Therefore, the genes in accessions 127 and 104 are potentialy novel genes that could be useful for future exploitation by leaf rust resistance breeding programs.

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Chapter 5

5. Genetic variation and path analysis of yield and yield-related traits among Langdon durum D-genome disomic substitution lines and Langdon durum

Abstract

The Langdon durum D-genome disomic substitution lines have an important role in the study of the genetics of tetraploid wheats. These lines, however, show morphological variation that complicate genetic analyses. This study was aimed at examining the genetic variation of some important agronomic traits and associations of yield and yield related traits among the 14 substitution lines and Langdon durum (*Triticum turgidum* var. *turgidum*). Considerable morphological variations was expressed among the substitution lines when compared with Langdon durum. A high heritability value (0.96) was calculated for heading date and kernel numbers per spike. Heritability was low (0.42) for the number of fertile tillers per plant. Substitution lines 2D2B, 7D7A, and 7D7B were the most extreme of all lines giving low values for most characters. Simple correlation analysis indicated that seed yield (SY) had a highly significant ($P<0.001$) negative correlation with heading date (HD) as well as highly significant positive associations with plant height (PH), number of spikelets per spike (SP), kernel numbers per spike (KS) and 200-kernel weight (KW). The path coefficient analysis suggested true associations of SY with KW and HD only. The direct path values from this analysis revealed that there was no true association between SY and PH, SP, and KS. Improved seed yield in the aneuploids can best be achieved by directly selecting for KW. It was further demonstrated from indirect path values that selection for KW would bring about simultaneous and favorable changes to KS, SP, and PH.

5.1 Introduction

There are various methods that have been employed for genetic analysis and diversity studies in a crop species. These include characterization of agro-morphological traits (Souza and Sorrells, 1991a; Van Beuningen and Busch, 1997b; Grzesik, 2000), cytogenetic methods (Sears, 1954; Joppa and Williams, 1977, 1983, 1988), pedigree analysis (Van Beuningen and Busch, 1997a), biochemical markers (Souza and Sorrells, 1991b; Tsegaye *et al.*, 1994; Labuschagne *et al.*, 2000; Metakovsky *et al.*, 2000), DNA based markers (Siedler *et al.*, 1994; Barrett and Kidwell, 1998; Bohn *et al.*, 1999) and seed storage proteins (Souza and Sorrells, 1991b, Gregova *et al.*, 1997; Labuschagne *et al.*, 2000). Each method of analysis has its own strengths and and weak points. For instance, a weakness of the molecular markers (RFLPs, RAPDs and AFLPs) is the absence of appropriate and sufficient polymorphisms in most crop hybrids. Wheat is one of the most recalcitrant crops for polymorphisms. The approach via the use of SSRs (microsatellite) markers, however, showed high levels of inter-varietal variation and it is seen as an important development for plant breeding (Röder *et al.*, 1995; Korzun *et al.*, 1997).

A study of agro-morphological traits for genetic analysis depends on the magnitude of differences in the characters. These traits have been widely used to discern genetic similarity estimates in agricultural crop species (Schut *et al.*, 1997). It is often assumed that phenotypic similarities with respect to morphological characters are accurate reflections of genotypic similarities of individuals in a crop species (Van Beuningen and Bush, 1997b).

Agronomic traits provide a true picture of the performance of an ideotype in a given environment. There are statistical packages for data analysis and interpretation of these characters. For this and other reasons these traits still continue to serve as the first useful steps in genetic variation studies (Van Beuningen and Busch, 1997b; Grzesik, 2000). Nevertheless there is criticism against morphological trait analysis describing this approach as a lengthy and costly process (Cooke, 1984). Besides, genetic control of some traits is

complex, often with epistatic genetic effect. The analysis requires a similar location and season to get valid conclusions and comparisons (Smith and Smith, 1989). Some of the agronomic traits are sensitive to genotype x environment interaction and hence require replicated tests (Yee *et al.*, 1999).

Two quantitative traits may vary independently or in association with each other. Knowledge of the nature of associations of such traits is important to carry out simultaneous selection and achieve a greater selection response in selection programs. This is particularly important when considering traits such as seed yield where its expression is controlled by several other components (Bos and Caligary, 1995; Asíns, 2002). In wheat improvement, increased grain yield is a desired trait. This trait is a product of a number of inter-related variables (yield components) for example plant height, number of spikelets per spike, number of kernels per spike, and average kernel weight (Moghaddam *et al.*, 1997; Dencic *et al.*, 2000). The direct and indirect influences of a character on yield could not be discerned from studying mutual associations without regard to cause (Sidwell *et al.*, 1976; Alexander *et al.*, 1984; Yildirim *et al.*, 1995). Consequently it could be a prerequisite to consider other trait(s) as indirect selection criteria. Grafius (1956) indicated that it was easier to increase yield in oats by selecting yield components, which presumably are more simply inherited than yield *per se*.

The degree of association between two quantitative traits can be expressed in terms of the correlation coefficient (r). A high correlation coefficient indicates that the variance for one trait is largely explained by the variance of the other trait. For studying associations of interrelated variables, simple correlation and path coefficient analysis are worthwhile. Path coefficient analysis is a statistical technique developed by Wright (1921) and later described by Wright (1923, 1934), Li (1948, 1956), Dewey and Lu (1959) and Bhatt (1973). The analysis has been widely used by animal breeders in developing selection indices. There have also been several studies regarding the use of path coefficient analysis in plant breeding (Dewey and Lu, 1959; Duarte and Adams, 1972; Sidwell *et al.*, 1976; Puri *et al.*, 1982; Kang *et al.*, 1983; Miligan *et al.*, 1990; Gravois and Helms, 1992; Samonte *et al.*, 1998).

Path coefficient is a standardized partial regression coefficient that helps to measure the direct influence of one variable upon another and permits the separation of the correlation coefficient into components of direct and indirect effects. Subsequently it will ease examining important relationships and discerning patterns among subsets of predictor variables. The use of this method requires cause and effect relationship among the variables, and the researcher must assign the causal system based on *a priori* grounds or experimental evidence (Dewey and Lu, 1959; Samonte *et al.*, 1998). The direct and indirect influences of a character on the response trait can not be discerned from simple correlation coefficients. Simple correlation measures mutual associations without regard to cause. The path coefficient analysis specifies the causes and measures their relative importance. Except for the work of Samonte *et al.* (1998) that treated first, second and third-order yield related traits, most of the path analyses on grain yield and yield components (Vlek *et al.*, 1979; Gravois and Helms, 1992; Gravois and McNew, 1993) considered only a few first order variables as yield components.

In bread wheat (2n=6x=42, AABBDD), the availability of suitable genetic stocks greatly enhanced cytogenetic studies. Many of the studies in hexaploid wheat have been carried out using sets of Chinese Spring (CS) aneuploids developed by Sears (1954). The same cytogenetic stocks can be used for genetic analysis in tetraploid wheats (*T. turgidum* L.). However, the use of a set of tetraploid wheat aneuploids would be more efficient and eliminate the confounding effect of the D-genome chromosomes from CS (Joppa and Williams, 1988).

Until the early 1980s tetraploid wheat aneuploids, such as nullisomics, monosomics, telosomics, and other aneuploids, were rarely employed in genetic analysis of tetraploid wheats. This is because of the inability of the species to tolerate the loss of one or more chromosome or part of a chromosome compared to hexaploid wheat. To circumvent this, Joppa and Williams (1977, 1983, 1988) have developed, characterized and discussed the uses of different aneuploid stocks of the durum cultivar Langdon. The stocks described by them include double-ditelosomics, dimonotelosomics, D-genome substitution-monosomics, D-genome disomic substitutions, intercultivar chromosome substitution lines, and

homozygous recombinant lines. The cytogenetic stocks can be used to determine the chromosomal location of genes, to transfer chromosomes from one cultivar or line of tetraploid wheat to another, to study the cytogenetics of tetraploid wheat, to determine gene linkages and to identify chromosomes involved in translocations (Joppa and Williams, 1988).

Joppa and Williams (1983) selected Langdon durum D-genome disomic substitution lines as segregates from the progenies of Langdon durum D-genome substitution monosomics. Detailed descriptions about Langdon durum D-genome substitution monosomics and the D-genome disomic substitutions have been given in chapters 2 and 4. The Langdon durum D-genome disomic substitution aneuploids have been used to determine the chromosomal location of genes controlling different traits in tetraploid wheats (Konzak and Joppa, 1988; Joppa and Cantrell, 1990; Cantrell and Joppa, 1991; Tsunewaki, 1992; Cai *et al.*, 1999). These aneuploids remain important in revealing the genetics of tetraploid wheats.

Considerable morphological variation exists among and within the different Langdon durum D-genome disomic substitution lines (Salazar and Joppa, 1981). Additionally the substitution lines appeared to be inferior in fertility compared to cytogenetic stocks of Chinese Spring monosomics (Joppa and Williams, 1988). Recently the substitution lines were grown under greenhouse conditions (see Chapter 4) in an attempt to locate leaf rust resistance genes in selected tetraploid wheat accessions. The lines were found heterogeneous with regard to phenological and seed characters. Variations between the lines could indicate a presence of weak compensation of the substituted D-genome chromosomes for the loss of its homoeolgue (Knott, 1989) or a loss/gain of single gene of major effect on the substituted chromosome. These variations will have shortcomings in using the aneuploids for genetic analysis. Thus provision of information from different environmental situations will be valuable to validate this variation and for further improvement. Therefore, this study was aimed at examining the genetic variation for some of the important agronomic traits among the substitution lines and Langdon durum (*Triticum turgidum* var. *turgidum*). Furthermore, path analysis was carried out to study the association of seed yield and yield-related

traits. Information on the true nature of the associations of traits will be beneficial as a basis to pinpoint the best selection criterion.

5.2 Materials and methods

5.2.1 Plant materials

The complete set of 14 Langdon durum D-genome disomic substitution lines and Langdon durum (2n=4x=28) were included in this study. The USDA/ARS (Northern Crop Science Lab, State University Station, Fargo, North Dakota, U.S.A) kindly supplied the lines. Table 4.1 of chapter 4 outlined the list, code and generation of Langdon durum D-genome disomic substitution lines. This study used seeds harvested one generation after growing the substitution lines. Therefore, except substitution line 3D(3B) that was an early generation (F_4) stock, other lines were advanced generations (F_8-F_{11}).

5.2.2 Growing conditions

Plants were grown in an air-conditioned glasshouse at the University of the Free State, South Africa. Ten seeds of each of the lines were planted in three 2-liters capacity pots with an appropriate soil mix. In each of the first two pots three seeds were sown and in the last pot four seeds. The day and night temperature of the glasshouse were maintained at 20 ± 5°C and 14 ± 5°C, respectively. Daylight was supplemented with 14 h of 120 $\mu molm^{-2}s^{-1}$ photosynthetically active radiation (PAR) that was emitted from cool white fluorescent tubes arranged directly above the plants. Two weeks after planting and every fortnight after that till maturity, 35 ml of 2 g/l Chemicult hydroponic nutrient solution was applied as a soil drench to each pot. Chemicult® contains macro elements (N, P, K, Ca, Mg, at respective percentages of 6.5, 2.7, 13.0, 7.7, 2.2) and microelements (Fe, Mn, B, Zn, Cu, Mo at percentages of 0.15, 0.024, 0.024, 0.005, 0.002, 0.001, respectively). For the control of aphids Metasystox® (2.5 ml/l) was sprayed once on the plants at late tillering stage.

5.2.3 Measurements

Heading date (HD) was recorded as the number of days from planting to the date when 50% of the spikes in the line were fully emerged from the flag leaf. Plant height (PH) was measured (cm) from the base of the plant to the tip of the spike, excluding the awns. Flag leaf length (FL) was measured (cm) from the base to the tip of a fully expanded flag leaf when 50% of the spikes of the line were fully emerged. Productive tiller numbers (TN) were counted on each plant during harvest. Spike length (SL) was measured from the base to the tip of the spike of the primary tiller. Spikelets per spike (SP) and kernels per spike (KS) were counted during harvest from the primary tiller. Kernel weight (KW) in grams was determined using a random sample of 200 kernels of each line. Seed yield (SY) was measured (g) per pot of both main and secondary spikes.

In nine of the substitution lines (1D1A, 2D2A, 2D2B, 3D3B, 4D4A, 5D5A, 5D5B, 6D6A, 7D7A) agronomic traits of all 10 plants were measured. Measurements in four lines (1D1B, 3D3A, 4D4B, 7D7B) were taken from nine plants where three plants survived per pot. Measurements on the substitution line 6D6B and cultivar Langdon were made on eight plants where the first two pots consisted of three plants each and the last pot contained only two plants. The pots represent replications that were completely randomized.

5.2.4 Analysis of data

All characters measured were subjected to an analysis of variance (ANOVA) procedure of the SAS statistical program (SAS, 1989). To conduct the ANOVA, individual measurements were averaged over the three pots (replications). Mean comparisons among the lines were carried out using Duncan's Multiple Range Test. Variance components were calculated to estimate the heritability of each character and genetic correlation among characters (Bos and Caligary, 1995; Falconer and Mackey, 1996). Heritability in the wider sense (H^2) was calculated as $H^2 = \frac{\sigma g^2}{\sigma g^2 + \sigma e^2}$ (Allard *et al.*, 1960; Falconer and Mackey, 1996).

The component, σ_g^2, is the genotypic variance (Vg) among the tested lines. The unbiased estimator of this component is given by $\sigma_g^2 = \frac{MSg - MSr}{J}$ (Bos and Caligary, 1995). In the equation, MSg represents the mean square of genotypes (lines), MSr is the mean square of residual/error from the analysis of variance and J is the number of pots. The MSr is an unbiased estimator of the environmental variance (Ve). The correlations were estimated as ratios of the covariance of two traits to the root of the product of the variances of the same traits. Genotypic and phenotypic variances and covariances were estimated using the *SAS* multivariate analysis (MANOVA) procedure. Phenotypic (r_p) and genotypic (r_g) correlations were calculated using the formula: $r_p = \frac{\delta_{pxy}}{\sqrt{\delta^2_{px} \times \delta^2_{py}}}$ and $r_g = \frac{\delta_{gxy}}{\sqrt{\delta^2_{gx} \times \delta^2_{gy}}}$, respectively (Griffing, 1956; Fisher, 1963; Falconer and Mackey, 1996). Where δ_{pxy} and δ_{gxy} are phenotypic and genotypic covariance, δ^2_{px} and δ^2_{py} are phenotypic variances and δ^2_{gx} and δ^2_{gy} are genotypic variances of trait *x* and *y*, respectively. Significance tests of the correlation coefficients were determined using the Student's t-test (Steel and Torrie, 1980): $t = r / \sqrt{1 - r^2 / n - 2}$, where *r* is the correlation coefficient and *n* is the number of observations. The degrees of association between the casual and response trait were expressed by the R-square values from the ANOVA. The R-square value is the explained variance, which is attributable by the dependent/response variable due to the independent/casual variable. A high R-square value indicates that the variance for one trait is largely explained by the variance of the other trait. The remainder of the R-square value is, $1-r^2$, which can be referred to as the unexplained variance. Part of this unexplained variation is caused by the experimental error (residual variance) and part is due to differences in the response variable independent of the causal variable (Falconer and Mackay, 1996; Lynch and Walsh, 1998). From separate ANOVA's conducted for each trait, the coefficient of variation (CV) was computed. The CV is a ratio of the standard deviation and the mean and is

expressed as a percentage $[CV = 100(\frac{Sx}{\bar{X}})]$. To calculate CV the grand mean ($\bar{X}$) and standard deviation (S_x) as the square root of MSr were considered. Low CV values show that experimental error was minimal (Snedecor and Cochran, 1989).

Direct and indirect path coefficients were calculated as initially proposed by Wright (1921, 1934) and later described by Dewey and Lu (1959), Li (1975) and Williams *et al.* (1990) using genotypic correlation coefficients.

The path analysis divides the genotypic correlations into direct effects (unidirectional pathways) and indirect effects (alternate pathways) of all traits upon the response trait. As more variables are considered in the correlations, the indirect associations become more complex, less obvious, and confusing. The path coefficient analysis, thus, provides an effective means of straightening out direct and indirect causes of associations and permits a critical examination of the specific forces acting to produce a given correlation and measures the relative importance of each causal factor (Williams *et al.*, 1990). The direct effect exerts the obvious direct influence on the response variable, with other variables held constant. The indirect effects play an important role and in some instances mask the direct influence (Samonte *et al.*, 1998).

For the present analysis seed yield (SY) was used as a response variable and heading date (HD), plant height (PH), flag leaf length (FL), tiller number (TN), spike length (SL), spikelet per spike (SP), number of kernels per spike (KS), and kernel weight (KW) as casual variables. Thus, the following relationships were established (see Fig. 5.1).

$$SY=HD + PH + FL + TN + SL + SP + KS + KW + R$$
$$=X1 + X2 + X3 + X4 + X5 + X6 + X7 + X8 + R,$$

where the Xs represents the eight characters and R is a residual variable that includes sampling error and it is assumed to be independent of the remaining variables. R measures the failure of the other components to account for seed yield (Williams *et al.*, 1990).

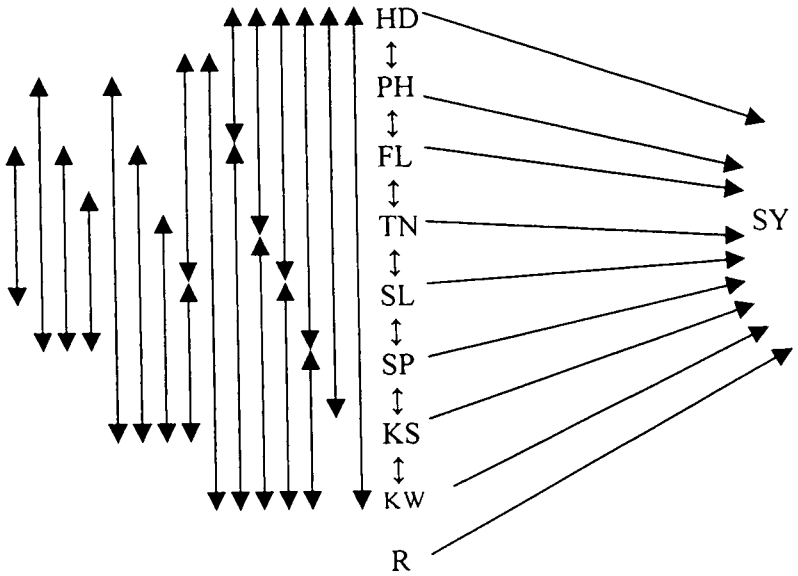

Fig. 5.1 Path diagram showing interrelationships between seed yield and selected yield predictor variables in tetraploid wheat aneuploids. Double arrowed lines indicate mutual associations as measured by correlation coefficients and the single arrowed lines represent direct influences as measured by path coefficients.

In statistics the correlation of a dependent variable Y on an independent variable X is given by:

$r(X_1, Y) = \frac{Cov(X_1, Y)}{\sqrt{V(X_1)V(Y)}}$. From the above relationship the formula could be iterated as

$$r(X_1, Y) = \frac{Cov(X_1, X1 + X2 + X3 + X4 + X5 + X6 + X7 + X8 + R)}{\sqrt{V(X_1)V(Y)}}$$

$$= \frac{Cov(X_1, X1)}{\sqrt{V(X_1)V(Y)}} + \frac{Cov(X_1, X2)}{\sqrt{V(X_1)V(Y)}} + \frac{Cov(X_1, X3)}{\sqrt{V(X_1)V(Y)}} + \frac{Cov(X_1, X4)}{\sqrt{V(X_1)V(Y)}} + \frac{Cov(X_1, X5)}{\sqrt{V(X_1)V(Y)}} + \frac{Cov(X_1, X6)}{\sqrt{V(X_1)V(Y)}} + \frac{Cov(X_1, X7)}{\sqrt{V(X_1)V(Y)}} +$$

$$\frac{Cov(X_1, X8)}{\sqrt{V(X_1)V(Y)}} + \frac{Cov(X_1, R)}{\sqrt{V(X_1)V(Y)}}$$

In the equation Cov(X1,X1)=V(X1), Cov(X1,R)=0, and Cov(X1,X2)= r(X1,X2)σX1σX2. Therefore,

$$r(X1, Y) = \frac{V(X1)}{\sqrt{V(X1)V(Y)}} + \frac{r(X1, X2)\sigma X1\sigma X2}{\sqrt{V(X1)V(Y)}} + \frac{r(X1, X3)\sigma X1\sigma X3}{\sqrt{V(X1)V(Y)}} + \frac{r(X1, X4)\sigma X1\sigma X4}{\sqrt{V(X1)V(Y)}} + \frac{r(X1, X5)\sigma X1\sigma X5}{\sqrt{V(X1)V(Y)}} + \frac{r(X1, X6)\sigma X1\sigma X6}{\sqrt{V(X1)V(Y)}} + \frac{r(X1, X7)\sigma X1\sigma X7}{\sqrt{V(X1)V(Y)}} + \frac{r(X1, X8)\sigma X1\sigma X8}{\sqrt{V(X1)V(Y)}}$$

$$= \frac{\sigma X1}{\sigma y} + r(X1, X2)\frac{\sigma X2}{\sigma y} + r(X1, X3)\frac{\sigma X3}{\sigma y} + r(X1, X4)\frac{\sigma X4}{\sigma y} + r(X1, X5)\frac{\sigma X5}{\sigma y} + r(X1, X6)\frac{\sigma X6}{\sigma y} + r(X1, X7)\frac{\sigma X7}{\sigma y} + r(X1, X8)\frac{\sigma X8}{\sigma y}$$

Where; $\frac{\sigma X1}{y}$='a', the path coefficient from X1 to Y; $\frac{\sigma X2}{y}$='b', the path coefficient from X2 to Y; $\frac{\sigma X3}{y}$='c'; the path coefficient from X3 to Y; $\frac{\sigma X4}{y}$='d'; the path coefficient from X4 to Y; $\frac{\sigma X5}{y}$='e', the path coefficient from X5 to Y; $\frac{\sigma X6}{y}$='f', the path coefficient from X6 to Y; $\frac{\sigma X7}{y}$='g', the path coefficient from X7 to Y; and $\frac{\sigma X8}{y}$='h', the path coefficient from X8 to Y.

Based on these expressions, the correlation of Y on variable X1 is given as: r(X1,Y)=a + r(X1X2)b + r(X1X3)c + r(X1X4)d + r(X1X5)e + r(X1X6)f +r(X1X7)g + r(X1X8)h. Thus the correlation between X1 and Y would be divided into eight parts:

(i) direct effect of X1 on Y which amounts to 'a'.
(ii) indirect effect of X1 on Y via X2 which amounts to r(X1X2)b.
(iii) indirect effect of X1 on Y via X3 which amounts to r(X1X3)c.
(iv) indirect effect of X1 on Y via X4 which amounts to r(X1X4)d .
(v) indirect effect of X1 on Y via X5 which amounts to r(X1X5)e.
(vi) indirect effect of X1 on Y via X6 which amounts to r(X1X6)f.
(vii) indirect effect of X1 on Y via X7 which amounts to r(X1X7)g and
(viii) indirect effect of X1 on Y via X8 which amounts to r(X1X8)h .

In the same manner the equations for r(X2,Y), r(X3,Y), r(X4,Y), r(X5,Y), r(X6,Y), r(X7,Y) and r(X8,Y) would be represented and a set of simultaneous equations set up:

r(X1,Y)=a + r(X1X2)b + r(X1X3)c + r(X1X4)d + r(X1X5)e + r(X1X6)f + r(X1X7)g + r(X1X8)h.
r(X2,Y)=r(X2X1)a + b + r(X2X3)c + r(X2X4)d + r(X2X5)e + r(X2X6)f + r(X2X7)g + r(X2X8)h.
r(X3,Y)=r(X3X1)a + r(X3X2)b + c + r(X3X4)d + r(X3X5)e + r(X3X6)f + r(X3X7)g + r(X3X8)h.
r(X4,Y)=r(X4X1)a + r(X4X2)b + r(X4X3)c + d.+ r(X4X5)e + r(X4X6)f + r(X4X7)g + r(X4X8)h.
r(X5,Y)=r(X5X1)a + r(X5X2)b + r(X5X3)c + r(X5X4)d + e.+ r(X5X6)f + r(X5X7)g + r(X5X8)h.
r(X6,Y)=r(X6X1)a + r(X6X2)b + r(X6X3)c + r(X6X4)d + r(X6X5)e + f + r(X6X7)g + r(X6X8)h.
r(X7,Y)=r(X7X1)a + r(X7X2)b + r(X7X3)c + r(X7X4)d + r(X7X5)e + r(X7X6)f + g + r(X7X8)h.
r(X8,Y)=r(X8X1)a + r(X8X2)b + r(X8X3)c + r(X8X4)d + r(X8X5)e + r(X8X6)f + r(X8X7)g.+ h.

Each normal equation represents a partitioning of the correlation coefficient of a predictor variable with response variable into the component terms; the direct effect or path coefficient for that predictor variable and seven indirect effects (alternate paths), each involving the product of a correlation coefficient between two predictor variables and the appropriate path coefficient in accordance to the path diagram (Fig. 5.1). The summed expressions in each equation can be interpreted as an explanation for the corresponding correlation coefficient between the response variable and that particular predictor variable.

The simultaneous equations were presented in a matrix notation as A=B*C. The A, B, and C vectors were presented as follows.

$$\begin{bmatrix} r(X1, Y) \\ r(X2, Y) \\ r(X3, Y) \\ r(X4, Y) \\ r(X5, Y) \\ r(X6, Y) \\ r(X7, Y) \\ r(X8, Y \end{bmatrix} = \begin{bmatrix} r(X1X1) + r(X1X2) + r(X1X3) + r(X1X4) + r(X1X5) + r(X1X6) + r(X1X7) + r(X1X8) \\ r(X2X1) + r(X2X2) + r(X2X3) + r(X2X4) + r(X2X5) + r(X2X6) + r(X2X7) + r(X2X8) \\ r(X3X1) + r(X3X2) + r(X3X3) + r(X3X4) + r(X3X5) + r(X3X6) + r(X3X7) + r(X3X8) \\ r(X4X1) + r(X4X2) + r(X4X3) + r(X4X4) + r(X4X5) + r(X4X6) + r(X4X7) + r(X4X8) \\ r(X5X1) + r(X5X2) + r(X5X3) + r(X5X4) + r(X5X5) + r(X5X6) + r(X5X7) + r(X5X8) \\ r(X6X1) + r(X6X2) + r(X6X3) + r(X6X4) + r(X6X5) + r(X6X6) + r(X6X7) + r(X6X8) \\ r(X7X1) + r(X7X2) + r(X7X3) + r(X7X4) + r(X7X5) + r(X7X6) + r(X7X7) + r(X7X8) \\ r(X8X1) + r(X8X2) + r(X8X3) + r(X8X4) + r(X8X5) + r(X8X6) + r(X8X7) + r(X8X8) \end{bmatrix} \begin{bmatrix} a \\ b \\ c \\ d \\ e \\ f \\ g \\ h \end{bmatrix}$$

Values for vectors A and B were formulated from Table 5.2. Values for vector 'C' (path coefficients) were obtained by multiplying both sides by inverse of "B" matrix (B^{-1}) using Microsoft® Excel 2000 Thus; $B^{-1}*A = B^{-1}*B*C$, since $B^{-1}*B=1$ then $C = B^{-1}*A$.

5.3 Results

5.3.1 Genetic variation of agronomic traits

The results from the ANOVA suggested that for all characters considered there were highly significant differences among entries but not between pots and for the interaction between pots and entries (Appendix IV). For this reason a second ANOVA in which pot effects and pot x entry interaction was excluded was carried out for each character. The latter ANOVA was computed on average measurements of each variable over three pots (replications). Results of the analysis viz. mean comparisons, mean square values, heritability

estimates and coefficients of variation (CV) of the various characters are presented in Table 5.1 and Appendix V (see Appendix VI for other statistics).

Heading date (HD)

The average heading date for the substitution lines was 52 days. There was considerable variation for heading date where 96% of the variation was explained due to differences among the substitution lines. HD had high heritability estimates (96%) and a very low CV (1.67%). Line 7D7B was late heading (62 days) followed by 6D6B and 7D7A which took 57 days for heads to emerge. The earliest heading date was reached after 48 days by lines 1D1B and 2D2A. There were no significant differences for HD between lines 1D1A, 1D1B, 2D2A, 3D3B, and 6D6A. Four substitution lines (2D2B, 4D4A, 4D4B and 5D5B) had the same heading date that was not significantly different from that of Langdon durum (Fig. 5.2A).

Plant height (PH)

The lines were on average 119.79 cm high (Table 5.1). Line 3D3A was extremely tall with a height of 150 cm which was significantly different from all other lines. Overall, 50% of the lines did not show a significant difference for PH when compared with Langdon durum (122.9 cm) (Fig. 5.2B). The broad sense heritability of the trait was estimated at 82%.

Flag leaf length (FL)

The FL of the lines ranged from 12 to 21 cm. Lines 4D4B and 2D2A showed the longest flag leaves, 21 and 19 cm, respectively. Langdon durum had a 13 cm long flag leaf which was not significantly different from 1D1A, 2D2B, 3D3A, 3D3B, 4D4A, and 7D7A (Fig. 5.2C). This trait had a heritability value of 73% (Tables 5.1).

Table 5.1 Results of mean comparisons, mean square values, heritability estimates, coefficients of variability and coefficient of variances of various agronomic characters of Langdon durum D-genome disomic substitution lines and Langdon durum.

Line	Character[a]								
	HD[b](days)	PH(cm)	FL(cm)	TN(no.)	SL(cm)	SP(no.)	KS(no.)	KW(g)	SY(g)
1D1A	48.97^{def}	117.73^{cdef}	12.02^{f}	3.36^{abc}	6.52^{cd}	18.17^{ab}	45.80^{b}	8.53^{cd}	24.48^{ab}
1D1B	47.99^{f}	110.20^{efg}	16.39^{d}	3^{abcde}	5.28^{fg}	17.11^{abcd}	58.44^{a}	10.93^{a}	25.47^{ab}
2D2A	47.55^{f}	115.95^{defg}	19.16^{ab}	4.08^{a}	6.51^{cd}	15.89^{cdef}	33.19^{cd}	8.13^{d}	21.55^{b}
2D2B	54.33^{c}	100.33^{h}	13.29^{ef}	3.86^{ab}	5.85^{ef}	13.11^{ghi}	22.05^{e}	7.02^{ef}	13.61^{c}
3D3A	50^{de}	149.99^{a}	13.67^{def}	3^{abcde}	7.14^{b}	16.56^{bcde}	33.33^{cd}	9.52^{b}	25.61^{ab}
3D3B	48.50^{ef}	106.44^{fgh}	12.26^{f}	2.50^{cde}	5.58^{ef}	14.83^{efg}	16.61^{fg}	9.09^{bc}	25.11^{ab}
4D4A	54.08^{c}	106.33^{fgh}	12.97^{ef}	3.17^{abcde}	4.97^{g}	15.56^{def}	15.58^{fg}	8.65^{bcd}	23.19^{b}
4D4B	53.33^{c}	133.67^{b}	20.78^{a}	2.78^{bcde}	6.44^{cd}	18.89^{a}	33.89^{cd}	10.9^{a}	22.16^{b}
5D5A	50.17^{d}	126.50^{bcd}	16.77^{bc}	3.78^{ab}	8.93^{a}	17.94^{abc}	9.36^{h}	9.08^{bc}	24.88^{ab}
5D5B	53.33^{c}	128.18^{bc}	16.27^{cd}	3.33^{abc}	6.49^{cd}	13.28^{gh}	14.83^{fg}	8.62^{bcd}	24.81^{ab}
6D6A	48.53^{ef}	128.67^{bc}	16.05^{cd}	2.14^{de}	4.08^{h}	14.33^{fgh}	32.08^{d}	10.91^{a}	26.61^{ab}
6D6B	57^{b}	131.72^{b}	16.30^{cd}	3.28^{abcd}	4.17^{h}	15.78^{def}	31.33^{d}	10.52^{a}	22.59^{b}
7D7A	56.72^{b}	105.58^{gh}	15.51^{cde}	3.11^{abcde}	5.63^{ef}	12.39^{hi}	19.14^{ef}	7.81^{de}	4.69^{d}
7D7B	61.89^{a}	112.56^{efg}	17.99^{bc}	1.99^{e}	5.95^{de}	11.11^{i}	13.33^{gh}	6.60^{f}	3.82^{d}
Langdon	53^{c}	122.94^{bcde}	12.92^{ef}	2.22^{cde}	6.58^{c}	19.06^{a}	37.78^{c}	10.85^{a}	29.34^{a}
Probability[c]	**	**	**	*	**	**	**	**	**
MS entry	50.15	547.37	20.45	1.18	4.31	17.66	555.01	6.21	176.79
MS error	0.77	37.74	2.22	0.37	0.11	1.32	7.88	0.26	7.75
H^2	0.96	0.82	0.73	0.42	0.93	0.80	0.96	0.88	0.88
CV (%)	1.68	5.13	9.63	20.07	5.52	7.37	10.10	5.55	13.13
R^2	0.96	0.87	0.81	0.59	0.94	0.86	0.97	0.92	0.91

[a] HD=Heading date, PH=Plant height, FL=Flag leaf length, TN=Tiller number, SL=Spike length, SP=Spikelet per spike, KS=Kernels per spike, KW=Kernel weight, SY=Seed yield

[b] means followed by the same letter in a column are not significantly different at 0.05 probability levels of Duncan's Multiple Range Test.

[c] * and ** denote significant and highly significant differences at 0.05 and 0.001 probability levels, respectively

Tiller number (TN)

The substitution lines were found to tiller poorly. The average productive tillers were three. Eleven of the 14 substitution lines produced a similar number of tillers to Langdon durum (Fig. 5.2D). The genotypic differences between the lines for tillering ability were minimal. This was displayed by the relatively low value of the coefficient of variation (59%) for TN among lines and low heritability value (42%).

Spike length (SL)

The length of spikes varied from 4 cm in line 6D6A to 9 cm in line 5D5A. There were high genotypic differences for SL with heritability estimated at 93%. Langdon durum and four of the substitution lines did not differ significantly for SL.

Number of spikelets per spike (SP)

On average the substitution lines were found to bear 16 spikelets per spike. This value for Langdon was 19, the highest compared with the rest of the lines. There were no significant differences between Langdon durum and lines 1D1A, 1D1B, 4D4B, and 5D5A (Fig. 5.2F). Only line 7D7B exhibited low numbers of spikelets/spike but was not significantly different from 2D2B and 7D7A. Almost 86% of the variation for SP was attributed to genotypic differences among the lines.

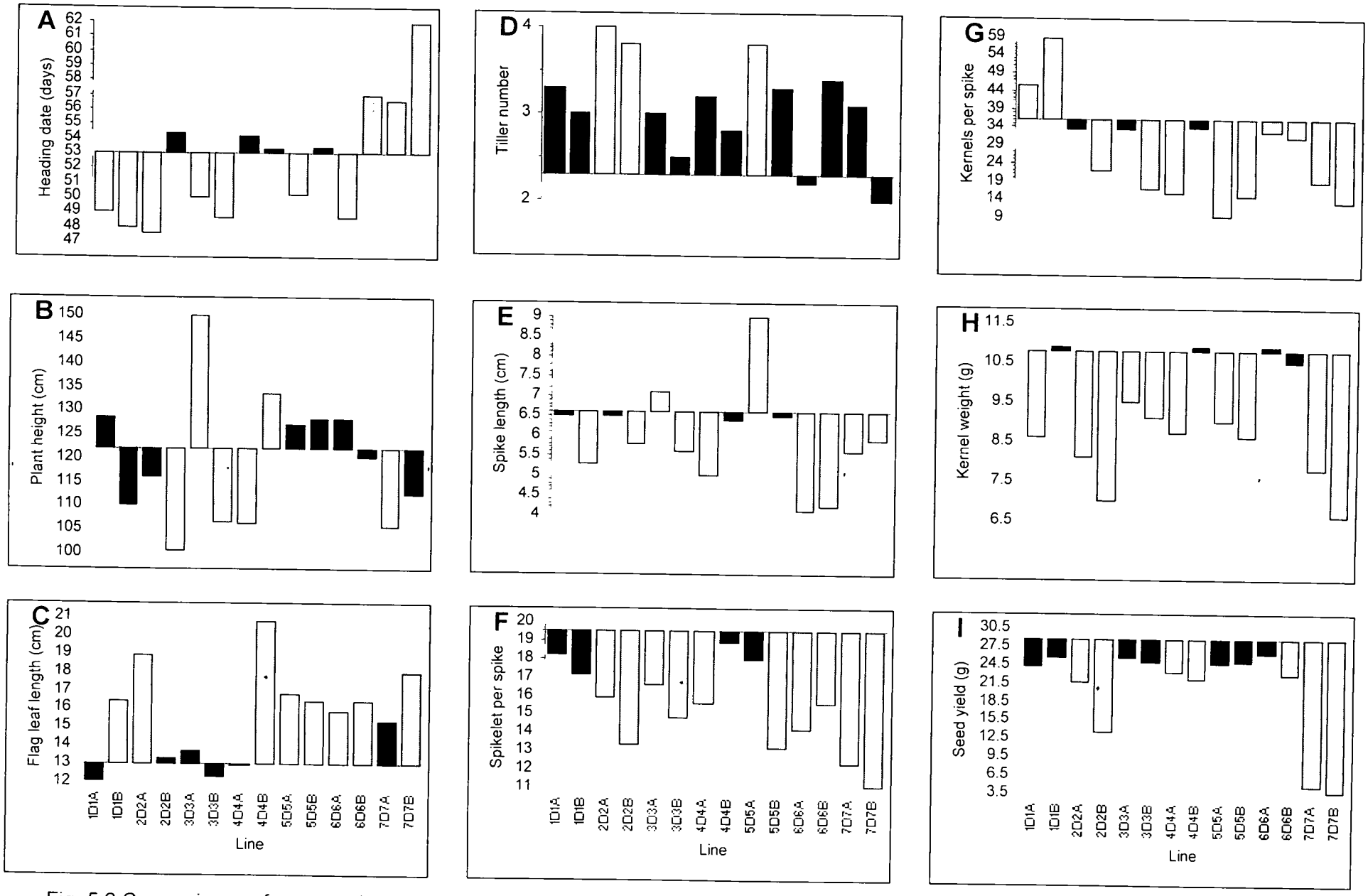

Fig. 5.2 Comparisons of agronomic traits among substitution lines and Langdon durum (LDN). The x-axis crosses at the mean value of LDN. Variation from this is indicated by up and downward bars. Blackened bars show non-significant differences from LDN.

Number of kernels per spike (KS)

Substitution lines 1D1A and 1D1B had the highest number of spikelets per spike (Table 5.1). These lines were significantly different from the rest of the substitution lines and Langdon durum. The recurrent parent was significantly different from 11 substitution lines. Line 5D5A that had longer spikes gave the lowest seed set per spike. The result indicated that KS was highly heritable. More explained variation (97%) in KS in comparison with other characters was attributed to differences among lines.

Kernel weight (KW)

Among the lines the average weight of 200 randomly sampled seeds was 9.14 g. The maximum being 11 g obtained from lines 1D1B, 4D4B, 6D6A, 6D6B and Langdon durum (Table 5.1). Lines 2D2B and 7D7B had a low KW (7 g). The heritability of KW was 88% with a CV of 5.55%.

Seed Yield (SY)

Eleven of the 14 sets showed non-significant differences for SY. Of these, seven did not differ significantly from Langdon durum. However, all of the substitution lines had low seed set compared to the recurrent parent (see Fig. 5.2l). Lines 7D7A and 7D7B showed low seed yield and did not differ significantly from each other. Seed yield had a high heritability value, estimated at 88%.

5.3.2 Correlation and path coefficient analysis

For all possible comparisons, simple phenotypic and genotypic correlations of agronomic characters of Langdon durum D-genome disomic substitution lines are presented in Table 5.2. The magnitudes of the phenotypic and genotypic correlation coefficients are nearly the same, suggesting that the environmental influence on the relationships was minimal.

There was a highly significant negative (-0.55) association between heading date and seed yield. The strong and negative association between HD and SY indicated that substitution lines that headed early had good seed yield (Table 5.2

and Fig. 5.3 A). Early headed genotypes, therefore, had longer time for grain filling and ripening time that contributed to increased seed yield. All lines were harvested at the same time. Characters PH, SP, KS, and KW

Table 5.2 Phenotypic and genotypic correlation coefficients (upper and lower diagonals respectively) for pair wise combinations of agronomic characters[a, b] of Langdon durum D-genome disomic substitution lines and Langdon durum.

	HD	PH	FL	TN	SL	SP	KS	KW	SY
HD		-0.28**	0.19*	-0.24*	-0.20*	-0.60***	-0.49***	-0.46***	-0.76***
PH	-0.11		0.20*	-0.07	0.39***	0.48***	0.22*	0.44***	0.48***
FL	0.18*	0.18*		-0.04	0.14	0.09	0.05	0.21*	-0.14*
TN	-0.18*	-0.03	-0.08		0.41***	0.33***	0.01	-0.18*	0.18*
SL	-0.08	0.21*	0.07	0.18*		0.37***	-0.25*	-0.26*	0.08
SP	-0.23*	0.23*	0.08	-0.02	0.24**		0.51***	0.61***	0.69***
KS	-0.38***	0.07	0.02	-0.06	-0.17*	0.33***		0.55***	0.37***
KW	-0.31***	0.34***	0.16	-0.09	-0.13	0.33***	0.46***		0.71***
SY	-0.55***	0.28**	-0.08	0.06	0.09	0.38***	0.29***	0.62***	

[a] HD=Heading date, PH=Plant height, FL=Flag leaf length, TN=Tiller number, SL=Spike length, SP=Spikelet per spike, KS=Number of kernels per spike, KW= Kernel weight, SY=Seed yield.

[b] *,**,*** significantly different at 0.05, 0.01 and 0.001 levels of probability, respectively.

were found to be highly and positively correlated with SY (Fig. 5.3 B, F, G and H). Highly significant and positive association between PH and SY showed that increased plant canopy was responsible for better seed yield in the substitution lines. This is, however, contrary to the notion of achieving a greater harvest index. The presence of a considerable number of spikelets per spike contributed to a strong positive association (0.62) with SY (Table 5.2 and Fig. 5.3 H). The notable difference of this association compared with other traits could be explained by the higher value of the regression coefficient/slope (3.34) and higher explained variance (38.9 %) (Fig. 5.3 H). Characters FL, TN, and SL were found to be poorly associated to SY of the main and secondary tiller spikes (Fig. 5.3 C, D and E).

From these associations it was evident that these traits had little influence on SY and might not be considered as selection criteria. The substitution lines produced unproductive tillers and some spikelets were infertile across the entire spike suggesting that TN and SL had no positive contribution to improved SY.

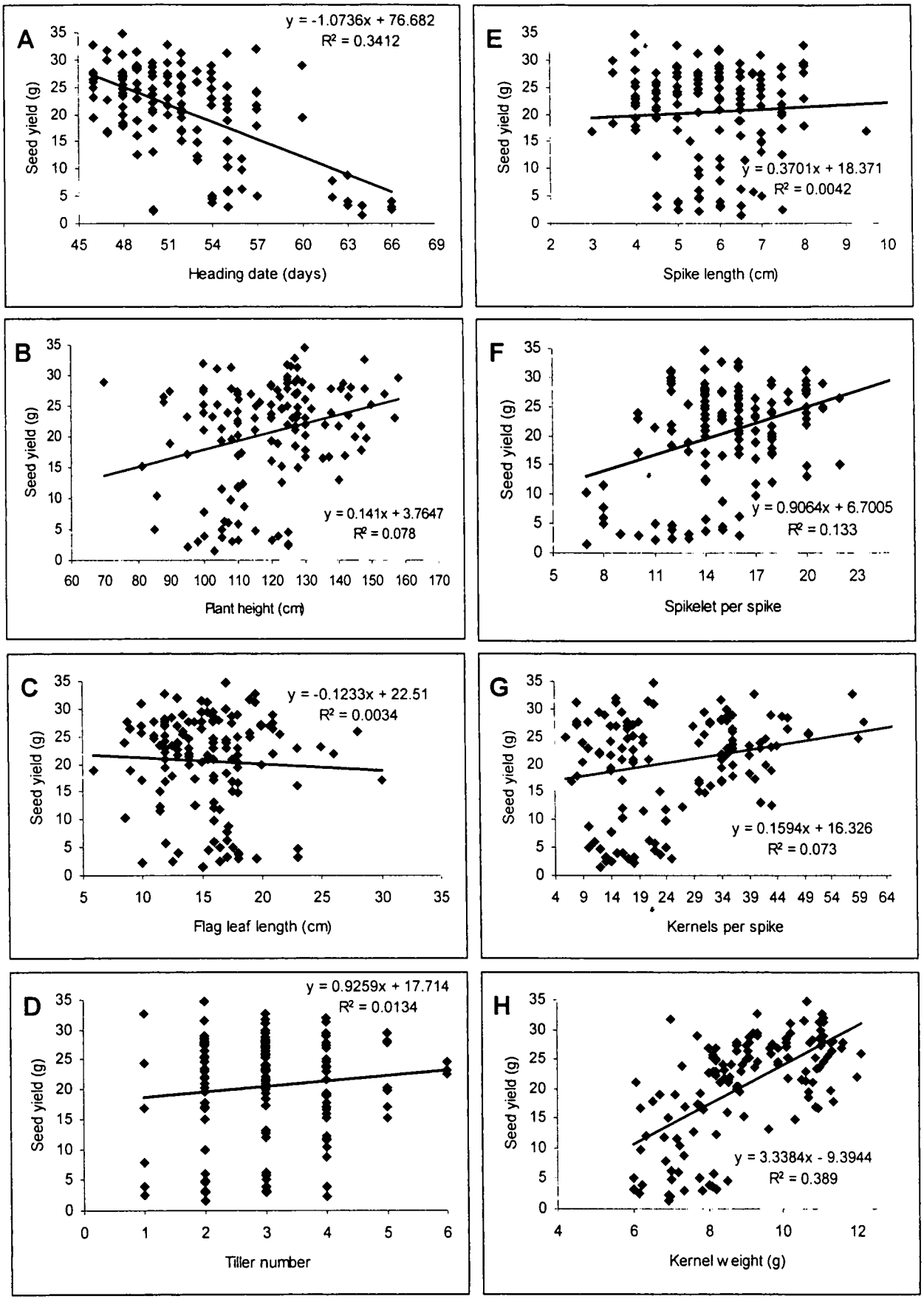

Fig.5.3 Association between seed yield and eight agronomic traits (A–H) of Langdon durum D-genome disomic substitution lines.

From the data set presented in Table 5.2 a genotypic correlation matrix was set up as A=B*C (Table 5.3a). In Table 5.3a vector "A" represents the genotypic correlation coefficients of seed yield (SY) against eight agronomic traits of Langdon durum D-genome disomic substitution lines. In the same table vector "B" is the genotypic correlation for all possible combinations among the eight traits and vector "C", the path coefficients.

The inverse of matrix B in Table 5.3a was calculated using the Matrix Inverse function (MINVERSE) of Microsoft Excel 2000 and is presented in Table 5.3b. The path coefficients were calculated as the product of vector A and each row of B^{-1} using the matrix multiplication (MMULT) function of the same software.

Table 5.3a. Matrix of the form A=B*C. The "A" vector represents the genotypic correlation coefficients of seed yield against eight agronomic traits of Langdon durum D-genome disomic substitution lines. Vector "B" is the genotypic correlations among the eight traits and vector "C", the path coefficients.

-0.55	=	1.00	-0.11	0.18	-0.18	-0.08	-0.23	-0.38	-0.31	a
0.28		-0.11	1.00	0.18	-0.03	0.21	0.23	0.07	0.34	b
-0.08		0.18	0.18	1.00	-0.08	0.07	0.08	0.02	0.16	c
0.06		-0.18	-0.03	-0.08	1.00	0.18	-0.02	-0.06	-0.09	d
0.09		-0.08	0.21	0.07	0.18	1.00	0.24	-0.17	-0.13	e
0.38		-0.23	0.23	0.08	-0.02	0.24	1.00	0.33	0.33	f
0.29		-0.38	0.07	0.02	-0.06	-0.17	0.33	1.00	0.46	g
0.62		-0.31	0.34	0.16	-0.09	-0.13	0.33	0.46	1.00	h

Table 5.3b. Inverse matrix of "B" vector from Table 5.3a.

1.38	0.03	-0.31	0.25	0.18	0.06	0.42	0.30
0.03	1.25	-0.13	0.05	-0.28	-0.09	0.12	-0.45
-0.31	-0.13	1.13	0.03	-0.11	-0.02	-0.04	-0.22
0.25	0.05	0.03	1.09	-0.18	0.06	0.07	0.08
0.18	-0.28	-0.11	-0.18	1.30	-0.40	0.28	0.32
0.06	-0.09	-0.02	0.06	-0.40	1.34	-0.35	-0.27
0.42	0.12	-0.04	0.07	0.28	-0.35	1.52	-0.45
0.30	-0.45	-0.22	0.08	0.32	-0.27	-0.45	1.63

Results of the path coefficient analysis are summarized in Table 5.4. This table shows direct path coefficient values (boldfaced main diagonals) and alternate/indirect path values of each trait on SY. All values of direct effects were below one, indicating that inflation due to multicolinearity was minimal.

Table 5.4 Direct (boldfaced main diagonals) and alternate/indirect path coefficient values of seed yield versus eight agronomic characters of Langdon durum D-genome disomic substitution aneuploids.

		Character								
		HD	PH	FL	TN	SL	SP	KS	KW	SY
Character	HD	**-0.37**	0.00	-0.02	0.00	-0.01	-0.03	0.05	-0.17	-0.55
	PH	0.04	**0.04**	-0.02	0.00	0.01	0.03	-0.01	0.18	0.28
	FL	-0.07	0.01	**-0.12**	0.00	0.00	0.01	0.00	0.09	-0.08
	TN	0.07	0.00	0.01	**0.02**	0.01	0.00	0.01	-0.05	0.06
	SL	0.03	0.01	-0.01	0.00	**0.07**	0.04	0.02	-0.07	0.09
	SP	0.08	0.01	-0.01	0.00	0.02	**0.15**	-0.04	0.18	0.38
	KS	0.14	0.00	0.00	0.00	-0.01	0.05	**-0.13**	0.25	0.29
	KW	0.11	0.01	-0.02	0.00	-0.01	0.05	-0.06	**0.53**	0.62

A high direct path coefficient value (0.53) and highly significant genotypic correlation was found between KW and SY. The true positive associations verified from the direct path value indicate that KW tends to serve as a principal selection criterion to improved SY in the substitution lines. The second highest direct path value was shown by number of spikelets per spike (0.15) with SY. However, a higher alternate path value of KW (0.18) indicates that selection for improved SY cannot be achieved by directly selecting SP *per se* it rather selected via KW. The low values of direct path coefficients for KS (-0.13) and PH (0.04) unlike their respective alternate path coefficients of 0.25 and 0.18, respectively, suggest that selection to achieving better SY could be attained via KW. Characters like FL, TN, and SL were relatively poorly associated with SY. This was reflected by both path values and suggests that they are unimportant selection criteria for improved SY.

The path coefficient analysis was relevant in elucidating the true association between KW and SY. Kernel weight had the strongest influence both directly and

indirectly upon seed yield. Improved seed yield in the substitution lines can best be achieved by directly selecting for improved KW. From the alternate path values it was also possible to conclude that selection for KW would bring about simultaneous and favorable changes towards KS, SP, and PH. In this particular environment, KW had a high heritability value (0.88) (Table 5.1).

5.4 Discussion

The analysis of variance for various agronomic characters studied in Langdon durum disomic substitution lines suggested that there was considerable variation among the substitution lines and the recurrent parent. To help maintain uniformity among the lines and thereby promoting synchronization of flowering and other agronomical traits, variation among the substitution lines needs to be minimized.

The study demonstrated that the earliest heading date in lines 2D2A and 1D1B has to be extended, whereas this trait needs to be reduced in Line 7D7B. The shortest PH in line 2D2B has to be increased together with selecting 3D3A line for reduced PH. Lines 4D4B and 2D2A need to be considered for reduced FL and TN, respectively. Relatively long spikelets are needed in lines 6D6A and 6D6B. Lines 7D7A, 7D7B, 2D2B, and 5D5B must be selected for increased SP. The reduced KS in lines 5D5A and 7D7B has to be improved. The KW and SY of lines 7D7B, 2D2B and 7D7A require further selection for improvement to move the present values until they reach that of the recurrent parent.

Substitution lines 2D2B, 7D7A, and 7D7B were found to be the most divergent by showing extreme values for at least three characters. It indicates that the degree of compensation of the D-genome chromosome for the homoeologus A- or B-genome chromosome is relatively week in these lines. It is thus suggested that repeated backcrossing to the recurrent parent and further selections are required to increase traits such as PH, SP, KW, and SY in line 2D2B. The same selection schemes are required to improve the number of SP, KS, KW, and SY in line 7D7B. Also, traits such as number of spikelets per spike, kernel weight and seed yield require further improvement in line 7D7A. The magnitudinal differences

observed among substitution lines in this particular environment agrees with that reported by Joppa and Williams (1988).

If simple correlation analysis only was considered, traits KS, SP, and PH would have been erroneously regarded as direct selection criteria. Simple correlation thus gave a misleading impression since the path coefficient analysis exposed KW as the major influence. These traits, however, were relatively poorly related to SY when analyzed by path coefficients. Therefore, when required, it is necessary to conduct path coefficient analysis in supplementation of simple correlation analysis. The result of the simple correlation analyses in this environment agree with that reported by Joppa and Williams (1988). The field and greenhouse data of Joppa and Williams (1988) on the substitution lines suggested that SY was negatively correlated with days to heading (-0.19) and it had strong positive correlation with number of seeds per spike (0.74), number of seeds per plant (0.76), plant height (0.62), and number of spikes per plant (0.39). Data on kernel weight was not reported by Joppa and Williams (1988) to make comparison to the present result.

The current result from the path analysis is in agreement with the reports of Sidwell *et al.* (1976) and Puri *et al.* (1982). In hard red winter wheat crosses (Sidwell *et al.*, 1976) and barley breeding (Puri *et al.*, 1982) path coefficient analysis indicated that kernel weight had substantial direct effects in determining grain yield. Selection for KW was found to be the most important and easiest trait to improve by direct selection and selection for this trait would be more effective in increasing grain yield than selection for other components or grain yield *per se*. It was further noted by the same reports that KW can be easily measured in a breeding program and appears to be worthy of further consideration as a selection criterion. Similar conclusions were drawn by Singh and Singh (1973) and Chaudhary (1977) from separate path analysis. However, a report from Gravois and Helms (1992) showed out that the direct effect of grain weight was of secondary and/or tertiary importance in determining rice yield.

From the path analysis we obtained information on the true associations of seed yield with kernel weight and heading date. This was in agreement with that

suggested by a simple correlation analysis. The direct path value of the path coefficient analysis exposed kernel weight as a key selection criterion to improve seed yield in the substitution lines. The alternate path values further indicated that selection for kernel weight would bring simultaneous selection of improved number of kernel per spike, spikelets per spike and plant height.

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Chapter 6

Plant breeding is an applied science and comprises all activities directed at the production of cultivars with an improved genetic constitution; improved with regard to human needs.

Achieving better yield is one of the main goals of wheat growers and consequently of most wheat breeding programs. In wheat, like many other crops, grain yield per ha is the most important selection criterion. The genetics of yield is mostly complex and one can discern several yield components (Asíns, 2002). For instance, the grain yield of wheat is determined by the number of spikes per hectare (ha), the number of seeds per spike and the average seed weight (usually expressed as 1000-seed weight). Moreover, potential yield cannot be achieved due to constraints by many factors such as pests and diseases, drought, heat, cold, earliness and height (Ekboir, 2002).

Yield stability is important for a grower to minimize the yield fluctuations over years. Due to large variations in the occurrence of pests and diseases and weather, the yields of wheat have been varying greatly from one year to another, even in favorable growing areas. Breeding for tolerance and resistance to such factors can reduce yield losses considerably.

Improved yield and yield stability are obtainable in various ways. Developing cultivars with improved resistance to pests and diseases can improve yield and stability. Such cultivars will also have spillover benefits. Spillover benefits are achieved when a wheat variety developed for one environment is directly used as a cultivar or its genes are partly used to breed a variety that is later grown in another environment. Spillover can be direct or indirect. A direct spillover appears when both parents of the variety are from the source environment and this variety is later grown in another/second environment. An indirect spill over occurs when one parent of the second environment variety is a variety from the first environment (Ekboir, 2002).

Because of the great variation in crops, ways of reproduction, aims and growing methods, no breeding program is identical to another one. There are, however, a number of steps that are eminent in all programs. The choice of the parents or starting populations is the first step in a breeding program.

There are various sources of genetic variation to select for desired parents and introgress genes for improved levels of pest and disease resistance (Jiang *et al.*, 1994; Friebe *et al.*, 1996, 1997). Among others are existing modern cultivars and landraces of wheat. If resistance genes cannot be found in these sources it is possible to search in less related material like primitive cultivars from the centers of diversity, wild or semi-wild material from the center of origin and related wild species. When crossing to primitive or wild material it is realized that besides the desired gene(s) a great deal of undesirable genetic material is introduced. To remove these undesirable genes a series of backcrosses are necessary.

Once parents with the target genes are identified, genetic analysis to locate quantitative trait loci (QTLs) and other useful monogenes or oligogenes such as disease resistance genes can be carried out using different techniques. Amongst these techniques are cytogenetic methods that employ aneuploid stocks and molecular techniques using RFLPs, RAPDs, and AFLPs. The molecular technique utilizes random recombinant inbred or homozygous lines for genetic analysis. The weakness of this analysis is the absence of appropriate and sufficient numbers of polymorphisms in most crop hybrids. Wheat is one of the most recalcitrant crops for polymorphisms. The recent approach via the use of SSRs (microsatellite) markers is showing promise by producing high levels of inter-varietal variation that is an important development for plant breeding (Korzun *et al.*, 1997; Law, 1997).

Cytogenetic methods, specifically the use of aneuploid techniques, facilitated genetic analysis of useful characters in plant breeding and genetic research. This method has been an important tool to reveal the genetic architecture of both tetraploid durum wheat and hexaploid bread/common wheat. The methods still have their place in informing plant breeders and geneticists how many

genes are involved, where these genes are located and whether they are linked or not (Law and Worland, 1996). Technical advances have improved the precision of cytogenetic analysis. For instance, the advent of laser microbeam equipment and computer aided scanners (Houben *et al.*, 1996) greatly facilitated the automated microdissection of chromosomes. Computer assisted systems have, furthermore, improved the precision of chromosome image analysis (Fukui, 1986; Ahne *et al.*, 1989; Ahne, 1994).

Wheat leaf rust, caused by the fungus *P. triticina* Eriks., is one of the most damaging diseases of wheat worldwide. It causes considerable grain losses that depend on environmental conditions and the stage of the crop development during the start of the initial rust infection. The cheapest, most effective and eco-friendly method to control this disease is the use of resistant cultivars. Development of resistant cultivars has been an important task of breeding programs. Resistance often breaks down due to the development of new and virulent pathotypes of leaf rust. Consequently it is necessary to constantly select for other sources of new resistance genes. Up to now nearly 50 leaf rust resistance (*Lr*) genes have been reported (McIntosh *et al.*, 1998, 1999, 2000, 2002).

In an effort to select leaf rust resistant germplasm, cereal rust researchers at the University of the Free State have identified two tetraploid wheat lines (2n=4x=28, AABB) among 353 *Triticum* accessions (Barnard, 1999). The selected sources of adult plant leaf rust resistance are: 104 (*Triticum turgidum* subsp. *dicoccum* variety *arras*) and 127 (*T. turgidum* subsp. *durum* variety *aestivum*). To exploit the candidate lines for use in future breeding programs, genetic analysis studies were conducted focusing on the following objectives:

- To identify the chromosomal location of leaf rust resistance genes in the two tetraploid wheat lines using cytogenetic stocks of CS monosomics and Langdon durum D-genome disomic substitution lines.
- To compare the results and determine which method of analysis works best for localizing genes in tetraploid wheat.

The Langdon durum D-genome disomic substitution lines were further investigated with additional objectives:

- To study the genetic variation for important agronomic traits among the lines and the recurrent parent Langdon.
- To test associations of yield and yield-related traits among Langdon durum D-genome disomic substitution aneuploids through path coefficient analyses.

The following conclusions were drawn and are presented below (see notes 1-5) in accordance to the stated objectives.

(1) Chromosomal location of leaf rust resistance genes ,using cytogenetic stocks of Chinese Spring (CS) monosomic lines

Employing A- and B-genome Chinese Spring (CS) monosomic stocks to produce and self F_1 monopentaploid hybrids (2n=5x-1=34, AABBD) the gene in accession 104 was localized on chromosome 1A. In accession 127 the gene was located on chromosome 4A. Earlier F_2 segregation analysis using pentaploid hybrids (2n=5x=35, AABBD) derived from crossing the AB-genome CS monosomics with accession 104 revealed that two genes governed inheritance in accession 104. Accession 127 displayed a typical dominant monogenic segregation ratio in the F_2.

The monopentaploid analysis had major drawbacks since most of the F_1 hybrids were sterile making the F_2 segregation analysis of accession 104 incomplete. Seven monopentaploid F_1 hybrids were sterile in the crosses between accession 104 and the 14 monosomic lines and sterility was observed in six F_1 hybrids of the crosses between accession 127 and the monosomics. Furthermore, it was found that seeds of one F_1 hybrid that resulted from crossing 104 with CS monosomic 6A failed to germinate. Also, seeds from three F_1 hybrids from crossing 127 with CS monosomic 2B, 5A and 6A failed to germinate and such crosses were not included in the F_2 analysis. The seeds of these hybrids were weak and shriveled.

To verify these findings and reach a credible conclusion we used another set of tetraploid cytogenetic stocks (see numbers 2 and 3 below).

(2) Chromosomal location of leaf rust resistance genes using Langdon durum D-genome disomic substitution lines

Inter-chromosomal gene mapping studies were carried out using Langdon durum D-genome substitutions (2n=4x-2+2). This method localized the gene in accession 104 on chromosome 6B and that in accession 127 on chromosome 4A. Thus two methods of analysis gave similar results for accession 127. However, the substitution analyses failed to locate the resistance gene in accession 104 on chromosome 1A. The inability of the substitution line to localize the second gene in this accession is attributed to a suppression gene brought from the D-genome chromosome of the substitution line 1D1A.

The present study confirmed the usefulness of the substitution lines for the chromosomal location of leaf rust resistance genes in tetraploid wheats. It is, however, worthwhile to carry out preliminary tests of the substitution lines with the known rust pathotypes to avoid gene interactions emerging from the substitution lines that subsequently may camouflage the phenotype of the desired parent under investigation.

(3) Comparative analysis of CS monosomics and Langdon durum D-genome disomic substitution lines for inter-chromosomal location of leaf rust resistance genes in tetraploid wheats

Hexaploid CS A- and B-genome monosomics and tetraploid Langdon durum D-genome disomic substitution lines were compared for their usefulness to determine chromosome locations of leaf rust resistance genes in tetraploid wheats.

Both stocks gave the same result in locating the gene for leaf rust resistance in accession 127. However, the gene localized on chromosome 1A in accession 104 by the CS analysis could not be confirmed using the Langdon durum D-genome

substitution analysis The CS analysis in turn failed to localize the gene in accession 104 on chromosome 6B as shown by the tetraploid aneuploids.

The present study demonstrated that the tetraploid aneuploids are more useful for genetic analysis of leaf rust resistance in the tetraploid wheats. The F_1 monopentaploid hybrids that resulted from crossing CS monosomics with the tetraploid wheats had a high degree of sterility and in some of the fertile hybrids seed germination was a problem. This rendered the F_2 segregation analysis incomplete and failed to locate the other gene on chromosome 6B in accession 104. The F_1 hybrids from the tetraploid cytogenetic stocks, however, did not show hybrid sterility and seed germination failure. Besides, the presence of relatively low numbers of chromosomes in the F_1 hybrids, from crosses between the tetraploid aneuploids and tetraploid disomic wheats, would make meiotic chromosome analysis easier than in crosses with hexaploid monosomics.

Accessions 104 and 127 are sources of resistance genes that could be further exploited in leaf rust resistance breeding programs. The cereal rust research group of the Department of Plant Sciences, University of the Free State, has transferred the resistance genes of the accessions to the susceptible bread wheat cultivar SST55 (*Triticum aestivum* L., 2n=6x=42). Currently early breeding generations are being studied and seed stocks are available for further research work on request to the Department .

To confirm these genes as new, however, it is essential to carry out linkage studies relative to earlier reported *Lr* genes that are located on the same chromosomes. Information on the linkage relationship of the genes will verify whether the genes are new or similar to earlier reported *Lr* genes. If the linkage analysis suggested that the genes are different from earlier identified ones, mapping of the genes will be undertaken with respect to known molecular markers. Following mapping of the genes, new gene symbols will be assigned for the genes in the accessions 104 and 127. Furthermore it is equally important to identify molecular markers flanking these genes in order to screen their presence in future breeding materials.

(4) Studies on genetic variation for important agronomic traits among Langdon durum D-genome disomic substitution lines and Langdon

Analysis of variance revealed considerable genetic variation among the substitution aneuploids when compared to the recurrent parent, Langdon. This variation is considered a shortcoming in employing the substitution lines in genetic analysis of tetraploid wheats.

Substitution lines 2D2B, 7D7A, and 7D7B were found to be the most divergent forms showing significant phenotypic variation for at least three characters investigated. The lines are reportedly backcrossed for 12 generations to Langdon. It appears, however, that there need to be further backcrossings to the recurrent parent and further targeted selections to increase traits such as plant height, number of spikelets per spike, kernel weight, and seed yield in line 2D2B. The same selection schemes are required to improve the number of spikelets per spike, number of kernels per spike, kernel weight, and seed yield in line 7D7B. Additionally traits such as number of spikelets per spike, kernel weight and seed yield require further improvement in line 7D7A.

(5) Path coefficient analyses on associations of yield and yield-related traits among Langdon durum D-genome disomic substitution aneuploids

Path coefficient analysis helps to portrait the direct influence of one variable upon another and permits the separation of the correlation coefficient into components of direct and indirect effects. Subsequently it will ease examining important relationships and discerning patterns among subsets of predictor variables. The direct and indirect influences of a character on the response trait may not be discernible from simple correlation coefficients.

From the path analysis study, information was obtained on the true associations of seed yield with kernel weight and heading date. This association was also supported by a simple correlation analysis. The direct path value of the path coefficient analysis exposed kernel weight as a key selection criterion to

improve seed yield in the substitution lines. The alternate path values further indicated that selection for kernel weight would bring simultaneous selection of improved number of kernel per spike, spikelets per spike and plant height.

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Chapter 7

Summary

Two sets of aneuploids were employed and compared to localize adult plant leaf rust resistance genes in tetraploid wheat accessions. One set was the hexaploid Chinese Spring (CS) A- and B-genome monosomics (2n=6x-1=41, AABBDD) and the other the tetraploid Langdon durum D-genome disomic substitutions (2n=4x-2+2=28). The tetraploid accessions (2n=4x=28, AABB) 104 (*Triticum turgidum* subsp. *dicoccum* var. *arras*) and 127 (*T. turgidum* subsp. *durum* var. *aestivum*) were selected as leaf rust-resistant after evaluating 353 *Triticum* accessions.

To study the chromosomal locations of the resistance genes, crosses were made between the complete sets of aneuploids (maternal parents) and the accessions. From both crosses F_1 hybrids were used for meiotic chromosome analysis and to select monosomic plants for F_2 segregation analysis. In the cross of the CS AB-genome monosomics with resistant lines, F_1 monopentaploid plants (2n=5x-1=34, AABBD) were selected. In the other crosses of the resistant accessions with the substitution lines, F_1 double monosomic plants were selected with 13 bivalent and two univalent chromosomes during metaphase I. The F_2 segregates of selfed monosomic plants were inoculated at the flag leaf stage with pathotype UVPrt2 of *Puccinia triticina*.

The CS monosomic analysis showed that in accession 104 a *Lr* gene occurs on chromosome 1A. Another gene in the accession was localized on chromosome 6B by Langdon durum substitution analysis. The second gene in this accession could not be localized from CS analysis since the F_1 monopentaploid hybrid of that cross was sterile making the F_2 segregation analysis incomplete. The gene localized on chromosome 1A in accession 104 by the CS analysis could not be localized by the substitution analysis owing to the presence of a suppressor gene brought from the D chromosome of substitution line 1D1A. In accession 127 the resistance gene was located on chromosome 4A using the two sets of aneuploids.

The study indicated that the tetraploid D-genome substitution lines are more commendable stocks than the hexaploid CS monosomics for chromosomal mapping of leaf rust resistance genes in tetraploid wheats. The trustworthiness of the tetraploid cytogenetic stocks is that the F_1 double monosomic hybrids resulting from crossing with the tetraploid did not show sterility or poor germination. These would furnish complete F_2 segregation analysis. Besides, the relatively few numbers of chromosomes in the F_1 hybrids would ease meiotic chromosome analysis. However, it would be necessary to consider the CS monosomic stocks during gene interaction from D-genome chromosomes of certain substitution lines on genes present on the A- or B-genome chromosomes of the tetraploid wheat under study.

The analysis of variance of important agronomic traits in the substitution lines suggested that three substitution aneuploids namely 2D2B, 7D7A and 7D7B were phenotypically divergent when compared to the other lines and the recurrent parent. These lines are reportedly backcrossed for 12 generations to Langdon. It appears, however, that further backcrossings to the recurrent parent and further targeted selections are necessary to increase traits such as plant height, number of spikelets per spike, kernel weight, and seed yield in line 2D2B. The same selection schemes are required to improve the number of spikelets per spike, number of kernels per spike, kernel weight, and seed yield in line 7D7B. Additionally traits such as number of spikelets per spike, kernel weight and seed yield require further improvement in line 7D7A. The path analysis revealed true associations of seed yield with kernel weight and heading date. This association was also supported by a simple correlation analysis. The direct path value of the path coefficient analysis exposed kernel weight as a key selection criterion to improve seed yield in the substitution aneuploids. The alternate path values further indicated selection for kernel weight would bring simultaneous selection of improved number of kernel per spike, spikelets per spike and plant height.

Opsomming

Twee aneuploïede reekse is vergelyk om volwasse blaarroesweerstandsgene in tetraploïede aanwinste te lokaliseer. Die reekse was die heksaploïede Chinese Spring (CS) A- en B-genoom monosome (2n=6x-1=41) en die tetraploïede Langdon durum D-genoom disomiese vervangingsreeks (2n=4x-2+2=28). Die tetraploïede koringaanwinste (2n=4x=28, AABB) beskryf as 104 (*Triticum turgidum* subsp. *dicoccum* var. *arras*) en 127 (*T. turgidum* subsp. *durum* var. *aestivum*), is geselekteer vir uitstekende blaarroesweerstand uit 353 *Triticum* aanwinste.

Beide aneuploïede reekse is as moederplante gebruik in kruisings met die weerstandbiede stuifmeelouers om die chromosomale posisies van die *Lr* gene vas te stel. Meiotiese chromosoomanalises van die F_1-basters is gebruik om monosomiese plante te selekteer vir die F_2-segregasie analises. Monopentaploïede (2n=5x-1=34, AABBD) is geselekteer uit die kruising tussen CS AB-genoom monosome en die weerstandbiedende ouers. In die kruisings tussen die disomiese vervangingslyne en die weerstandbiede ouers, is dubbelmonosomiese F_1-plante geselekteer met 13 bivalente en 2 univalente tydens metafase I. Selfbestuiwing van die geselekteerde monosome is tydens die vlagblaarstadium geïnokuleer met patotipe UVPrt2 van *Puccinia triticina*.

Die CS monosoomanalises dui daarop dat die *Lr*-geen op chromosoom 1A geleë is in die kruising met aanwins 104. 'n Verdere geen op chromosoom 6B is waargeneem met die Landon durum vervangingslynanalise. Die tweede geen kon nie by die CS analise waargeneem word nie, omdat die F_1-monopentaploïed steriel was en F_2-segregasie-analise dus nie gedoen kon word nie. Die lokalisering van die *Lr*-geen op chromosoom 1A by aanwins 104 en CS monosoomanalise kon nie deur die vervangingsanalise bevestig word nie, moontlik weens onderdrukking van 'n ander *Lr*-geen op die D-genoom chromosoom van die Langdon durum vervangingslyn, 1D(1A). Altwee

reekse aneuploïdes vind die *Lr*-geen op chromosoom 4A in kruisings met aanwins 127.

Hierdie studie toon dat chromosomale kartering van *Lr* gene in tetraploïede korings beter is met tetraploïede D-genoom vervangingslyne as sitogenetiese materiaal, as met die heksaploïede CS monosome. Die voordeel van die tetraploïede vervangingsmateriaal is dat die F_1-dubbelmonosome (die resultaat van die kruising met die tetraploïede korings) nie steriel is en geen ontkiemingsprobleme veroorsaak nie. 'n Volledige F_2-segregasie-analise kan dus uitgevoer word. Daarbenewens bied die relatiewe lae chromosoomgetal van die F_1-basters 'n makliker meiotiese analise. 'n Komplimentêre heksaploïede analise is slegs geregverdig indien die tetraploïede analise beïnvloed word deur geeninteraksie met die D-genoom.

Die variansie-analise van belangrike agronomiese kenmerke in die vervangingslyne dui daarop dat die vervangingslynaneuploïdes, 2D2B, 7D7A en 7D7B, fenotipies betekenisvol verskil van die ander lyne en die spilouer. Dit noodsaak herhaalde terugkruisings na die spilouer en verdere seleksies is nodig vir kenmerke soos planthoogte, aantal blompakkies per aar, saadmassa en saadopbrengs in lyn 2D2B. Dieselfde seleksieprosedure is nodig vir 'n verbetering in die aantal blompakkies per aar, aantal sade per aar, saadmassa, en saadopbrengs in lyn 7D7B. Kenmerke soos die aantal blompakkies per aar, saadmassa en saadopbrengs benodig verdere verbeterings in lyn 7D7A. Baananalises dui aan dat saadopbrengs geassosieerd is met saadmassa en aarverskyning. Hierdie assosiasie word ondersteun deur 'n eenvoudige korrelasie analise. Die direkte baanwaarde van die baankoëffisiëntanalise dui op saadmassa as die sleutel seleksiemaatstaf vir die verbetering van saadopbrengs in die vervangingsaneuploïedes. Die alternatiewe baanwaardes dui daarop dat seleksie vir saadmassa gelyktydig seleksie vir die verbetering van aantal sade per aar, blompakkies per aar en planthoogte sal meebring.

Appendix

I Major infection type classes for stem and leaf rust (Roelfs, 1988b; McIntosh *et al.*, 1995a)[1].

Infection type	Host response	Symptoms
0	Immune	No visible uredia
;	Very resistant	Hypersensitive flecks
1	Resistant	Small uredia with necrosis
2	Resistant to moderately resistant	Small to medium sized uredia with chlorosis or necrosis
3	Moderately resistant/ moderately susceptible	Medium sized uredia with or without necrosis
4	Susceptible	Large uredia without chlorosis or necrosis
X	Resistant	Heterogeneous, similarly distributed over the leaves
Y	Resistant	Variable size with larger uredia towards the tip
Z	Resistant	Variable size with larger uredia towards the leaf base

[1] see citation in Chapter 2

II Avirulence/virulence formula of pathotypes of *Puccinia triticina* based on infection types on South African differential sets and their selective hosts.

Pathotype	Avirulence/virulence	Selective host
UVPrt2	*Lr1, Lr2a, Lr2b, Lr3ka, Lr11, Lr15, Lr17, Lr20, Lr24, Lr26, Lr30/Lr2c, Lr3a, Lr3bg, Lr10, Lr14a, Lr16*	Zaragoza
UVPrt3	*Lr3a, Lr3bg, Lr3ka, Lr10, Lr11, Lr14a, Lr16, Lr17, Lr20, Lr26, Lr30/Lr1, Lr2a, Lr2b, Lr2c, Lr15, Lr24*	Agent
UVPrt9	*Lr2a, Lr2b, Lr3bg, Lr15, Lr16, Lr17, Lr26, Lr30/Lr1, Lr2a, Lr2b, Lr2c, Lr10, Lr14a, Lr15, Lr17, Lr24*	Karee
UVPrt13	*Lr3a, Lr3bg, Lr3ka, Lr11, Lr16, Lr20, and Lr30/Lr1, Lr2a, Lr2b, Lr2c, Lr10, Lr14a, Lr15, Lr17, Lr24, Lr26*	Gamtoos

III Adult plant disease reaction of Chinese Spring A- and B-genome monosomics and Langdon durum D-genome substitution lines, after inoculation with four pathotypes of *Puccinia triticina*.

Line	Pathotype			
	UVPrt 2	UVPrt3	UVPrt9	UVPrt13
CS Monosome				
CSM 1A	3C	1	2C	1
CSM 1B	3	2C	2	2C
CSM 2A	3N	2	1	2
CSM 2B	3	1N	3	1
CSM 3A	4	3	4	1
CSM 3B	3C	3	3	1N
CSM 4A	3	2C	4	1
CSM 4B	3	3	2C	1N
CSM 5A	4	3	3	3
CSM 5B	3	3C	2	3
CSM 6A	3	2C	3	3
CSM 6B	3N	4	3	4
CSM 7A	3N	3	3	3
CSM 7B	2^{+}	3	3	2N
D-genome substitution and Langdon durum				
LDN 1D(1A)/*12LDN	1N	1N	1N	1
LDN1D(1B)/*12LDN	3C	3	3C	3C
LDN2D(2A)/*12LDN	3	3C	3	3
LDN2D(2B)/*12LDN	3	2C	2	1N
LDN3D(3A)/*12LDN	4	4	4	1N
LDN3D(3B)/3Daddition line	3C	2C	2C	1
LDN4D(4A)/4D(4A)	3	1N	3	1
LDN4D(4B)/*12LDN	4	3	2C	1N
LDN5D(5A)/*12LDN	4	4	4	3
LDN5D(5B)/*12LDN	4	3C	3	3
LDN6D(6A)/*12LDN	3	1N	3	1
LDN6D(6B)/6D(6A)//6D(6B)	3N	2C	3	1
LDN7D(7A)/*12LDN	3N	2C	1	3
LDN7D(7B)/*12LDN	3	3	3	1N
Langdon durum	4	3	3C	2C
Susceptible checks				
Zaragosa	4			
Agent		4		
Karee			3	
Gamtoos				4
Resistant accessions				
104	1N	;N	;	;
127	2C	1N	1N	1

IV ANOVA after SAS procedure for nine agronomic traits of 14 Langdon durum D-genome substitution lines and Langdon durum. Mean square values for the main effects of entries, pots and their interaction and probability values are given. The degrees of freedom for entry, pot, entry x pot and error were 14, 2, 28, and 97, respectively.

Trait/variable	Mean square				Pr > F		
	Entry	Pot	Entry X pot	Error	Entry	Pot	Entry x pot
Heading dates (days)	154.05	12.42	1.78	6.68	0.0001	0.1612	0.9999
Plant height (cm)	1690.2	118.99	116.64	126.78	0.0001	0.3947	0.5853
Flag leaf length (cm)	63.48	3.98	7.40	11.49	0.0001	0.7078	0.9077
Tiller number	3.47	1.43	1.14	0.76	0.0001	0.1589	0.0792
Spike length (cm)	13.91	0.09	0.37	0.96	0.0001	0.9026	0.9973
Spikelets/spike	56.98	0.30	4.31	8.28	0.0001	0.9636	0.9745
Kernels/spike	1746.4	9.54	24.76	35.97	0.0001	0.7675	0.8700
200-Kernel weight (g)	18.83	1.09	0.75	0.93	0.0001	0.3165	0.7372
Seed yield (g)	547.98	1.28	23.42	21.58	0.0001	0.9426	0.3716

V ANOVA after SAS procedure for nine agronomic traits of 14 Langdon durum D-genome substitution lines and the Langdon durum. Entries were tested over three pots. The degrees of freedom for entry and residual were 14 and 30, respectively.

Trait/variable	Sum of squares		F value	Pr > F
	Entry	Error		
Heading dates (days)	702.15	23.16	64.89	0.0001
Plant height (cm)	7663.24	1132.12	14.5	0.0001
Flag leaf length (cm)	286.26	66.79	9.19	0.0001
Tiller number	16.56	11.17	3.18	0.0038
Spike length (cm)	60.40	3.30	39.21	0.0001
Spikelets/spike	247.28	39.68	13.35	0.0001
Kernels/spike	7770.18	236.32	70.46	0.0001
200-Kernel weight (g)	86.98	7.72	24.14	0.0001
Seed yield (g)	2475.09	232.36	22.83	0.0001

VI Means and standard deviations of agronomic traits of 14 Langdon durum D-genome substitution lines and Langdon durum. N represents number of replications.

Level of		HD		PH		FL	
ENTRY	N	Mean	SD	Mean	SD	Mean	SD
1D1A	3	48.97	0.29	117.74	11.58	12.02	0.75
1D1B	3	47.99	0.58	110.20	13.96	16.39	1.84
2D2A	3	47.55	0.39	115.95	4.44	19.17	2.32
2D2B	3	54.33	0.88	100.33	3.43	13.29	0.42
3D3A	3	50.00	0.00	149.99	1.53	13.67	1.04
3D3B	3	48.50	0.87	106.44	5.17	12.26	0.23
4D4A	3	54.08	1.13	106.33	2.31	12.97	1.07
4D4B	3	53.33	0.34	133.67	2.91	20.78	0.35
5D5A	3	50.17	0.29	126.50	5.77	16.77	0.89
5D5B	3	53.33	0.67	128.18	1.36	16.27	2.69
6D6A	3	48.53	0.65	128.67	5.37	16.06	1.36
6D6B	3	57.00	0.00	131.72	3.25	16.30	1.34
7D7A	3	56.72	1.06	105.58	3.48	15.51	1.81
7D7B	3	61.89	2.37	112.56	6.55	17.99	2.47
LANGDON	3	53.00	0.67	122.94	5.76	12.92	0.72

Level of		TN		SL		SP	
ENTRY	N	Mean	SD	Mean	SD	Mean	SD
1D1A	3	3.36	0.63	6.52	0.15	18.17	0.44
1D1B	3	3.00	0.88	5.28	0.25	17.11	0.51
2D2A	3	4.08	1.09	6.51	0.30	15.89	0.51
2D2B	3	3.86	0.55	5.85	0.25	13.11	1.84
3D3A	3	3.00	0.33	7.14	0.41	16.56	1.26
3D3B	3	2.50	0.17	5.58	0.15	14.83	0.44
4D4A	3	3.17	0.29	4.97	0.21	5.56	0.77
4D4B	3	2.78	1.07	6.44	0.54	18.89	0.38
5D5A	3	3.78	0.69	8.93	0.66	17.94	0.59
5D5B	3	3.33	0.33	6.49	0.35	13.28	0.75
6D6A	3	2.14	0.55	4.08	0.14	14.33	1.53
6D6B	3	3.28	0.68	4.17	0.17	15.78	1.57
7D7A	3	3.11	0.19	5.63	0.31	12.39	1.14
7D7B	3	1.99	0.32	5.95	0.25	11.11	1.02
LANGDON	3	2.22	0.19	6.58	0.36	19.06	2.28

Level of		KS		KW		SY	
ENTRY	N	Mean	SD	Mean	SD	Mean	SD
1D1A	3	45.80	2.33	8.53	0.56	24.48	1.46
1D1B	3	58.44	6.29	10.93	0.27	25.47	2.12
2D2A	3	33.19	1.89	8.13	0.26	21.55	0.86
2D2B	3	22.05	1.11	7.02	0.28	13.62	2.08
3D3A	3	33.33	4.05	9.52	0.36	25.61	2.21
3D3B	3	16.61	1.14	9.09	0.12	25.11	1.26
4D4A	3	15.58	1.18	8.65	0.97	23.19	4.24
4D4B	3	33.89	1.26	10.90	0.52	22.16	3.86
5D5A	3	9.36	1.48	9.08	0.58	24.88	2.98
5D5B	3	14.83	1.42	8.62	0.25	24.81	1.85
6D6A	3	32.08	2.79	10.91	0.06	26.61	2.71
6D6B	3	31.33	4.73	10.52	0.37	22.59	3.11
7D7A	3	19.14	2.17	7.81	0.28	4.69	0.76
7D7B	3	13.33	1.45	6.60	0.39	3.82	0.53
LANGDON	3	37.78	2.55	10.85	1.09	29.34	5.92