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Chromosomal location and molecular mapping of tan spot resistance genes in common wheat (*T. aestivum* L.)

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2. Tadesse, W., S.L.K, Hsam, G. Wenzel, and F.J. Zeller 2006a. Identification and monosomic analysis of tan spot resistance genes in synthetic wheat lines (*Triticum turgidum* L. x *Aegilops tauschii* Coss.). Crop Sci. 46:1212-1217.
3. Tadesse, W., S.L.K. Hsam, and F.J. Zeller. 2006b. Evaluation of common wheat cultivars for tan spot resistance and chromosomal location of a resistance gene in the cultivar 'Salamouni'. Plant Breed. 125:318-322.

These manuscripts are attached in the Appendix part of this dissertation for detail reference.

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List of Abreviations

AFLP	Amplified length polymorphism
APS	Amonium persulphate
bp	base pair
cM	centi Morgan
CS	Chinese Spring
CTAB	Cethyltrimethyl amonium bromide
CIMMYT	International center for wheat and maize improvemnet
dNTP	Deoxy nucleoside triphosphate
DNA	Deoxyribonuclic acid
DTR	Dreschslera tritici-repentis
EDTA	Ethylenediaminetetraacetic acid
GWM	Gatersleben wheat microsatellite
LOD	Log of odds
MAS	Marker assisted selection
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
Ptr	*Pyrenphora tritici-repentis*
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment lenth polymorphisms
SSR	Simple sequence repeat (microsatelites)
TEMED	Tetramethyl ethylene diamine

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1. INTRODUCTION

Common wheat (*Triticum aestivum* L.) is believed to be one of the first of the grains domesticated by humans since the Neolithic period ~10,000 years B.C., and todate it counts one of the most important cereal grain feeding the increasing world population (Feldman *et al.,* 2001). The wheat flour is important not only for making bread, biscuit and pastry products, but also for the production of commercial starch and gluten. According to the FAO report (2006), about 620 million metric tons of wheat were produced in a total area of 217 million hectares in the year 2005/06 worldwide with an average yield of 2.85 metric tons per hectare.

The production of wheat is affected by different abiotic and biotic factors. The biotic factors, mostly fungal pathogens causing leaf diseases, account for the major yield loss in wheat. One of such pathogens which is becoming important since the years 1970s is the fungus *Pyrenophora tritici- repentis* (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoem) (DTR), which causes tan spot of wheat (Hosford, 1971, 1982; Wolf and Hoffmann, 1993). Tan spot of wheat is spreading world-wide at an increasing rate, and can cause a yield loss of up to 50 % in susceptible wheat cultivars (Hosford, 1974; Wolf and Hoffmann, 1993; Riede *et al*., 1996; Duveiller *et al*., 2005). Adoption of new farm management practices such as minimum or zero tillage, banning of stubble burning, and intensive wheat after wheat cultivation systems have contributed to the fast spread of the pathogen *Pyrenophora tritici-repentis* (Rees, 1982; Kohli *et al*., 1992; Fernandez *et al*., 1998; Tekauz *et al*., 2004).

The development and use of resistant cultivars is regarded as the most cost effective, socially feasible and ecologically safe means of controlling tan spot. Because of the co-evolution of the host and pathogen, however, the deployment of individual resistance genes leads to the emergence of new virulent pathogen mutants. Hence, identification of new resistance sources and pyramiding of more resistance genes in a cultivar are of paramount importance for effective and better genetic control. However, selection of genotypes with such gene combinations via classical genetics and breeding methods is very time consuming and difficult due to the lack of pathogen isolates with specific virulence genes.

On the other hand, the development of molecular markers that are closely associated with the respective resistance genes would enable to pyramidize genes of interest effectively and successfully through marker assisted selection (Gupta *et al*., 1999; Huang *et al*., 2000).

Todate, however, unlike powdery mildew and rust resistance genes of wheat, only very few sources of tan spot resistance genes are identified and mapped (Faris *et al*., 1996, 1997; Friesen and Faris, 2004; Cheong *et al*., 2004; Singh *et al*., 2006). This study was undertaken with the following major objectives.

1. Screening of landraces/cultivars, synthetic lines, and wild species of *Triticum* in order to identify sources of resistance against tan spot
2. Compare seedling and adult plant resistance
3. Determine the inheritance of tan spot resistance genes
4. Determine the chromosomal location of the resistance genes through monosomic analysis
5. Molecular mapping of the resistant genes using SSR markers

2. LITERATURE REVIEW

2.1 Evolution and origin of *Triticum*

2.1.1 Evolution

All the cultivated wheats belong to the genus *Triticum*, which in turn was divided into three major taxonomic groups: einkorn, emmer, and dinkel by Schultz (1913). This classification was supported by the pioneering cytological study of Sakamura (1918), who found that Schultz's three wheat groups also differ in their chromosome number; the einkorns are diploids (2n = 2x = 14), the emmers are tetraploids (2n = 4x = 28) and the dinkels are hexaploids (2n = 6x = 42), all with the basic chromosome number x = 7. Soon after, based on cytogenetic analysis, Kihara (1924) designated the genome formulae for the cultivated einkorn (*T. monococcum* L., 2n = 2x = 14), emmer (*T. turgidum* L. 2n = 4x= 28) and dinkel (*T. aestivum*, 2n = 6x = 42) as AA, AABB and AABBDD, respectively.

The diploid einkorn wheat, *Triticum monococcum* (2n = 2x = 14, A^mA^m), has been domesticated directly from its wild form, *T. aegilopoides* (2n = 2x = 14, A^mA^m). Similarly, the cultivated emmer wheat, *Triticum dicoccum* (2n = 2x = 28, AABB), is adapted from the wild emmer, *Triticum dicoccoides* (2n = 2x = 28, AABB), which is an allopolyploid, arose by amphiploidy between *Triticum urartu* (2n = 2x = 14, AA) and *Aegilops speltoids* (2n = 2x = 14, BB) (Johnson and Dhaliwal, 1976; Feldman and Levy, 2005). There is also another tetraploid wheat, *Triticum timopheevii* (2n = 4x = 28, A^tA^tGG), which, however, has been cultivated in a very limited extent. It is believed to be domesticated from the wild emmer wheat, *T. dicoccoides ssp armeniacum* (Feldman, 2001). According to Naranjo (1990) and

Jiang and Gill (1994), a species-specific translocation involving chromosomes $6A^t$, 1G and 4G distinguishes *T. timopheevii* from *T. turgidum*, which contains a translocation involving chromosomes 4A, 5B and 7B (Devos *et al*., 1995).

The evolution of the common wheat, *Triticum aestivum* (2n = 6x = 42, AABBDD), has been the subject of many investigations and intense discussions for several decades. As indicated in Fig. 1, it is now considered certain that hexploid wheat was formed from a hybrid between the tetraploid wheat speceis *T. turgidum* (2n = 4x = 28, AABB) and the diploid speceis *Ae tauchii var. strangulata* (2n = 2x = 14, DD) (McFadden and Sears, 1946; Riley *et al*., 1958; Dvorak *et al*., 1998). Genome analyses by Kihara (1919) and Sax (1922) on the pairing behaviour of interspecific hybrids between 2x/4x and 4x/6x wheats indicated that *T. monococcum* and *T. turgidum* share one genome in common, and *T. turgidum* and *T. aestivum* share two genomes in common. However, the cytological data did not discriminate between *T. monococcum* (2n = 2x = 14, A^mA^m) and *T. urartu* (2n = 2x = 14, AA) genomes (Johnson and Dhaliwal, 1976), but the molecular evidence showed that *T. urartu* actually is the A genome donor of both tetraploid and hexaploid wheats (Dvorak *et al*., 1993). The other haxaploid wheat, *T. zhukovsky* (2n = 6x = 42, $A^tA^tA^mA^mGG$), arose from the hybridization of *Triticum timopheevii* (2n = 4x = 28, A^tA^tGG) with *T. monococcum* (2n = 2x = 14, A^mA^m) (Upadhya and Swaminanthan, 1963).

There has been much controversy regarding the origin of the B and G genomes of polyploid wheats since the early proposal of Sarkar and Stebbins (1956) supported by Riley *et al*. (1958) that *Ae. speltoides* was the donor of the second genome of tetraploid wheats. Recent molecular evidence, however, is convincing that the B and G genomes of polyploid wheats were donated by *Ae. speltoides* (Dvorak and Zhang, 1990; Daud and Gustafson, 1996; Petersen *et al*., 2006). Furthermore, the cytoplasmic genome heterogenity within *Ae. speltoides* indicated that it may be the maternal (cytoplasmic) donor of all polyploid wheats (Wang *et al*., 1997; Gill and Friebe, 2001).

Chromosome pairing in polyploid *Triticum* species occurs in a diploid-like fashion between homologous chromosomes and not between homoeologues (partially homologous chromosomes of the different genomes). This is due to the supressor *Ph1* (Riley and Chapman, 1958; Sears, 1976; Vega and Feldman, 1998) and *Ph2* (Mello-Sampayo, 1971; Dong *et al*., 2002) genes. Therefore, in plants lacking these genes, particularly the *Ph1* gene,

multivalents were observed during meiosis due to pairing among the homoeologus chromosomes, resulting in partial sterility of plants, indicating the crucial role of the *Ph1* gene

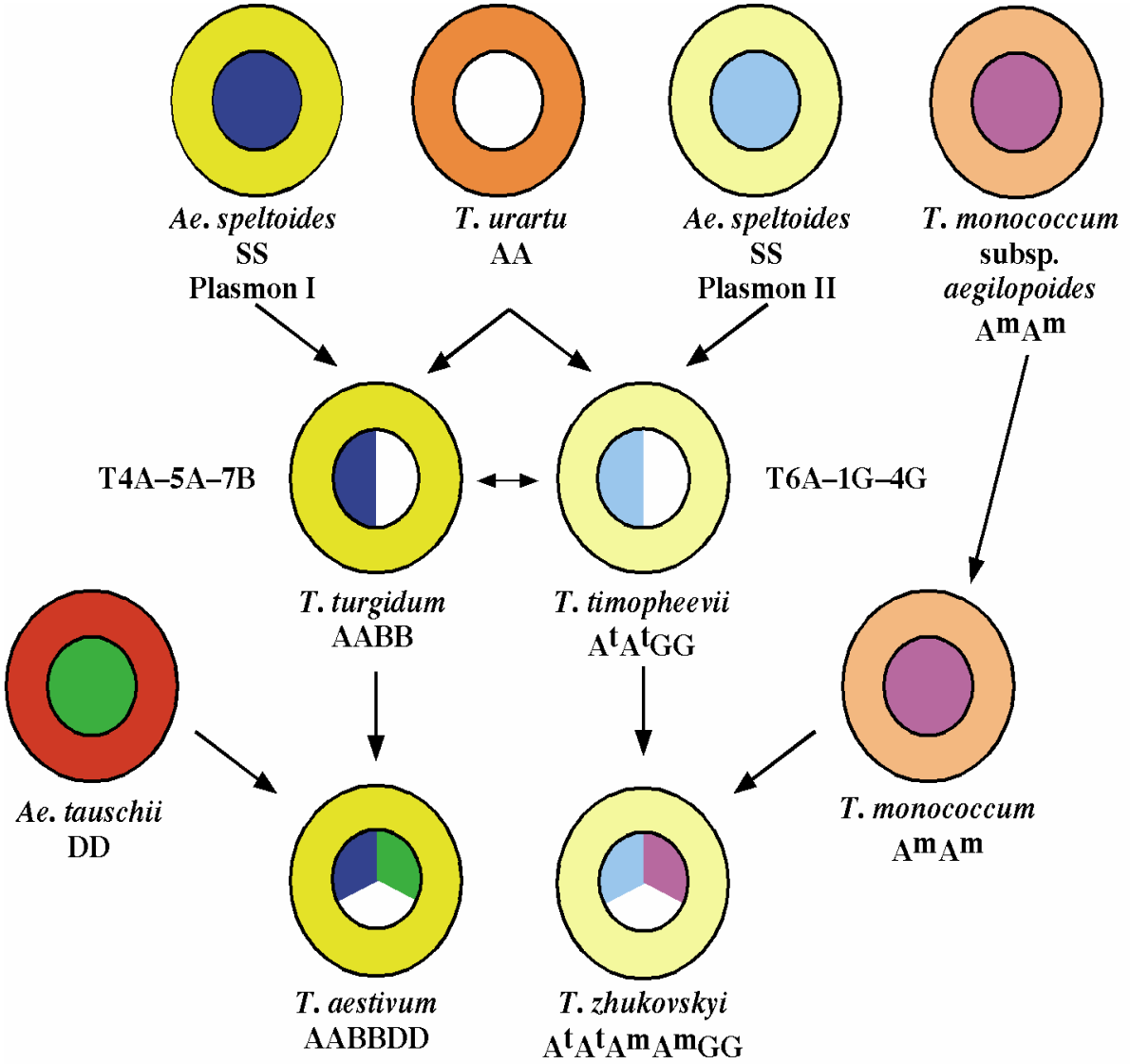


Fig 1 Current theory of *Triticum* evolution (Gill and Friebe, 2001)

for diploid-like chromosome pairing and for the evolution of polyploid wheats and their domestication (Riley and Chapman, 1958; Sears, 1976, 1977; Koebner and Shepherd, 1986).

2.1.2 Origin and distribution of *Triticum*

The origin and evolution of a cultivated plant can be best studied following the identification of its wild progenitor and the current and past distribution of its progenitor. This may indicate

the changes that led to domestication as well as the site of the initial cultivation. However, when such a wild progenitor is not found, or is extinct, understanding the complete history of that cultivated plant is greatly impaired (Feldman, 2001).

Archaeological and botanical studies of both wild and cultivated forms have indicated that the Fertile Crescent (Fig. 2) is the birth place of cultivated wheats about 8000 to 10000 years ago (Gill and Friebe, 2001; Mujeeb-Kazi and Villareal, 2002). Among diploid wheats, eincorn wheat (*Triticum monococcum* L.) is still cultivated to a limited extent, and its wild form, *T. aegilopoides*, is widely distributed in the Middle East (Johnson, 1975; Heun *et al*., 1997).

The tetraploid hulled wheat, *T. turgidum ssp. dicoccum* (emmer wheat), was one of the ancient cultivated wheats. However, it is the free-threshing macaroni or durum wheat, which arose by few mutations from primitive emmer wheats that are widely cultivated in the present times (Gill and Friebe, 2001). The remains of the cultivated emmer (*Triticum turgidum* ssp. *dicoccum*) have been discovered at several archaeological sites in Syria dating to 7500 BC (Zohary and Hopf, 1993; Zohary, 1999). The other cultivated tetraploid wheat, *Triticum timopheevii* (2n = 4x = 28, AAGG), is of little economic importance. The wild forms of both tetraploid wheats, *Triticum turgidum ssp dicoccoides and T. timopheevii ssp armeniacum,* are widely distributed in the Fertile Crescent. *T. diccoccides* is found exclusively in Israel, Syria, and Lebanon, while *T. armeniacum* is dominantly found in Azerbaijan and Armenia, and yet both overlap in Turkey, northern Iraq and possibly Iran (Gill and Friebe, 2001; Feldman, 1995, 2001). The hexaploid species, *T. aestivum* (2n = 6x = 42, AABBDD) and *T. zhukovsky* (2n = 6x = 42, AABBGG) have no wild progenitors, and are only found in cultivated forms in farmers´fields by hybridisation between cultivated tetraploid wheat and wild diploid species (Feldman, 2001).

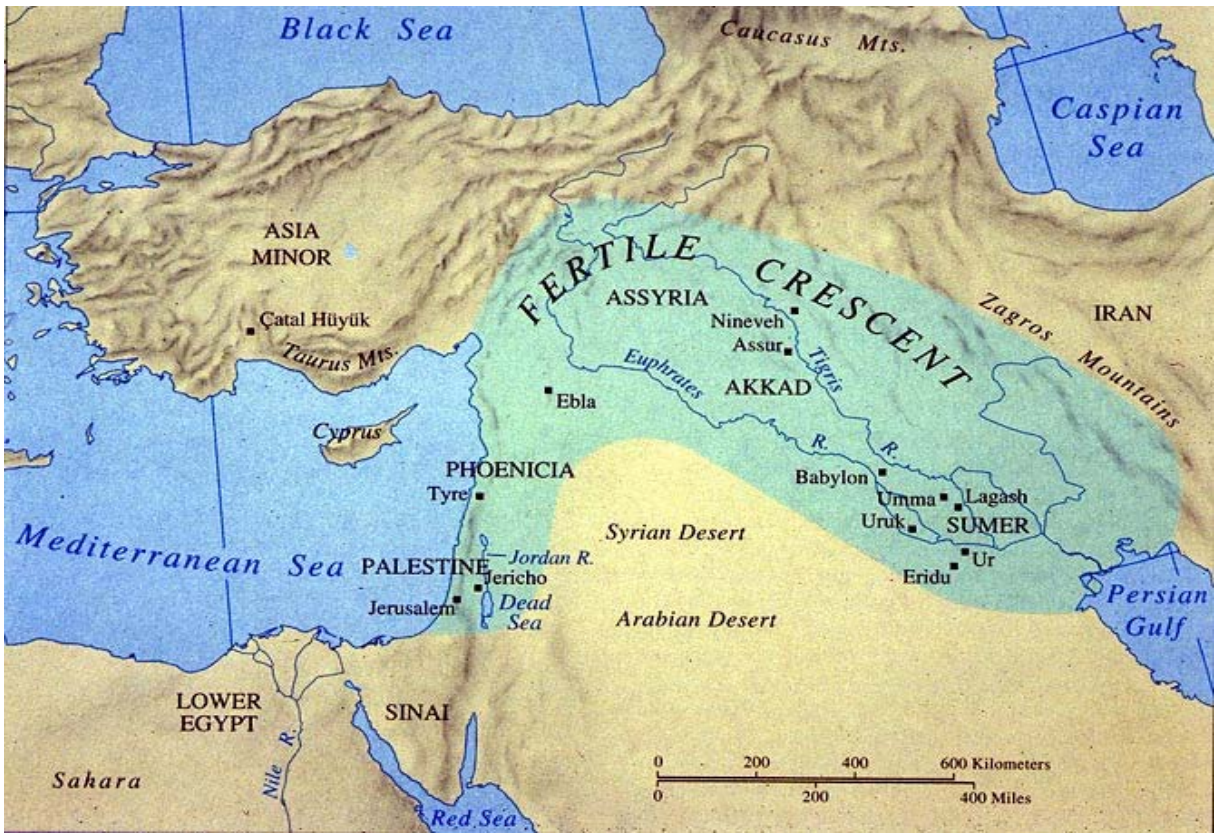


Fig 2 Geographic origin of *Triticum*

Bread wheat arose farther north west, away from the Fertile Crescent, in the corridor extending from Armenia in Transcaucasia to the south west coastal areas of the Caspian Sea in Iran (Dvorak *et al*., 1998). In this region, *Ae. tauschii* var. *strangulata* is predominant, which evidently hybridized with cultivated emmer to produce *T. aestivum*.

There are five *T. aestivum* (2n = 6X = 42, AABBDD) subspecies based on spike morphology, namely: *T. aestivum* ssp *aestivum* (*QQ cc S1S1*), *T. aestivum* ssp *compactum* (*QQ CC S1S1*), *T. aestivum* ssp *spelta* (*qq cc S1S1*), *T. aestivum ssp macha* (*qq CC S1S1*) and *T. Aestivum* ssp *sphaerococcum* (*QQ CC s1s1*) which differ principally due to allelic variations of single major genes: *q* (the speltoid gene) and its dominant allele *Q* (which confers free-threshing grain and tough rachis) on chromosome 5A and 2D; *c* and its dominant compact-ear producing allele *C* on chromosome 2D; *S* and its recessive spherical-grain producing *s* allele on chromosome 3D (Miller, 1987). The first bread wheats may have looked similar to *T. aestivum ssp. spelta* found growing in Iran from which free-threshing types were derived by mutation (McFadden and Sears, 1946). According to Ohtsuka (1998) and Yan *et al*. (2003), the European spelt wheats may have been derived secondarily from a hybridization involving

T. compactum and emmer wheat. More recently, Matsuoka and Nasuda (2004), on the other hand, have suggested durum wheat (*T. durum ssp. durum*) as a candidate for the female progenitor (AABB) genome of bread wheat after embryo rescue-free crossing of the durum wheat cultivar Langdon with *Ae. tauschii* line and successfully producing fertile triploid F_1 hybrids which spontaneously (with out colchicine treatment) set hexaploid F_2 seeds at average selfed seed rate of 51.5%. Currently, common wheat (*T. aestivum*, 2n = 6X = 42, AABBDD), is the world´s most widely cultivated crop grown in all temperate and in most subtropical countries with altitude levels ranging from below sea level near the Dead Sea and the Imperial Valley of California to more than 4500 m in Tibet (Stoskopf, 1985).

2.2 Tan spot (*Pyrenophora tritici-repentis*) of wheat

2.2.1 Biology and life cycle

Tan spot (syn. yellow spot) of wheat is caused by the fungus *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* (Died.)Shoem.), a homothallic ascomycete (Hosford, 1971). It survives as a saprophyte on infected host debris between crops. Psuedothecia, approximately 0.2-0.35 mm in diameter, are produced in abundance on straw lying on soil during the autumn and winter. Ascospores are released in the spring during wet weather and serve as primary inoculum (Fig 3).

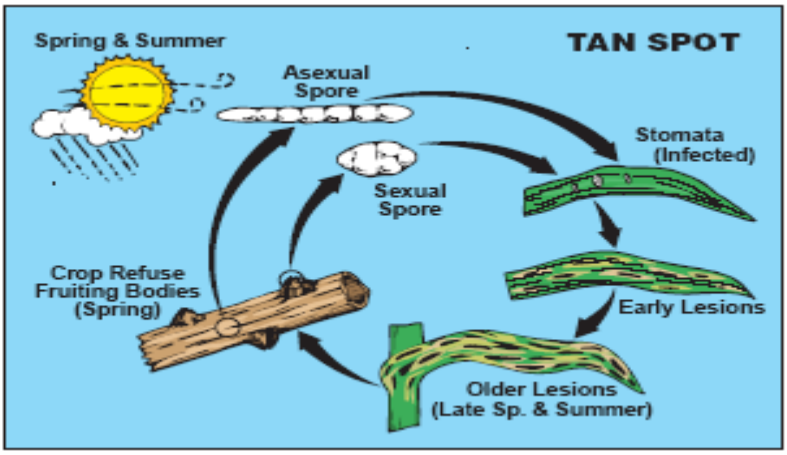


Fig 3 Life cycle of *Pyrenophora tritici-repentis (*McMullen, 2003)

Other sources of primary inoculum include mycelium from infected seed and conidia produced on colonized straw, other grass hosts and volunteer plants. Larez *et al*. (1986) indicated that the conidiospores (conidia) of *Pyrenophora tritici-repentis* germinate to produce germ tubes (the infecting units of the pathogen) from basal (polar) and intercalary

cells. The germ tubes grow over the leaf surface randomly or follow the contours (depressions between adjoining epidermal cells) of the leaf surface. The conidia can produce multiple germ tubes which can form a club-shaped or round appressorium over a target cell. Appressoria adhere firmly to the cuticle of the host by means of an extracellular sheath and develop above the junction of the epidermal cells, on hair cells or trichomes and over stomatal complexes (Dushinicky *et al.*, 1996; Larez *et al.*, 1986). This finally develops into a large brown lens-shaped necrotic lesions surrounded by a chlorotic halo with a small black point in the center of the lesion on susceptible cultivars, which is a typical symptom of tan spot (Hosford, 1982; Mielke and Reichelt, 1999; De Wolfe *et al.*, 1998). Under favourable conditions (temperatures ranging from 20-28 °C with frequent rains), susceptible cultivars would be severely infected, leading to leaf death (Lamari and Bernier, 1989a). Conidia produced on primary lesions during wet weather serve as secondary inoculum and are disseminated by wind to other parts of the same plant or other plants as indicated in Fig. 3.

2.2.2 Physiological races and their toxins

Most of the early tan spot rating systems were based on quantitative parameters, such as lesion size (Cox and Hosford, 1987), percent infection (Nagle *et al.*, 1982) or a combination of these (Luz and Hosford, 1980). Later, Lamari and Bernier (1989a, 1991) identified two qualitative types of symptoms, tan necrosis (nec +) and extensive chlorosis (chl +) produced by *P. tritici-repentis* on selected differential wheat cultivars. Four pathotypes were identified based on this criteria. Pathotype 1 (nec+chl+) causes both necrosis and chlorosis; pathotype 2 (nec+chl-) causes necrosis only; pathotype 3 (nec-chl+) causes chlorosis only; and pathotype 4 (nec-chl-) cuase neither necrosis nor chlorosis symptoms (Lamari and Bernier 1989a). This symptom based classification was limited to a maximum of four categories and could not accommodate new virulence patterns.

To overcome this problem, Lamari *et al*. (1995) introduced a race designation scheme based on the virulence of isolates on a set of differential lines or cultivars. Currently, at least eight races of *P. tritici-repentis* have been identified based on the type of toxin they produce and their ability to induce necrosis and/or chlorosis on a set of wheat differential cultivars (Strelkov and Lamari, 2003). *Ptr* ToxA, produced from race 1 is the most well-characterized host-selective proteinaceous toxin that causes necrotic symptoms in susceptible wheat cultivars. *Ptr* ToxB, isolated from race 5 (Orolaza *et al.,* 1995; Friesen and Faris, 2004) and *Ptr* ToxC isolated from race 1 cause chlorosis symptom. Isolates of race 3 which putatively

produce only *Ptr* ToxC, are virtually non-existent in hexaploid wheats and very rare (< 1%) in durum wheats (Effertz *et al*., 2002).

2.2.3 Economic importance of tan spot and its control measures

Tan spot occurs in all of the major cereal growing areas of the world, but it is more common and destructive in areas having relatively warm and wet weather during the cereal growing season. The pathogen can attack both durum (*Triticum turgidum* L. var *durum*) and common wheat (*Triticum aestivum* L.), as well as numerous other grass species (Hosford, 1971; Krupinsky, 1982, 1992; Ali and Francl, 2003). The incidence of tan spot and its economic importance is dramatically increasing since 1970s all over the world (Rees and Platz, 1979; Hosford, 1974, 1982; Wiese, 1987; Perello *et al*., 2002). Losses in grain yield are primarily the result of reduced kernel size, and ranges 3 to 50 % in susceptible varieties in Canada and USA (Hosford, 1974). In Germany reduction of grain yield due to this disease could range from 10 to 36 % (Wolf and Hoffmann, 1993). Kohli *et al*. (1992) has alsao indicated the fast spreading and destructive nature of the disease in the southern Cone region of South America including Argentina, Brazil, Chile, Paraguay and Uruguay.

The fast spread of the pathogen *Pyrenophora tritici-repentis* is attributed to its stubble-borne nature, to a shift towards soil conservation practices such as minimum and zero tillage, and the trend away from stubble burning (Sutton and Vyn, 1990; Bockus and Claasen, 1992; Bailey, 1996; Fernandez *et al*., 1998) and an intensive wheat after wheat production. These practices retain crop residues on the soil surface, resulting in an increase of inoculum as the pathogen survives from one season to the next on wheat and grass stubble. Many of the semi-dwarf wheat varieties introduced in Australia after 1960 have a high susceptibility to the disease (Rees *et al*., 1988) indicating that changes in cultivar genotypes may have also played a role in the increased incidence of tan spot.

Cultural practices such as crop rotation with non hosts, deep tillage and removal or destruction of infested residue, are effective in controlling tan spot. Effective control of tan spot can be also achieved with foliar fungicides such as propiconazole and tebuconazole (Watkins *et al*., 1982), but costs may be prohibitive in addition to the negative ecological impact. Lamari and Bernier (1989a) and Friesen and Faris (2004) indicated that the best approach for controlling tan spot is incorporation of genetic resistance into adapted cultivars since it is cost effective, socially feasible and ecologically safe. Both *Ptr* toxins and conidia

can be used for germplasm screening against tan spot. According to Friesen *et al*. (2002), however, toxin insensitive wheats are susceptible to isolates of a common race of the fungus suggesting that breeders aimed at developing tan spot resistant wheats should not rely on toxin reactions as they could select for toxin-insensitive wheats that are tan spot susceptible.

2.3 Genetics of host plant resistance

Host pathogen resistance to biotic stresses can be classified as qualitative or quantitative. Qualitative resistance also called monogenic resistance, race specific resistance, vertical resistance, complete resistance etc., refers to Mendelian genes of large effect that clearly interact on a gene-for- gene basis with the pathogen, wherein for every host resistance gene, there is a corresponding infecting gene from a pathogen (Flor, 1955; Van der Plank, 1963, 1968); whereas, quantitative resistance also called polygenic resistance, non-race specific resistance, horizontal resistance, partial resistance, field resistance, etc., describes resistance that shows continuous variation and is usually incomplete in expression (Van der Plank, 1963, 1968; Black, 1970). It is governed by many genes with minor effects, which are designated as 'Quantitative Trait Loci (QTL). Each QTL is supposed to have an additive effect to the resistance (Lindhout, 2002).

The main feature of qualitative resistance is the hypersensitive reaction to the attacking organism, whereby a number of biochemical reactions occur like oxidative burst, PR protein accumulation, production of phytoalexins etc. (Black, 1970; Lindhout, 2002). The pathogen is surrounded with necrotic cells around the point of invasion and local necrotic lesions are formed. The imprisoned pathogen is unable to survive and further spread is avoided. There are also other mechanisms of defence for such race specific resistance. However, race specific resistance does not ensure a lasting protection since each new resistant cultivar increases the selection pressure on the prevalent races of the pathogen to which it is resistant. Consequently, the frequency of the prevalent races goes down, other races become prevalent, to which the cultivar is not resistant. Further more, new races can occur as a result of mutation or gene recombination, which have not existed at the time of the development of the cultivar (Parlevliet, 1977; Castro *et al*., 2003). Qualitative resistance can be measured as the reaction of either seedling or adult plant to inoculation, and its use for the development of new cultivars can be straight forward.

On the other hand, quantitative resistance allows invasion of the pathogen but in a restricted way. In other words, quantitative resistance does not warrant a complete protection but it does warrant a lasting protection. It is often determined in the field and requires extensive field testing at multiple growth stages.

Both quantitative and qualitative resistances have been reported in the wheat x *P. tritici-repentis* system. Some researchers (Nagle *et al.,* 1982; Elias *et al*., 1989; Faris *et al*., 1997, Effertz *et al*., 2002) reported quantitative resistance, while others (Lee and Gough, 1984; Lamari and Bernier, 1989b, 1991; Gamba and Lamari, 1998) have found that resistance of tan spot is qualitative, controlled by single major recessive genes. More recently, Lamari *et al*. (2003) proposed that a one- to- one relationship existed in the wheat *x P. tritici repentis* system. This relationship is a mirror image of the one described by the classical gene-for-gene model (Flor, 1955). The main difference resides in the fact that compatibility is the basis of specificity in tan spot of wheat (interaction between a host specific toxin and its putative receptor) as opposed to incompatibility in the classical gene-for-gene model. Thus conceptually, the gene-for-gene model could be extended to pathosystems involving multiple host specific toxins (Heath, 1994).

2.4 Chromosomal location and molecular mapping of genes

2.4.1 Chromosomal location through monosomic analysis

Aneuploidy is an increase or decrease in chromosome number that does not involve an entire genome, and occurs in polyploidy species. The development of several sets of wheat aneuploids including monosomics (2n = 41), nullisomics (2n = 40), trisomics (2n = 43), tetrasomics (2n = 44) in the hexaploid wheat cultivar Chinese Spring (CS) by Sears (1953, 1954) has revolutionised the wheat genetic studies. The change in chromosome number may involve a single chromosome arm, as in the case of telosomics, or more than one chromosome, as in the case of the nullisomics-tetrasomics. These aneuploids have been used to locate genes on chromosomes, to map gene to centromere distances, to transfer chromosomes from one cultivar or species to another, and to identify chromosome homoeologies (Sears, 1966; Knott, 1989, Friebe *et al*., 1994).

Monosomic analysis has been most commonly used to locate genes for disease resistance or other traits of interest on chromosomes. It works best when resistance is governed by only one or two genes, and the inheritance of the resistance gene/s is known (Knott, 1989). When genes

determining phenotypes of interest occur in wheat for which an aneuploid series is not available, crosses can be made to a monosomic series in a cultivar with the contrasting phenotype. If the 21 monosomics of a cultivar are crossed with another cultivar, homozygous for the gene to be located, and monosomic hybrids are selected from their resulting progeny, then the hemizygous chromosome in each of the crosses must be derived from the donor cultivar under investigation. In the case of disease resistance, if the gene is recessive and hemizygous effective to the allele carried by the recipient monosomic cultivar, then one of the monosomic hybrids will segregete into resistant and susceptible plants and all the other monosomic hybrids will be susceptible. The chromosome carrying the recessive gene can thus be determined. This gene can also be determined from the F_2 segregation ratio, in which case the 20 non critical crosses will segregate into 1 resistant and 3 susceptible (1:3) ratio, while in the critical cross only 3% of the plants which are nullisomic (--), will be susceptible, and hence this cross deviates significantly from the 1:3 expected ratio indicating that the gene is located on this chromosome.

On the other hand, when the gene is dominant, all of the F_1 monosomic hybrids will be resistant, but differences between the hybrid monosomic families will appear in the F_2 generation. For 20 of the F_2 monosomic families, the usual 3:1 ratio (resistant: susceptible) will be obtained; whereas for the critical monosomic family, which will identify the chromosome carrying the dominant gene, almost all of the progeny will be resistant (Sears, 1953; McIntosh, 1987). Studies on transmission rates of univalents have shown that the deficient gametes are transmitted on average at the rate of 75% through females and 4% through males when a monosome is selfed or crossed with a disome resulting in 24% disomic, 73% monosomic and 3% nullisomic plants in the population (Sears, 1954). Knott (1989) has indicated that the susceptible plants in the critical cross during monosomic analysis are the nullisomics and the occasionally occurring univalents which lost the arm carrying the resistance gene.

Using these methods, many resistance genes have been identified and localized for various wheat diseases such as rusts (The *et al*., 1979; McIntosh *et al*., 1995; McIntosh *et al*., 2002; Marais *et al*., 2005), powdery mildew (The *et al*., 1979; Zeller *et al*., 1993; Lutz *et al*., 1995; Peusha *et al*., 1996; Zeller and Hsam, 1996, 1998; Hsam *et al*., 2001; Zeller *et al*., 2002; Singrün *et al*., 2003, 2004), septoria (Simon *et al*., 2001) and helminthosporium leaf blight (Ragiba *et a*l., 2004). The tan spot resistance gene *tsn1* (Stock *et al*., 1996) has been also

localized using monosomic analysis. Substitution lines, which are developed through crossing and repeated back-crossing with the monosomic series of the cultivar Chinese Spring, have been also used to determine the location of genes for disease resistance and other traits of interest (Mentewab *et al*., 2000; Rubiales *et al*., 2000; Hussien *et al.*, 2005).

2.4.2 Molecular mapping of resistance genes

Identification and differentiation of all known resistance genes and phenotypic selection for combinations of highly effective resistance genes are not readily feasible via classical genetics and breeding methods. The development of molecular markers that are closely associated with the respective resistance genes, on the other hand, would enable to pyramidize genes of interest effectively (Gupta *et al*., 1999; Huang *et al*., 2000; Castro *et al*., 2003) for the fact that these markers are faster, repeatable, not influenced by the environment and can be scored at all stages of plant growth.

Common wheat (*Triticum aestivum*, 2n = 6x = 42) has a genome size of 1.7 x 10^{10} bp (Ma and Lapitan, 1998), which is 35 and 110 times larger than rice and Arabidopsis, respectively (Bennett and Smith, 1976). However, the gene containing fraction of the wheat genome is estimated to be less than 2.7% with more than 80% repetitive DNA, which makes identification and marking of the gene containing regions possible by using different markers (Gupta *et al*., 2005). Among the different molecular markers developed todate, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified length polymorphisms (AFLPs) and micro satellites also called simple sequence repeats (SSRs) have been used for gene mapping in wheat. Although each marker system is associated with some advantages and disadvantages, the choice of marker system is determined to a large extent by the intended application, convenience and the cost involved (Gupta *et al*., 1999)

2.4.2.1 RFLP

Restriction fragment length polymorphism (RFLP) analysis was the first technology developed which enabled the detection of polymorphisms at the sequence level. The approach involves digesting DNA with restriction enzymes, separating the resultant DNA fragments by gel electrophoresis, blotting of the fragments to a filter, and hybridizing probes to the separated fragments. A probe is a short sequence of oligonucleotides which share homology and are thus able to hybridize with a corresponding sequence or sequences in the genomic

DNA. Among the various molecular markers developed todate RFLPs were developed first and were initially used for human genome mapping (Bostein *et al*., 1980). Later these markers were adopted for mapping plant genomes (Helentjaris *et al*., 1986; Weber and Helentjaris, 1989) including those of bread wheat *Triticum aestivum* (Chao *et al*., 1989; Liu and Tsunewaki, 1991; Anderson *et al*., 1992; Marino *et al*., 1996) and *Aegilops tauschii* (syn. *Triticum tauschii*, D genome) (Kam-Morgan *et al*., 1989; Lagudah *et al*., 1991; Gill *et al*., 1991, 1993). RFLP is found to be relatively more useful for the selection of chromosomal regions carrying useful genes derived from the wild relatives (Hartl *et al*., 1993; Jia *et al*., 1996). The use of heterologous RFLP probes across species boundaries also permits analysis of genome synteny (Gupta *et al*., 1999). It has been also used in wheat genome mapping (Devos *et al*., 1992; Devos and Gale, 1993), varietal identification, characterization of wheat-rye recombinants and identification of homoeologous chromosome arms (Helentjaris *et al*., 1985; Tanksley *et al*., 1989; Devos *et al*., 1993). In an effort to map the tan spot resistance gene *tsn1*, Faris *et al*. (1996) have identified closely linked RFLP markers. Using RFLP markers, Faris *et al*. (1997) have also identified major QTLs located on chromosome 1AS and minor QTLs on 4AL and 2DS for resistance to the chlorosis component of wheat tan spot. Recently, *tsc2*, a tan spot resistance gene to *Ptr* race 5 and insensitive to *Ptr* ToxB, was identified and mapped on the short arm of chromosome 2B using RFLP Markers (Friesen and Faris, 2004).

RFLP analysis, however, has got its own limitations. It is not only time consuming and labour intensive but also it has low polymorphism in wheat may be due to the polyploid nature, the high proportion of repetitive DNA, large genome size and recent origin of wheat (Chao *et al*., 1989; Gupta *et al*., 1999). The technique itself is dependant on the utilization of radioactive substances, expensive and too slow for the rapid evaluation of the large number of progenies commonly used in a commercial breeding program (Gale *et al*., 1995).

2.4.2.2 Randomly Amplified Polymorphic DNAs (RAPDs)

The molecular markers based on PCR offer the potential to reduce the time, effort and expense required for molecular mapping. In particular, RAPDs involving the use of a single DNA primer to direct amplification of discrete random sequences (Williams *et al*., 1990) have shown promise in many crops, including cereals. RAPDs have been used for a variety of purposes including the construction of genetic linkage maps (Reiter *et al*., 1992), gene tagging, identification of cultivars (Nybom, 1994), assessment of genetic variations in

populations (Chalmers *et al*., 1992; Devos and Gale, 1992) and species (Nesbit *et al*., 1995), study of phylogenetic relationships among species, subspecies and cultivars (Landry *et al*., 1994), and for many other purposes in a large number of plant species including wheat. Stock (1996) has utilized RAPD markers to map the tan spot resistance gene (*tsn1*) in common wheat and identified two loosely linked RAPD markers. In general, its application in bread wheat was found to be very limited partly owing to the low level of polymorphism detected and lack of reproducibility of results (Kojima *et al*., 1998; Gupta *et al*., 1999).

2.4.2.3 Amplified Fragment Length Polymorphism (AFLP)

AFLP is based on selective PCR amplification of restriction fragments generated by specific restriction enzymes. In this technique, specific double stranded DNA adapters are ligated to the DNA restriction fragments (Vos *et al*., 1995) so that the sequences of adaptors and the adjacent restriction sites serve as primer-binding sites. Although the AFLP kit was initially optimized for plants that have small genomes (5 x 10^{8}bp to 6 x 10^{9} bp), it was later also used successfully in bread wheat, despite its large genome size (1.7 x 10^{10} bp) (Ma and Lapitan, 1998). A single primer combination detected up to eight times more polymorphism than a polymorphic RFLP marker. AFLP has been used to map resistance genes in common wheat (Hartl *et al*., 1999; Huang *et al*., 2000, Singrün *et al*., 2004; Schmolke *et al*., 2005; Mohler *et al*., 2005). Recently, Haen *et al*. (2004) have used AFLP markers to develop high-resolution map of the tan spot resistance gene *tsn1*. However, most AFLP markers are dominant and require large amount of DNA (Mackill *et al*., 1996; Gupta *et al*., 1999).

2.4.2.4 Microsatellites or SSRs

Microsatellites are simple sequence repeats (SSRs) of only a few base pairs (2-6). These repeats are highly polymorphic, even among closely related cultivars, due to mutations causing variation in the number of repeat units. This kind of polymorphism at specific loci is easily detected using specific primers in the flanking regions of such loci and subsequent amplification via the polymerase chain reaction (Litt and Luty, 1989).

The usefulness of SSRs as genetic markers in plants has been demonstrated for several species, including soybean (Akkaya *et al*., 1995), rice (Wu and Tanksley, 1993), maize (Senior and Heun, 1993), *Arabidopsis* (Bell and Ecker, 1994), barley (Saghai Maroof *et al*., 1994) and hexaploid wheat (Plaschke *et al*., 1995; Röder *et al*., 1998; Ward, 2003). SSRs have been proven to be efficient markers especially for self-pollinating species with a low level of intraspecific polymorphisms such as barley and wheat. SSR markers have been used

effectively to map powdery mildew and fusarium resistance genes of wheat (Huang *et al.*, 2000, Singrün *et al*., 2004; Chen *et al*., 2005; Mohler *et al*., 2005; Schmolke *et al*., 2005). Recently, Singh *et al*. (2006) have mapped the tan spot resistance gene (*tsn2*) in durum wheat using SSR markers. SSR Markers are genome specificand detect only a single locus in one of the three genomes (A, B and D) of bread wheat (Röder *et al*., 1995, Stephenson *et al*., 1998). The locus-specificity, repeatability, codominance nature, evenly distribution over the genome, abundance, and high level of polymorphism associated with microsatellites make them the markers of choice for practical wheat breeding (Gupta *et al*., 1999; Hammer *et al*., 2000).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant materials

Wheat cultivars (2n = 6x = 42, AABBDD): Salamouni, Glenlea, Katepwa, Red Chief, 6B365, XX41, Chinese Spring and Kanzler were used as differential cultivars (Table 1). The cultivars Salamouni, Glenlea, Red Chief and 6B365 were kindly provided by Dr. L. Lamari, University of Manitoba, Canada. The disomic cultivar Chinese Spring (2n = 6x = 42) and its 21 monosomic lines (2n = 6x = 41) were obtained from the late Dr. E.R. Sears, University of Missouri, USA.

Table 1. List of differential wheat cultivars tested

Cultivar	Ploidy level	Origin	Source
Salamouni	6X	Lebanon	Dr. L. Lamari, University of Manitoba
Katepwa	6X	Canada	" " " " " "
Red Chief	6X	USA	" " " " " "
6B365	6X	Canada	" " " " " "
XX41	6X	Germany	Department of Plant Breeding, TUM
Kanzler	6X	Germany	Department of Plant Breeding, TUM
Chinese Spring	6X	China	Dr. E.R. Sears, University of Missouri
Glenlea	6X	Canada	Dr. L. Lamari, University of Manitoba

A total of 467 genotypes (Table 2) consisting of 98 synthetic wheat genotypes (2n = 6x = 42, AABBDD) which are amphiploids developed from the hybrid between tetraploid wheat (*Triticum turgidum* L., 2n = 4x = 28, AABB) and *Aegilops tauschii* Coss. (2n = 2x = 14, DD), 179 common wheat cultivars from different countries (2n = 6x = 42, AABBDD), 72 spelt wheat lines/cultivars and 118 cultivars of Ethiopian origin (90 common wheat, 2n = 6x = 42,

AABBDD and 28 durum wheat, *Triticum durum* ssp *aethiopicum*, 2n = 4x = 28, AABB) were used for this study. The synthetic lines starting with Syn were obtained from the International Maize and Wheat Improvement Center (CIMMYT) and others which start with XX were developed by the Department of Plant Breeding, Technical University of Munich, Germany. The 71 common wheats (*Triticum aestivum*, 2n = 2x = 42, AABBDD) and 28 durum wheat (*T. durum* ssp *aethiopicum*) genotypes of Ethiopian origin were obtained from Gatersleben Gene Bank, Germany, while the 19 commercial bread wheat (*T. aestivum*) cultivars were obtained from Adet Research Center, Bahir Dar, Ethiopia.

Table 2. Summary of wheat genotypes screened for tan spot resistance

Wheat group	Ploidy level	Number of genotypes	Source
Synthetic wheats	6X	98	TUM and CIMMYT
Common wheats	6X	269	Gatersleben, TUM and Ethiopa
Spelt wheat	6X	72	Gatersleben and TUM
Durum wheat	4X	28	Gatersleben

3.1.2 *Ptr* Isolates

Three isolates: ASC1a, ASC1b and 86-124 were kindly provided by Dr. L. Lamari, University of Manitoba. Isolates Cz1-2 and DW-16 were provided by Dr. J. Sarova, Czech University of Agriculture, Prague, Czech Republic. DTR1/2000 and DTR12/2000 were supplied from the Bavarian State Research Center for Agriculture (LFL), Germany, while the remaining two isolates, NunBr-1 and Rog5/04, were developed during the course of this study following the method described by Lamari and Bernier (1989a) from infected leaf samples collected in Nürnberg and Roggenstein areas of southern Germany, respectively (Table 3). The isolates were screened for their effectiveness using standard cultivars and cultivar Chinese Spring. Three of the most virulent isolates: ASC1a and ASC1b (race 1) and DW-16 (race 5) were used for screening of germplasms, F_2 and F_3 populations of the monosomic and allelic crosses.

Table 3. List of *Ptr* isolates tested

Isolate	Race	Source
DW-16	5	Dr. L. Lamari, University of Manitoba
ASC1a	1	" " " " " "
ASC1b	1	" " " " " "
86-124a	2	" " " " " "
Cz1-2	unknown	Dr. J. Sarova, Czech University of Agriculture
NuBr-1	unknown	Present study
Rog5/04	unknown	Present study
DTR1-2000	unknown	LFL, Germany
DTR12-2000	unknown	LFL, Germany

3.2 Methods

3.2.1 Evaluation of germplasms for tan spot resistance

3.2.1.1 Greenhouse evaluation for seedling resistance

3.2.1.1.1 Inoculum production

Inoculum production followed the method of Lamari and Bernier (1989a) and Raymond *et al*. (1985). A single medium consisting of 150 ml Vegetable (V8) juice (Campbell Soup Company, Camden, NJ), 10 g Potato Dextrose Agar (PDA), 3 g CaCo3, 10 g Bacto agar and 850 ml distilled water was prepared and poured into petri-dishes. Small plugs with 0.5 cm diameter from a seven day old culture of *P. tritici- repentis* were transferred singly into the above mentioned plates. The cultures were then incubated in the dark for about eight days, flooded with sterile distilled water and the mycelia were flattened using a sterilized glass rod. Water was decanted from the plates and the cultures were transferred to a regime of 24 h light at room temperature followed by 22 h of darkness at 15 °C. The light period enables for the formation of conidiophores while the dark period induces the formation of conidia. After 22 h of darkness, conidia were harvested by flooding the plates in sterile distilled water and scraping the spores from the plates. The concentration was adjusted approximately to 3000 spores ml^{-1}.

3.2.1.1.2 Conidial inoculation and rating

Cultivars and individual lines of each F_2 and $F_{2:3}$ families were planted at a rate of about 10 seeds per row in two rows per pot using pots of 13 cm diameter containing peat moss. The pots were arranged on a box (40 pots/box) and placed on a bench in the greenhouse at a

temperature of 20-23 ^{o}C with 16 h photoperiod. Water was supplied by capillary action via holes in the base of the pots. After two weeks, the second leaf from each plant was cut and the first leaf of each line was inoculated. Nine *Ptr* isolates (Table 3) were used to test differential cultivars, while only the most virulent isolates: ASC1a, ASC1b, DW-16 and Rog-5/04 were used for germplasm screening and monosomic analysis. After inoculation, plants were placed into a 2 m x 1.5 m x 1m portable plastic tent inside the greenhouse. The tent was further covered by a black plastic sheet to ensure complete darkness. A relative humidity of 100 % was maintained using a humidifier. After 24 h of leaf-wetness period in the dark as indicated above, the plants were transferred into a growth chamber at a temperature of 22 ^{o}C and photoperiod of 12 h /day for about seven days. The plants were evaluated for their resistance to tan spot seven days after inoculation following the 1-5 rating scale developed by Lamari and Bernier (1989a) as indicated in Table 4 and Fig 4.

Table 4. Tan spot rating scale

Scale	Class	Symptom
0*	immune	-
1	highly resistant	small dark brown to black flecks with very little chlorosis or tan necrosis
2	moderately resistant	small dark brown to black spots with very little chlorosis or tan necrosis
3	moderately resistant to moderately susceptible	small dark brown to black spots completely surrounded by a distinct chlorotic or tan necrotic ring; lesions generally not coalescing
4	moderately susceptible	small dark brown or black spots completely surrounded with chlorotic or tan necrotic zones; some of the lesions coalescing
5	susceptible	the dark brown or black centres may or may not be distinguishable; most lesions consist of coalescing chlorotic or tan necrotic zones

* used only in the screening of synthetic lines

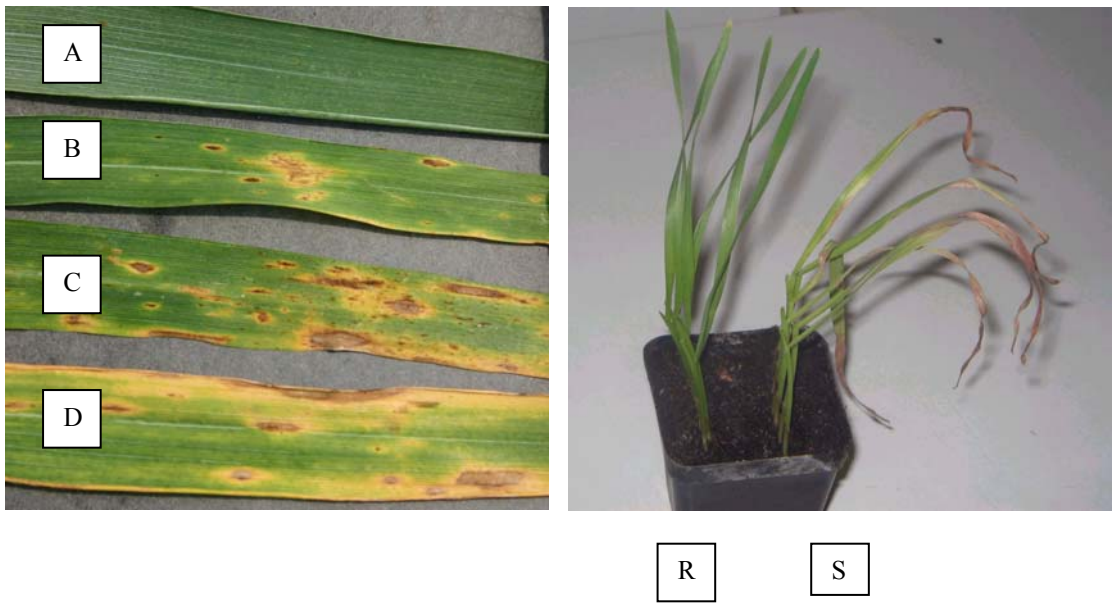


Fig 4. Rating scales: A = 0-1; B = 2; C = 3-4 D=5 and R (Resistant) and S (Susceptible) checks

3.2.1.2 Field evaluation for adult plant resistance

A total of 12 winter wheat cultivars was evaluated for three years (2004-2006) at Roggenstein, southern Germany. The varieties were planted in October each year at a rate of 240 seeds/m^2 in a plot size of 10 m^2 (5m length x 2m width) using a randomised complete block design with three replications. No artificial inoculation of the pathogen was applied since the area is hot-spot for *Pyrenophora tritici repentis* maintained through wheat after wheat production system. Standard wheat agronomic practices were followed during the trial period. Disease evaluation was carried out at heading stage, when the disease pressure is believed to be maximum, following the 1-5 qualitative scale developed by Lamari *et al*. (1989a). Analysis of variance and correlation between seedling and dult plant resistance were deterimned using SAS soft ware (SAS Insititute, 2004).

3.2.2 Genetics of resistance

To study the inheritance of tan spot resistance, the resistant synthetic lines (XX41, XX45 and XX110), and resistant common wheat cultivars (Salamouni, Arina, Red Chief) were crossed

with the susceptible cultivar Chinese Spring. Further more, the resistant cultivars from Ethiopia: HAR604, HAR2562 and Dashen were crossed with the susceptible cultivar Glenlea. Allelic crosses among the resistant synthetic lines (XX41/XX45, XX41/XX110, XX45/XX110), and the Ethiopian cultivars (HAR604/HAR2562, HAR604/Dashen, HAR2562/Dashen) were made for allelism tests.

The F_2 and $F_{2:3}$ families of the CS/synthetic crosses for the inheritance study, and the F_1 and F_2 allelic crosses among the resistant synthetic lines were inoculated using ASC1b isolate. The F_1 and F_2 plants of the other crosses for inheritance and allelism studies were inoculated using *Ptr* isolates ASC1a (race 1) and DW-16 (race 5) in two sets of inoculations. Evaluation was made using 1-5 scale as described above in the disease screening, and reaction classes of 1 to 2 were grouped as resistant while 3 to 5 were grouped as susceptible. Chisquare (χ^2) analysis was carried out for the F_2 segregation ratios of 1:3 for R/S (resistant/susceptible) and 7:9 for R/R (resistant/resistant) crosses following the method of Snedecor and Cochran (1989) which stated as: $\chi^2 = \Sigma^{k}_{i=1} = (O_i - E_i)^2/E_i$, where O_i and E_i are the observed and expected frequencies of resistant and susceptible plants, respectively and k is the number of classes whose contributions are summed to calculate χ^2.

3.2.3 Monosomic analysis

A total of eight resistant lines: Three synthetic lines (XX41, XX45 and XX110), two winter cultivars (Arina and Red Chief), and three spring cultivars (Salamouni, HAR604, HAR2562) were crossed with the monosomic series of the wheat cultivar Chinese Spring (CS), which was used as the female parent in all the crosses. The synthetic lines were crossed only with seven monosomic lines of the D genome, while the other resistant cultivars were crossed with all 21 monosomic lines of Chinese Spring. The 21 monosomic lines of Chinese Spring and the F_1 crosses were screened for monosomy (2n = 41) using chromosome counts from squashes of root- tip cells pretreated with mono-bromnaphthalin and stained by the Feulgen method as indicated in Lutz *et al*., 1995.

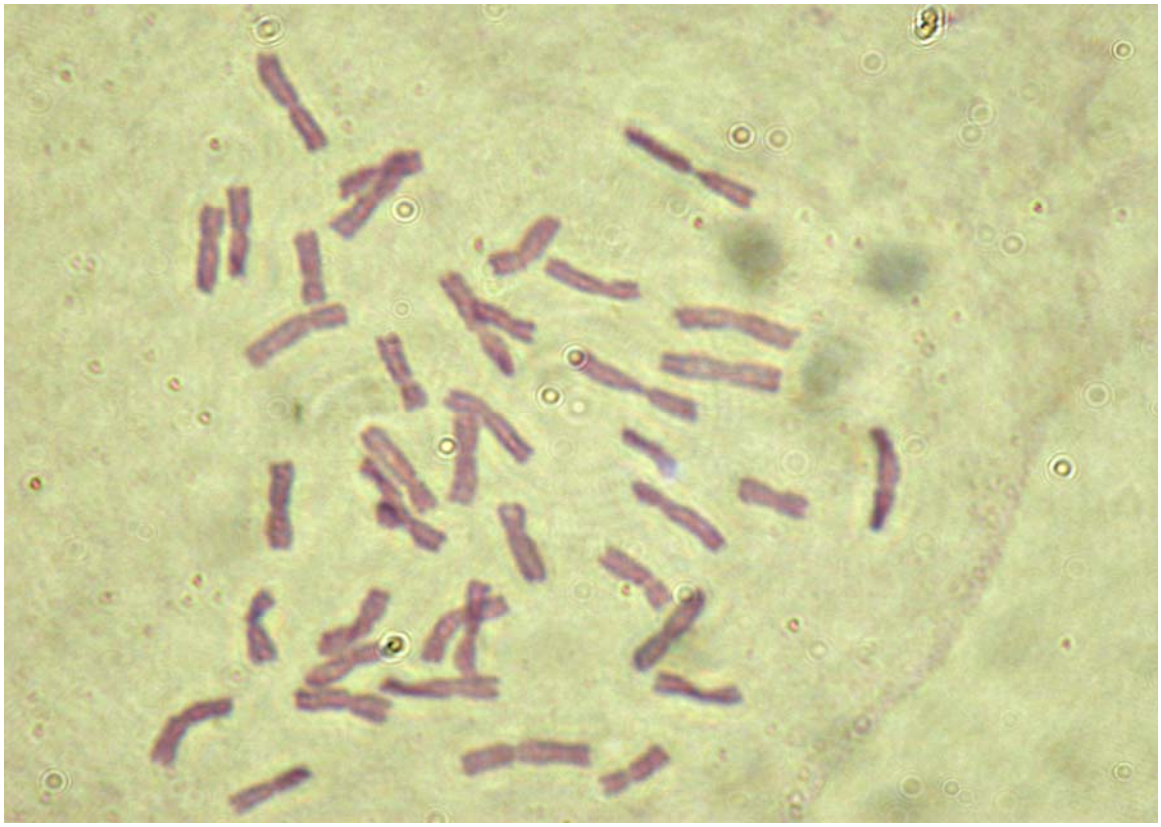


Fig 5 Mitotic chromosomes of a monosomic wheat line (2n = 41)

A minimum of eight seeds were germinated in a petridish with a labelled and moist filter paper at room temperature for four days. Two root tips (1 cm long) from a seed were cut and inserted into a tube to which 2 ml mono-bromnaphthalin solution, prepared by mixing 12-14 drops of mono-bromnaphthalin per 100 ml of water, was added and incubated at room temperature for 5 hours. This process shortens the chromosomes, enables them to move to the metaphase plate, and hinders the formation of spindle fibres. After 5 h of incubation, the bromnaphthalin solution was decanted and roots were transferred into new tubes. Half (0.5) ml of 100% acetic acid was added and incubated at 4 °C for at least 15 minutes to fix the root tips. Then after decanting the acetic acid, 1 ml of 1 N HCl was added, and incubated at 60 °C for 10 minutes. This softens the tissue and leads to separation of purine from sugar. Finally, the HCl solution was decanted and 0.5 ml of Schiff reagent was added and kept at room temperature until the root tips are lilly coloured. The coloured root tip was cut and used for squashing on a slide by adding a drop of stain (Orcein) and covering with a slide cover. Chromosome count was made using 100X lens of the Zeiss Axioplan Microscope.

Only confirmed 2n = 41 chromosome seedlings of the monosomic series of Chinese Spring and the F_1 hybrids were planted (three seedlings per pot) in 50 cm diameter pot and raised in the greenhouse following standard wheat agronomic practices. Crosses of disomic cultivar Chinese Spring with XX41, XX45, XX110, Salamouni, Red Chief, HAR604, and HAR2562 were made as controls to study the segregation and inheritance of tan spot resistance. The monosomic families were screened in three sets of inoculations using *Ptr* isolate ASC1a (race 1) and/or DW-16 (race 5) *Ptr* isolates. For each set of inoculation, seventeen days old seedlings were raised by planting F_2 seeds in three pots at a rate of ten seeds per pot for each combination depending on the availability of seeds since the amount of seed harvested from each crosses was different. For some of the crosses, F_1 plants were also inoculated for comparison. Inoculum production, inoculation techniques and rating scales used for the screening were also applied here. Evaluation was made on single plant basis, and score values of 1 and 2 were grouped as resistant while 3, 4 and 5 were grouped as susceptible. The number of resistant and susceptible plants in each set of inoculation was summed up to get the total frequency of susceptible and resistant F_2 plants per each combination, which was then subjected to χ^2 analysis as described above.

3.2.4 Microsatellite analysis

3.2.4.1 DNA Extraction

Genomic DNA was extracted from the second leaf of two weeks old seedlings using the cetyltrimethyl amonium bromide (CTAB) method as described by Saghai-Maroof *et al*. (1984) for the CS/XX41, CS/XX45 and CS/XX110 $F_{2:3}$ populations. The mini-CTAB method (Doyle and Doyle, 1987) was used for the CS/Red Chief F_2 population and the 34 cultivars (which were used for marker validation) as indicated below. Leaf samples were put in labelled 2 ml eppendorf tubes and dried using a freeze-drying machine. After drying, two metall balls were added in each eppendorf tube and the leaf sample was fine powdered using a rotary mill for 2 minutes. In each of the tubes containing the fine powder, 1 ml of the extraction buffer [1.5% CTAB (w/v); 100 mM Tris-HCl pH 8.0; 20 mM EDTA pH 8.0; 1.05 M NaCl] and 10 µl of 1% ß-mercaptoethanol were added, and then incubated in a shaking water bath (GFL 1083) at 60 °C for 60-90 minutes. After incubation, the samples were cooled on ice for about 5 minutes. One ml of Chloroform-Isoamyl alcohol mixture (24:1) was added in each tubes and mixed up-side-down for 30 minutes using Heidolph Reax2 rotator. This was then centrifuged at 13000 rpm for 20 minutes at 4 °C. The liquid phase was taken and added in

new eppendorf tubes each containing 5 µl RNaseA and incubated at room temperature for 30 minutes. After incubation, 1 ml of Isopropanol (stored at –20 °C) was added and centrifuged at 13000 rpm for 20 minutes at 4 °C. The pellet was decanted and 1 ml of 75% ethanol was added for washing purpose. Ethanol was then decanted and DNA was dried using vacuum concentrator. Finally, DNA was resuspended by adding 50-200 µl TE buffer [10 mM Tris pH 8.0, 1 mM EDTA (ethylene diamine tetra cetic acid) pH 8.0], and kept at 4 °C. The concentration of DNA was then determined along with the molecular weight standard λHindIII by running on 0.8% agarose gel stained with 0.5 µg/ml ethidium bromide using 1X TAE buffer (40 mM Tris acetate, 1 mM EDTA) at 50 volts for about 90 minutes. The gel was visualised using UV light and photgraphed. Finally, DNA was diluted to 50 ng/µl.

3.2.4.2 PCR

A total of 12 SSR markers from wheat chromosome 3D, and 8 SSR markers from chromosome 3A was screened for polymorphism (Table 5) following the procedure of Huang *et al*. (2000).

Table 5. Description of SSR markers tested

Locus	Anealing Temperature (°C)	SSR motif	Chromosome arm
Xgwm2	50	$(CA)_{18}$	3DS
Xgwm52	55	$(GT)_4AT(AT)_{20}$	3DL
Xgwm161	60	$(CT)_{15}$	3DS
Xgwm314	55	$(CT)_{25}$	3DL
Xgwm3	55	$(CA)_{18}$	3DL
Xgwm497	55	$(GT)_{29imp}$	3DL
Xgwm645	55	$(GT)_{28}$	3DL
Xbarc1040	55	$(ATCT)_8$	3DS
Xwmc366	55	$(CA)_{12}$	3DL
Xbarc42	55	$(TTA)_{12}$	3DL
Xbarc52	55	$(ATCT)_5$	3DL
Xgwm114	55	$(GA)_{53}$	3DS
Xgwm2	50	$(CA)_{18}$	3AC
Xgwm5	50	$(TC)_{23}(T)_4(GT)_{12}(GA)_{10}$	3AS
Xgwm30	60	$(AT)_{19}(GT)_{15}$	3AC
Xgwm155	60	$(CT)_{19}$	3AL
Xgwm218	60	-	3AS
Xgwm369	60	$(CT)_{11}(T)_2(CT)_{21}$	3AS
Xgwm480	60	$(CT)_{16}(CA)_{13}$	3AL
WMC379	60	$(CT)_{15}(GT)_5$	3AC

PCR reactions were performed in a PE 9600 thermal cycler (Perkin Elmer) in a total volume of 20 µl containing 2 µl of 10X PCR buffer (50 mM of KCl, 10 mM of Tris-HCl, 1.5 mM of $MgCl_2$, pH 8.3), 2.5 mM of each dNTPs, 2.5 mM of each labelled and unlabelled primer, 1 U Taq DNA polymerase (Qiagen) and 50 ng template DNA. The PCR was programmed at an

initial denaturation step of 3 min at 95 °C followed by 35 cycles of 1 min denaturation at 95 °C, annealing at 50, 55 or 60 °C (depending on the primer) for 1 min, initial extension at 72 °C for 1 min and final extension at 72 °C for 15 min. The PCR product was checked along with the molecular weight standard λ*Hind*III and a non-template control by running on 1.5% agrose gel containing 5 µg/µl of ethidium bromide for about 30 min at 5 v/cm. Depending on the intensity of the bands, PCR products were diluted with double distilled water at 1:3 or 1: 4 ratio. The samples were mixed with 0.15 µl GenScan-500 TAMRA internal size standard (PE Biosystems) and 0.85 µl formamide dye (98% formamide, 0.01% dextran blue), denatured at 95 °C for 2 min and chilled on ice.

Samples were loaded on 5% denaturing polyacrylamide gel (Long Ranger TM, FMC Bioproducts) in 1X TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3). Electrophoresis was carried out in an ABI Prism^{TM} 377 DNA sequencer (Applied Biosystems) at 1200 V for 1.5 h. ABI collection software version 1.1 was used for raw data collection. Microsatellite fragments were analysed using GENSCAN^{TM} analysis software version 2.1.

3.2. 4.3 Gene mapping

Linkage between SSR markers and the *tsn* loci was established with MAPMAKER/EXP, version 3.0b using a LOD value of 3.0 and a maximum distance of 50 cM. (Lander *et al*., 1987). The Kosambi function was applied to convert recombination fractions into map distances (Kosambi, 1944), and linkage maps drawn using the Mapchart software (Voorrips, 2002).

4. RESULTS

4.1 Screening of wheat germplasms for tan spot resistance

Before screening the available wheat germplasm for tan spot resistance, development of differential cultivars and *Ptr* isolates has to be carried out. Accordingly, iolates were developed and tested against differential cultivars. As indicated in Table 6, the cultivars responded differentially towards *Ptr* isolates possessing different virulence. Salamouni showed moderately susceptible response to isolates ASC1b, DTR1-2000 and DTR12-2000. Glenlea, Katepwa and Kanzler were susceptible to most of the isolates. The cultivar Red Chief and XX41 showed resistant response across all the isolates. The cultivars Chinese Spring and Glenlea were susceptible to ASC1a, ASC1b (race 1) and DW-16 (race 5) *Ptr* isolates showing both necrosis and chlorosis symptoms.

Table 6. Response of eight wheat cultivars to differential *Ptr* isolates

Wheat cultivar	Isolates								
	DW-16	ASC 1a	ASC 1b	86-124a	Cz1-2	NuBr-1	Rog 5/04	DTR 1-2000	DTR 12-2000
Salamouni	MR[2]	R	MR	R	R	R	R	MS	MS
Glenlea	S	S	S	MS	MS	R	MS	S	S
Katepwa	S	S	S	MS	MS	MS	MS	S	S
Red Chief	R	R	R	R	R	R	R	R	R
6B365	S	R	S	R	MR	R	R	R	R
Kanzler	S	S	S	R	MS	MS	S	S	S
XX41	R	R	R	R	R	R	R	R	R
Chinese Spring	S	S	S	R	R	R	MS	MS	MS

S = susceptible (4-5 in 1 – 5 scale), MS = moderately susceptible (3), R= resistant (1), MR = moderately resistant (2).

4.1.1 Synthetic wheats

A total of 98 synthetic wheat lines were screened using *Ptr* isolate ASC1b (race 1) for their seedling resistance against tan spot caused by *P. tritici-repentis*. The response of the genotypes to *Ptr* ASC1b ranged from 0 (immune) to 5 (highly susceptible) with a mean value of 2.2 in 0-5 scale (Table 7). Two genotypes (syn 38 and syn 44) were found to be immune and twenty genotypes were highly resistant. The majority of the genotypes were moderately resistant. In the present study, XX41 and XX45 were confirmed to be highly resistant while XX110 was moderately resistant. Parental lines of the three resistant synthetic lines: XX41 (Langdon durum and *Aegilops tauchii*, CI 00017), XX45 (Langdon durum and *Aegilops tauschii*, RL 5565) and XX110 (*T. dicoccum,* A38 and *Aegilops tauschii,* CI 33) were evaluated using *Ptr* islolate ASC1b so as to identify the source of resistance in the respective synthetic lines. The tetraploid parents Langdon durum and *T. dicoccum* (A38) were susceptible while the diploid *Ae. tauschii* parents (CI 00017 and RL 5565) were highly resistant (1) and CI 33 was moderately resistant (2), indicating that the source of resistance in the synthetic lines were the diploid *Ae. tauschii* (2n = 2x = 14, DD) lines.

Table 7. Evaluation of synthetic wheat accessions for tan spot resistance using isolate ASC1b

Acc.	Pedigree	M + S.E
Syn 38	Fgo/USA2111// *Ae. tauschii* (658)	0.0 + 0.0
Syn 44	68-11/RGB-U//Ward/3/FGO/4/RABI/5/ *Ae. tauschii* (878)	0.0 + 0.0
Syn 45	68-11/RGB-U//Ward/3/FGO/4/RABI/5/ *Ae. tauschii* (878)	1.0 +0.0
Syn 47	68-11/RGB-U//Ward/3/FGO/4/RABI/5/ *Ae. tauschii* (882)	1.0 + 0.0
Syn 48	Sora/ *Ae. tauschii* (884)	1.0 + 0.0
Syn 60	Scoop 1/ *Ae. tauschii* (358)	1.0 + 0.0
Syn 73	Gan/ *Ae. tauschii* (897)	1.0 + 0.0
Syn 84	Green/ *Ae. tauschii* (458)	1.0 + 0.0
Syn 87	SCA/ *Ae. tauschii* (409)	1.0 + 0.0
XX 41	Langdon durum/ *Ae. tauschii* (CI 00017)	1.0 + 0.0
XX 45	Langdon durum/*Ae. tauschii* (RL 5565)	1.0 + 0.0
XX 111	*T. dicoccum (*119)/*Ae. tauschii* (33)	1.0 + 0.0
XX 195	*T. turgidum* (88)*/Ae. tauschii* (BGRC 1458)	1.0 + 0.0
XX 227	*T. turgidum* (89)/ *Ae. tauschii (*26)	1.0 + 0.0
Syn 1	Altar 84/ Ae. tauschii (188)	1.0 + 0.0
Syn 11	D67-2/P66.270// *Ae. tauschii* (213)	1.0 + 0.0
Syn 30	68112/Ward// *Ae. tauschii* (369)	1.0 + 0.0
XX 202	*T. turgidum* (01)/ *Ae. tauschii (*CI 18)	1.3 + 0.3
XX 183	*T. durum* (22912)/*Ae. tauschii* (CI 0221)	1.3 + 0.3
Syn 32	Doy1/ *Ae. tauschii* (447)	1.3 + 0.3
XX 235	*T. turgidum* (90)/ *Ae. tauschii* (26)	1.3 + 0.3
Syn 42	Yar/ *Ae. tauschii* (783)	1.3 + 0.3
XX 110	*T. dicoccum* (A 38)/ *Ae. tauschii* (CI 33)	1.7 + 0.3
Syn 76	Falcin/ *Ae. tauschii* (312)	1.7 + 0.3
XX 205	*T. turgidum* (01)/ *Ae. tauschii*(CI 33)	1.7 + 0.3
XX 206	*T. turgidum* (235)/ *Ae. tauschii(* RL 5688)	1.7 + 0.3
Syn 55	Gan/*Ae.tauschii* (180)	1.7 + 0.3
Syn 70	Snipe/Yav79//Dack/Teal/3/ *Ae. tauschii* (700)	1.7 + 0.3
Syn 49	68-11/RGB-U//Ward/3/FGO/4/RABI/5/ *Ae. tauschii* (890)	1.7 + 0.3
Syn 33	Yav3/sco//JO69/cra/3/Yav79/4*Ae. tauschii*(498)	1.7 + 0.3
Syn 92	Ceta/ *Ae. tauschii* (1024)	1.7 + 0.3
Syn 69	D67-2/P66-270// *Ae. tauschii* (659)	2.0 + 0.0
Syn 91	Croc 1/ *Ae. tauschii* (517)	2.0 + 0.0
Syn 75	Arlin/ *Ae. tauschii* (283)	2.0 + 0.0
Syn 88	CPI/Gediz/3/GOO//JO69/CRA/4/ *Ae. tauschii* (409)	2.0 + 0.0
Syn 9	Altar 84/ *Ae. tauschii* (211)	2.0 + 0.0
Syn 63	Yar/ *Ae. tauschii* (518)	2.0 + 0.0
Syn 26	Aco89/ *Ae. tauschii* (309)	2.0 + 0.0
Syn 39	Croc 1/ *Ae. tauschii* (725)	2.0 + 0.0
Syn 72	Snipe/Yav79//Dack/Teal/3/ *Ae. tauschii* (877)	2.0 + 0.0
Syn 14	YUK/ *Ae. tauschii* (217)	2.0 + 0.0
Syn 10	D67-2/P66.270// *Ae. tauschii* (211)	2.0 + 0.0
Syn 34	Doy1/ *Ae. tauschii* (511)	2.0 + 0.0
XX 233	*T. turgidum* (01)/ *Ae. tauschii (*CI 33)	2.0 + 0.0
XX 175	*T. durum* (22909)/*Ae. tauchii* 202229-c	2.0 + 0.0
XX 208	*T. turgidum* (01)/ *Ae. tauschii(*CI 38)	2.0 + 0.0
XX 216	*T. turgidum* (61)/ *Ae. tauschii (*CI 18)	2.0 + 0.0
XX 218	*T. turgidum* (61)/ *Ae. tauschi (*AE 724/82))	2.0 + 0.0
XX 222	*T. turgidum* (235)/ *Ae. tauschii* (RL 5686)	2.0 + 0.0
Syn 85	Ceta/ *Ae. tauschii* (174)	2.3 + 0.3
Syn 86	Doy1/ *Ae. tauschii* (372)	2.3 + 0.3
XX 50	Langdon durum/*Ae. tauschii* (268210)	2.3 + 0.3
XX 220	*T. turgidum* (61)/*Ae. tauschii (*CI 33)	2.3 + 0.3
XX 234	*T. turgidum* (90)/ *Ae. tauschii (*PI 210987)	2.3 + 0.3

Table 7 continued...

Syn 7	Altar 84/ *Ae. tauschii* (205)	2.3 + 0.3
Syn 35	68.111/RGB-U//Ward/3/ *Ae. tauschii* (511)	2.3 + 0.3
Syn 46	Croc 1/ *Ae. tauschii* (879)	2.3 + 0.3
XX 201	*T. turgidum* (89)/ *Ae. tauschii*(CI 33)	2.3 + 0.3
XX 220	*T. turgidum* (61)/*Ae. tauschii (*CI 33)	2.3 + 0.3
XX 234	*T. turgidum* (90)/ *Ae. tauschii (*PI 210987)	2.3 + 0.3
Syn 43	Yuk/ Ae. tauschii (864)	2.3 + 0.3
XX 198	*T. carthlicum*/ *Ae. tauschii* (RL5320)	2.3 + 0.3
Syn 85	Ceta/ *Ae. tauschii* (174)	2.3 + 0.3
Syn 86	Doy1/ *Ae. tauschii* (372)	2.3 + 0.3
Syn 95	Doy1/ *Ae. tauschii* (1030)	2.3 + 0.3
XX229	*T. turgidum* (89)/*Ae. Tauschii* (RL5670)	2.7 + 0.3
XX 200	*T. turgidum* (61)/ *Ae. tauschii* (AE 432/80)	2.7 + 0.3
XX 194	*T. turgidum* (89)/*Ae. tauschii* (AE457/78)	2.7 + 0.3
Syn 2	Doy1/ *Ae. tauschii* (188)	2.7 + 0.3
Syn 12	ROK/KML// *Ae. tauschii* (214)	2.7 + 0.3
Syn 23	D67-2/P66-270// *Ae. tauschii* (223)	2.7 + 0.3
Syn 25	Altar 84/ *Ae. tauschii* (224)	2.7 + 0.3
Syn 62	Sca/ *Ae. tauschii* (518)	2.7 +0.3
Syn 74	YAV/TEZ// *Ae. tauschii* (895)	2.7 + 0.3
Syn 90	Altar 84/ *Ae. tauschii* (502)	2.7 + 0.3
XX 52	Longdon durum/*Ae. tauschii* (RL 5392)	3.0 + 0.0
XX 196	*T.durum* (488)/ *T. turgidum* (88)	3.0 + 0.0
XX 231	*T. turgidum* (80)/ *Ae. tauschii* (AE 431/83)	3.0 + 0.0
Syn 57	LCK59-61/ *Ae. tauschii* (313)	3.0 + 0.0
Syn 59	SRN/ *Ae. tauschii* (358)	3.0 + 0.0
Syn 61	Gan/ *Ae. tauschii* (408)	3.0 + 0.0
Syn 77	Rascon /*Ae. tauschii* (312)	3.0 + 0.0
Syn 94	Ceta/ *Ae. tauschii* (1027)	3.0 + 0.0
XX 186	*T. turgidum* (90)/*Ae. tauschii* (BGRC 1458)	3.3 + 0.3
XX 193	*T. turgidum* (88)/*Ae. tauschii*(BGRC 1457)	3.3 + 0.3
XX 197	*T.durum* (488)/ *T. turgidum* (89)	3.3 + 0.3
Syn 29	68-11/RGB-U//Ward/3/ *Ae. tauschii* (326)	3.3 + 0.3
Syn 31	68112/Ward// *Ae. tauschii* (369)	3.3 + 0.3
Syn 56	D67-2/P66-270/ *Ae. tauschii* (257)	3.7 + 0.3
XX 203	*T. turgidum* (01)/ *Ae. tauschii (*AE 432/80)	4.0 + 0.0
Syn 5	Altar 84/ *Ae. tauschii* (198)	4.0 + 0.0
Syn 64	Botno/ *Ae. tauschii* (617)	4.0 + 0.0
Syn 65	Botno/ *Ae. tauschii* (620)	4.0 + 0.0
XX 224	*T. turgidum* (90)/ *Ae. tauschii* (AE 141/78)	4.3 + 0.3
Syn 4	Altar 84/ *Ae. tauschii* (193)	4.7 + 0.3
Syn 54	Ceta/ *Ae. tauschii* (895)	4.7 + 0.3
XX 199	*T. turgidum*(61)/ *Ae. tauschii* (AE 724/82)	5.0 + 0.0
Red Chief	early Red Clawson/Red Arcadian	1 .0+ 0.0
Glenlea	Pembina *2/Bage//CB-100	5.0 + 0.0
Mean		2.2 + 0.1

4.1.2 Common wheat cultivars

Including the standard checks, 179 cultivars were screened using two of the most virulent *Ptr* isolates, ASC1a (race 1) and DW-16 (race 5). The cultivars showed disease reactions ranging from 1 to 5 with mean values of 3.02 and 3.42 for race 1 (ASC1a) and race 5 (DW-16) *Ptr* isolates, respectively (Table 8). Out of 179 genotypes, 18 (9.6 %) and 9 (5.1 %) were highly resistant (1 in 1-5 scale) to ASC1a and DW-16 isolates, respectively. Forty nine (27.5 %) and 35 (19.7 %) of the genotypes showed moderately resistant (2) response to ASC1a and DW-16 isolates, respectively, while the other genotypes were susceptible with score levels ranging from 3-5 in 1-5 scale. Cultivars Red Chief (USA), Salamouni (Lebanon), Armada (United Kingdom), Empire (United Kingdom), Ibis, Toronto (Canada), Albrecht (Germany), Solitär (Germany), Arina (Switzerland), Kronjuvel, Ohio, Yindus, Casten VIII (Germany) and Heines VII (Germany) were resistant (scores of 1-2) to isolate ASC1a.

4.1.3 Spelt wheat

As shown in Table 9, a total of 72 spelt wheat lines/cultivars was evaluated using *Ptr* race 1 (ASC1a) and race 5 (DW-16) isolates. Out of these genotypes, 16 (23.2%) and 5 (6.9%) were highly resistant (1 in 1-5 scale) to ASC1a and DW-16 isolates, respectively. Twenty eight (38.9%) and 15 (20.8%) of the genotypes showed moderately resistant (2) response to ASC1a and DW-16 isolates, respectively, while the other genotypes were susceptible with score levels ranging from 3-5 in 1-5 scale. Cultivars Ceralion, Hercule, Schwabenkorn and lines GL-22 and EPIH-1 showed highly resistant response to both race 1 and race 5 isolates. Among the commercial cultivars: Roter Schwabenpelz, and Waggershauser Hohenheimer were highly suceptible to both race 1 (Asc1a) and race 5 (Dw-16) isolates.

Table 8. Evaluation of 179 winter wheat genotypes for tan spot resistance using two *Ptr* isolates

Cultivar	ASC1a	Dw-16	Cultivar	ASC1a	DW-16
Cassten VIII	1	1	Idol	2	3
Albrecht	1	2	Joutzhis grossoru	2	2
Arina	1	1	Kamza-21	2	2
Armada	1	3	Kavkas	2	4
Druchamp	1	1	Marina	2	2
Empire	1	1	Mecc x David-1-11	2	3
Ibis	1	1	Pantus	2	5
Kronjuwel	1	2	Piko	2	3
Ohio	1	2	Qualibo	2	3
Red Chief	1	1	Saunders	2	3
Reliance	1	4	Taka	2	2
Salamouni	1	2	Thorogi	2	3
Solitär	1	2	Topper	2	4
TA 1161	1	3	Travix	2	2
Toronto	1	1	Vilmorin 23	2	2
Vergas	1	4	Vorosibuskaja	2	4
Yindos	1	2	Zorba	2	3
Zenith	1	2	73PL113	3	2
6B365	2	4	Abo	3	3
Achiat	2	3	Agronom	3	3
Akteur	2	4	Bert	3	4
Carifen-12	2	2	Bussard	3	5
Astron	2	2	Cardos	3	4
Bersee normal	2	3	Caribo	3	5
Browick	2	3	Apolo	3	2
Camino	2	4	Cassten V	3	3
Cappele-Desprez	2	2	Castens Dickkopf	3	3
Capitole	2	1	Champtal	3	4
Centauro	2	2	Champtol	3	4
Compair	2	2	Cheyene	3	4
Contra	2	3	Clan	3	3
Creative	2	4	Complet	3	4
Cubus	2	3	Crievener 104	3	4
Dragon	2	2	Dream	3	3
Erik	2	3	Els	3	5
Euris	2	4	Etoile de Choisy	3	4
Except	2	4	G18/90	3	3
Fones Fife	2	2	Gigant	3	2
Friedland	2	4	Grunbachar	3	-
Frodin	2	2	Habchit	3	5
Fronthatch	2	3	Heine	3	3
Gaston	2	2	Heines Germania	3	3
Gb16.92	2	4	Heines Japlet	3	4
Geverson Dickkopf	2	2	Herman	3	2
Granus	2	5	Heta	3	4
Guderig	2	2	Hybride 40	3	4
Halle stamm	2	3	Hybride 46	3	4
Hatifde weltinen	2	4	Kador	3	4
Heines VII	2	1	Kamza-20	3	5

Table 8 continued...

Cultivar	ASC1a	DW-16	Cultivar	ASC1a	DW-16
Hussar	2	3	Redman	4	3
Minhardi	3	3	Rüso	4	4
Ralle	3	2	Septre	4	5
Romanus	3	5	TA 1921	4	4
Ruive	3	3	Thesee	4	3
Squarehead Master	3	4	Ushio komugi	4	4
Sunnan	3	5	Virest	4	5
SW Maxi	3	2	Xiayans	4	2
TA 1684	3	4	Annapurna 1	5	5
Tenor	3	2	Bandit	5	3
Terminillo	3	3	Blaukorn	5	5
Termir	3	3	Estrella	5	3
Tom pauce Barbu	3	3	Farmer	5	4
Tommi	3	3	Geverson senbloit	5	5
Vostok	3	3	Heines Koibri	5	5
Kenya Civet	3	5	Kanzler	5	5
Vuka	3	4	Katepwa	5	5
Yamhill	3	3	Lynx	5	5
Altos	4	4	Mara	5	5
Amazon	4	5	Mewa	5	3
Asketis	4	3	Normandie	5	5
Aspect	4	4	Regent	5	4
Atys	4	5	Rendezvous	5	5
Benno	4	3	Strampelli	5	4
Centrum	4	5	Strube 56	5	2
Chinese Spring	4	4	TA 1926	5	5
CWW 926	4	4	Monos	5	5
Damier	4	3	Tamaro	5	4
Diamant	4	4	Vicam 71	5	5
Enorm	4	5	Volkom	5	4
Ferto	4	4	**Mean**	**3.02**	**3.42**
Flair	4	5	**SD**	**1.3**	**1.4**
Forlani	4	5			
Freisinger Landweizen	4	4			
Gambros	4	2			
Glockner	4	4			
Greif	4	5			
Heines Noc	4	3			
Jubilar	4	2			
Kanred	4	5			
Karpos	4	5			
Kaspar	4	4			
Kolban	4	4			
Konini	4	4			
Kontra	4	4			
Madson	4	4			
Marquillo	4	3			
Moisson	4	3			
Neepawa	4	4			
Prins	4	4			
Redford	4	3			

Table 9. Evaluation of 72 spelt wheat genotypes for tan spot resistance using two *Ptr* isolates

Cultivar	Response to		Cultivar	Response to	
	ASC1a	DW-16		ASC1a	DW-16
GL 40	-	5	GL 3	3	4
GL 46	-	3	GL 31	3	4
Steiners Roter Tiroler	-	2	GL10	3	4
Bauländer Spelz	1	2	GL15	3	5
Ceralion	1	1	GL 44	3	4
EPIH-21	1	1	GL27	3	5
GL 16	1	2	GL 47	3	5
GL 19	1	2	GL 48	3	5
GL 22	1	1	GL 9	3	5
GL 34	1	3	Erbe-Weizen	3	4
GL 39	1	3	Frankenkorn	3	2
Hercule	1	1	Neugger Weißkorn	3	2
Rouquin	1	2	Rep-29	3	3
Schwabenkorn	1	1	T. spelta grey	3	4
Sirino	1	2	Albin	4	3
Spelz v. Gotland	1	3	GL 11	4	5
Tamro	1	3	GL 17	4	4
Winter spelz	1	3	GL 30	4	5
Zuger Dinkel	1	3	GL 4	4	5
AEPOH 33	2	2	GL 5	4	4
Altgold Rotkorn	2	3	Waggershauser Hohenheimer	4	4
Blaspelz	2	3	GL 42	5	4
Extra Square Head	2	4	GL 43	5	3
GL 1	2	3	GL 45	5	3
GL 13	2	4	Roter Schwabenspelz	5	5
GL 14	2	3	**Mean**	**2.37**	**3.23**
GL 18	2	3	**S.E.**	**0.13**	**0.13**
GL 2	2	3			
GL 20	2	2			
GL 21	2	2			
GL 25	2	3			
GL 26	2	4			
GL 35	2	5			
GL 36	2	4			
GL 41	2	4			
GL 8	2	4			
Mili 57	2	2			
Oberkulmer Rotkorn	2	3			
Ostro	2	2			
ReinersweiserSchlegel	2	3			
Roter Schlegel Dinkel	2	3			
Spelt Schwaben	2	4			
Squarehead II	2	5			
Titan	2	4			
V.RechbergersFrüher Dinkel	2	3			
V.RechbergersBrauner Dinkle	2	2			
Zenit	2	2			

4.1.4 Germplasm from Ethiopia

A total of 118 genotypes (bread and durum wheats) was screened for resistance using *Ptr* isolates ASC1a and DW-16 (Table 10). Out of the total 90 bread wheat genotypes tested, 33 (36.7%) and 26 (28.9%) were resistant (scores with 1-2 in 1-5 scale) to ASC1a (race 1) and DW-16 (racs 5) *Ptr* isolates, respectively. The bread wheat genotypes had disease reactions ranging from 1 to 5 for both isolates, with average reactions of 2.8 and 3.1 for ASC1a and DW-16, respectively. HAR604, HAR2562 and Dashen, with reaction levels of 1-2 (in 1-5 scale) against ASC1a, were the most resistant genotypes among the commercial Ethiopian bread wheat cultivars. These cultivars also showed 1-2 response towards race 5 (DW-16) isolate. Paven-76, 16300/88, 16293/90, 16167/88, 15085/86, 16294/96, 16192/89, 16168/88, 15440/90 and 16152/90 were resistant to both isolates. The durum wheat genotypes showed disease reactions ranging from 2 to 5 with mean values of 4.1 and 3.8 for race 1 (ASC1a) and race 5 (DW-16) *Ptr* isolates, respectively. Acc. 15360/89 was the only resistant durum wheat genotype to both isolates in the present study.

Table 10. Response of 118 Ethiopian bread and durum wheat genotypes for tan spot resistance using race 1 (ASC1a) and race 5 (DW-16) isolates in 1- 5 scale

Bread wheat accessions						Durum wheat accessions		
Accessions	ASC1a	DW-16	Accessions	ASC1	DW-16	Accession	ASC1a	DW-16
15043/86	1	4	15845/87	3	2	15360/89	2	2
15090/86	1	3	16233/89	3	4	15371/90	2	4
15525/87	1	3	16287/89	3	1	7295/82	2	3
15573/87	1	4	16772/89	3	4	15519/89	3	3
15832/89	1	3	HAR 1407	3	3	15657/89	3	4
15832/89	1	3	HAR 1522	3	4	15859/88	3	3
16126/89	1	4	HAR 1595	3	3	16278/90	3	3
16131/88	1	3	HAR 1868	3	4	15024/95	4	5
16148/88	1	4	HAR 1899	3	4	15506/89	4	3
16167/88	1	2	HAR 2192	3	4	15601/90	4	4
16168/88	1	2	HAR 2501	3	3	15687/89	4	3
16192/89	1	2	HAR 2536	3	4	15760/89	4	3
16196/89	1	3	HAR2508	3	3	15849/90	4	3
16220/89	1	4	14797/86	4	1	15855/89	4	4
16300/88	1	1	14831/86	4	5	14770/89	5	4
16752/89	1	3	15011/86	4	5	14771/89	5	4
17873/97	1	5	15012/89	4	5	14807/86	5	5
Dashen	1	1	15087/89	4	3	15023/99	5	5
HAR 2562	1	2	15361/89	4	4	15061/86	5	5
13134/88	2	3	15462/87	4	4	15070/96	5	5
15016/87	2	3	15516/90	4	2	15089/86	5	4
15085/86	2	2	15538/88	4	4	15254/90	5	4
15086/86	2	4	15543/90	4	4	15412/89	5	4
15440/90	2	2	15597/87	4	3	15570/90	5	4
15511/89	2	3	15608/87	4	3	15572/88	5	5
15540/88	2	3	15725/92	4	3	15721/89	5	4
16152/90	2	2	15785/87	4	2	15838/89	5	4
16161/89	2	3	15805/89	4	1	17830/97	5	5
16293/90	2	2	15809/90	4	1	Mean	**4.1**	**3.8**
16294/96	2	2	15831/87	4	4	S.E	**0.19**	**0.15**
HAR 604	2	2	15831/87	4	4			
HAR1685	2	3	16137/96	4	5			
Paven-76	2	2	16138/97	4	2			
3708/89	3	1	16240/88	4	4			
14803/90	3	4	3427/75	4	3			
15030/86	3	4	ET13-02	4	5			
15044/86	3	3	HAR 1775	4	3			
15094/86	3	2	HAR 1920	4	5			
15446/87	3	5	HAR 2029	4	4			
15518/87	3	4	HAR 2149	4	4			
15527/89	3	3	14792/88	5	4			
15572/90	3	2	15041/86	5	1			
15579/87	3	2	15444/90	5	4			
15609/96	3	3	15476/91	5	5			
15810/90	3	4	3484/75	5	5			
Mean				**2.8**	**3.1**			
S.E				**0.13**	**0.12**			

4.2 Comparison of seedling and adult plant resistance

Twelve winter wheat cultivars were tested for their seedling and adult plant resistance under green house and field conditions, respectively. The seedling resistance was carried out by artificial inoculation using the most virulent isolate (Rog5/04). There was highly significant difference in tan spot resistance among cultivars (Table 11). Cultivar Arina was highly resistant while Cubus showed moderately resistance both under field and greenhouse tests. Tommi showed moderately resistance response across the three years under field conditions, but it was moderately susceptible under greenhouse tests to *Ptr* isolate Rog5/04.

Table 11. Mean response of 12 winter wheat cultivars for seedling resistance against Rog5/04 *Ptr* isolate and adult plant resistance against *Pyrenophora tritici-repentis* from 2004-2006 at Roggenstein, Germany

Cultivar	Mean seedling resistance to *Ptr* isolate Rog5/04	Adult resistance across years (1-5 scale)			
		2004	2005	2006	Mean
Apollo	3.0	2.3^{c}	4.0^{ab}	3.0^{c}	3.1^{b}
Arina	1.0	1.0^{d}	1.7^{d}	1.0^{e}	1.2^{c}
Bandit	4.5	4.0^{ab}	4.7^{a}	4.3^{a}	4.3^{a}
Bussard	3.3	3.7^{ab}	3.7^{ab}	4.0^{ab}	3.8^{ab}
Camino	2.3	2.0^{c}	4.3^{ab}	3.7^{abc}	3.3^{b}
Centrum	3.3	4.0^{ab}	3.3^{abc}	2^{d}	3.1^{b}
Cubus	2.0	2.3^{c}	1.7^{d}	1.3^{de}	1.8^{c}
Greif	3.5	4.3^{a}	3.3^{abc}	3.7_{abc}	3.8^{ab}
Habicht	3.8	4.3^{a}	4.7^{a}	4.3^{a}	4.4^{a}
Hybrid	3.3	3.0^{bc}	3.0^{bcd}	3.0^{c}	3.0^{b}
Tamaro	3.0	2.3^{c}	4.0^{ab}	3.3^{bc}	3.2^{b}
Tommi	2.5	2.0^{c}	$2c^{d}$	1.3^{de}	1.8^{c}

Means followed by the same letter are not significantly different at 5%

As indicated in Fig.6, clear difference was observed for tan spot resistance between the resistant cultivar(Arina) and the susceptible cultivar (Habicht). Arina was also found to be highly resistant to ASC1a (race 1) and DW-16 (race 5) isolates (Table 8).

Fig 6. Response of Arina and Habicht to *Ptr* at Roggenstein, 2005

Varieties which were resistant under filed condition showed similar resistance level under greenhouse condition for seedling resistance. A highly singinficant positive correlation (r= 0.864) was obtained between seedling resistance and adult plant resistance indicating the possibility of screening large number of wheat germplasms against *P. tritci-repentis* at seedling stage in growth chambers effectively and rapidly. This reduces the cost invloved in multilocation trials and avoids the risk of weather associated problems for adult plant resistance screening under field conditions.

4.3 Genetics of tan spot resistance

The inheritance of tan spot resistance was determined using cultivars of different origin. The F_2 populations from the crosses between the disomic Chinese Spring (susceptible parent) and XX41 and XX110 (resistant parents), segregated into 34 and 110 susceptible, and 11 and 42 resistant plants, respectively, fitting a 1:3 (resistant: susceptible) Mendelian ratio indicating that resistance in these two synthetic lines to tan spot is controlled by a single recessive gene. On the other hand, the F_2 populations from crosses between disomic Chinese Spring and XX45 segregated into 97 resistant and 41 susceptible plants (Table 12), indicating that the resistance gene is dominant. Inoculation of $F_{2:3}$ seedlings (F_2 derived F_3 families) of each of

the three populations (CS/XX41, CS/XX45 and CS/XX110) with the same *Ptr* race 1 isolate ASC1b has resulted in a segregation ratio of 1:2:1 conforming that tan spot resistance in these synthetic lines is controlled by a single gene.

The recessive gene in CS/XX41 population segregated into 14 homozygous susceptible, 31 segregating and 17 homozygous resistant families, a satisfactory fit for segregation at a single locus ($\chi^2_{1:2:1}$ =0.29, P =0.865 at 2 df). Similarly, the CS/XX110 population segregated into 12 homozygous susceptible, 37 segregating and 11 homozygous resistant families ($\chi^2_{1:2:1}$ = 3.07, P = 0.215 at 2 df). On the other hand, the CS/XX45 population segregated into 12 homozygous resistant, 40 segregating and 14 homozygous susceptible ($\chi^2_{1:2:1}$ = 1.71, P = 0.425 at 2 df). The detail is indicated in manuscript III (Appendix).

Table 12. Response of F_2 seedlings and $F_{2:3}$ families of the CS/XX41, CS/XX45 and CS/XX110 populations against ASC1b

Population	Segregation in F_2		χ^2 (1:3 or 3:1 ratio)	Segregation in $F_{2:3}$ families				
	R*	S		HR	Seg	HS	χ^2 (1:2:1, df 2)	P
CS/XX41	11	34	0.008	17	31	14	0.29	0.865
CS/XX45	97	41	1.6.3	12	40	14	3.07	0.215
CS/XX110	42	110	2.771	11	37	12	1.71	0.425

* R = Resistance, S = Susceptible, HR = Homozygous resistance; Seg. = Segregating; HS = Homozygous Susceptible

Inheritance of tan spot resistance in three resistant Ethiopian wheat cultivars (HAR604, HAR 2562 and Dashen) was studied by crossing them with the susceptible parent Glenlea. The F_1 lines of all the crosses (HAR604/Glenelea, HAR2562/Glenlea and Dashen/Glenlea) showed susceptible reactions to both races 1 and 5 isolates, while the F_2 lines segregated into 1 resistant and 3 susceptible (1:3) ratio indicating that tan spot resistance in these Ethiopian wheat cultivars is controlled by a single recessive gene (Table 13). Similar results were obtained for the CS/HAR604, CS/HAR2562, CS/Salamouni, CS/Arina and CS/Red Chief F_2 populations as indicated in the respective monosomic analyses.

Table 13. Response of F_1 and F_2 plants to isolates ASC1a (race 1) and DW-16 (race 5), and Chisquare tests of F_2 segregation ratios

Crosses	Number of F_1 & F_2 plants observed after inoculation with ASC1a (race 1)				χ^2 for F_2	Number of F_1 & F_2 plants observed after inoculation with DW-16 (race 5)				χ^2 for F_2
	F_1 plants		F_2 Plants			F_1 plants		F_2 plants		
	R	S	R	S	1:3 ratio	R	S	R	S	1:3 ratio
HAR 604/Glenlea	0	12	60	150	1.427	0	9	40	130	0.196
HAR 2562/Glenlea	0	11	42	145	0.642	0	8	56	128	2.894
Dashen/Glenlea	0	10	37	122	0.253	0	11	62	143	3.01

4.4 Chromosomal location of resistance genes

4.4.1 The *tsn3* genes in synthetic wheat lines

The results of the F_2 monosomic analyses of the crosses between the D genome Chinese Spring monosomic lines (1D-7D) and the three resistant synthetic genotypes, XX41 (a hybrid between Langdon durum and *Ae. tauschii* (CI 00017), XX45 (Langdon durum /*Ae. tauschii*, RL 5565) and XX110 (*T. dicoccum* (A38)/*Ae. tauschii*, CI 33) are presented in Tables 14, 15 and 16, respectively. The χ^2 analyses of the segregation ratio from the three populations indicated that the combinations of mono-3D segregated differently and significantly ($p<0.001$) from the expected 1:3 and 3:1 (resistant: susceptible) ratios indicating that these were the critical crosses. The segregation patterns in the critical crosses were 60:5, 74:4 and 70:7 resistant : susceptible plants in CS/XX41, CS/XX45 and CS/XX110 F_2 populations, respectively. The results clearly indicated that the resistance genes are located on chromosome 3D. The recessive genes from XX41 and XX110 are designated as *tsn3a* and *tsn3c*, respectively, and the dominant gene from XX45 is named *Tsn3b*.

Table 14. Frequencies of resistant and susceptible seedlings in crosses of Chinese Spring monosomics and XX41 tested with isolate ASC1b

Crosses	Number of plants in the F_2 populations			
	Resistant	Susceptible	χ^2 Ratio	P for 1:3
mono-1D/XX41	26	55	2.321	0.1276
mono-2D/XX41	17	53	0.018	0.8912
mono-3D/XX41	**60**	**5**	**157.048**	**<0.0001****
mono-4D/XX41	27	60	1.869	0.1937
mono-5D/XX41	20	52	0.296	0.5864
mono-6D/XX41	24	58	0.796	0.3723
mono-7D/XX41	15	38	0.308	0.5789
Disomic/XX41	11	34	0.008	0.9287

** Significant at P = 0.001.

Table 15. Frequencies of resistant and susceptible seedlings in crosses of Chinese Spring monosomics and XX45 tested with isolate ASC1b

Crosses	Number of plants in the F_2 populations			
	Resistant	Susceptible	χ^2 Ratio	P for 3:1
mono-1D/XX45	-	-	-	-
mono- 2D/XX45	-	-	-	-
mono-3D/XX45	**74**	**4**	**16.4268**	**0.000****
mono-4D/XX45	56	17	0.1141	0.7355
mono-5D/XX45	54	21	0.360	0.5485
mono-6D/XX45	52	20	0.2962	0.5862
mono-7D/XX45	56	25	1.4853	0.2229
Disomic/XX45	97	41	1.6330	0.2012

** Significant at P = 0.001.

Table 16. Frequencies of resistant and susceptible seedlings in crosses of Chinese Spring monosomics and XX110 tested with isolate ASC1b

Crosses	Number of plants in the F_2 populations			
	Resistant	Susceptible	χ^2 Ratio	P for 1:3
mono-1D/XX110	15	40	2.321	0.1514
mono-2D/XX110	25	50	2.777	0.0956
mono-3D/XX110	**70**	**7**	**136.380**	**0.000****
mono-4D/XX110	23	52	1.824	0.1768
mono-5D/XX110	21	49	0.933	0.3340
mono-6D/XX110	17	55	0.074	0.7855
mono-7D/XX110	24	58	0.796	0.3722
Disomic/XX10	42	110	2.771	0.0960

** Significant at P = 0.001.

4.4.2 Chromosomal location of the resistance gene in cultivar Salamouni

Evaluation of the F_2 plants of the hybrids between the 20 monosomic lines of wheat cultivar Chinese Spring and the resistant cultivar Salamouni was carried out using *Ptr* isolate ASC1a to determine the chromosomal location of the resistance gene. The cross with monosomic line 5A failed. The disomic Chinese Spring/Salamouni F_2 population segregated in a 1:3 Mendelian ratio (Table 17) indicating that resistance in Salamouni was controlled by a single recessive gene.

Table 17. Frequencies of resistant and susceptible F_2 seedlings in crosses of Chinese Spring monosomics and Salamouni tested with *Ptr* isolate ASC1a

F_2 populations	Resistant	Susceptible	χ^2 (1 : 3)
1A/ Salamouni	16	45	0.049
2A/ Salamouni	18	42	0.800
3A/ Salamouni	**70**	**7**	**178.4****
4A/ Salamouni	20	40	2.215
5A/ Salamouni	-	-	-
6A/ Salamouni	11	49	1.416
7A/ Salamouni	16	45	0.049
1B/ Salamouni	11	49	1.416
2B/ Salamouni	13	47	0.356
3B/ Salamouni	13	47	0.356
4B/ Salamouni	11	51	1.741
5B/ Salamouni	9	54	1.416
6B/ Salamouni	15	50	0.128
7B/ Salamouni	10	50	2.226
1D/ Salamouni	16	44	0.089
2D/ Salamouni	20	55	0.111
3D/ Salamouni	17	50	0.004
4D/ Salamouni	15	54	0.391
5D/ Salamouni	15	43	0.023
6D/ Salamouni	17	45	0.193
7D/ Salamouni	11	63	1.910
Disomic	35	90	0.600

** Significant at P = 0.001.

As indicated in Table 17, the 3A/Salamouni F_2 population segregated into 70 resistant and 7 susceptible plants, deviating significantly ($P < 0.001$) from the expected 1 resistant: 3 susceptible ratio. This indicated that the resistance gene in Salamouni, designated as *tsn4*, is located on chromosome 3A. (For details, refer manuscript II in Appendix).

4.4.3 Chromosomal location of the resistance gene in the winter wheat cultivars Red Chief and Arina

In this investigation, the resistance cultivars Red Chief and Arina were crossed with the 21 monosomic lines of the susceptible cultivar Chinese Spring in order to localize the resistance gene through monosomic analysis. As indicated in Tables 18 and 19, all the monosomic F_1 crosses, except mono3A/Red Chief and mono3A/Arina, respectively were susceptible. The mono 3A /Red Chief F_1 cross segregated into 8 resistant and 3 susceptible plants while the mono 3A /Arina F_1 cross segregated into 9 resistant and 4 susceptible plants to isolate ASC1a, indicating that mono 3A/Red Chief and mono3A/Arina are the critical crosses.

This was further confirmed by testing all the F_2 crosses in which the 20 monosomic F_2 crosses and the disomic F_2 populations of the respective crosses segregated into resistant and susceptible plants of 1:3 ratio indicating that the resistance gene in Red Chief and Arina to race 1 *Ptr* isolate ASC1a is recessive. The mono3A/Red Chief F_2 crosses segregated into 98 resistant and 9 susceptible plants, deviating significantly ($\chi^2 = 252.9$, P = 0.000) from the 1:3 ratio. Similarly, the mono 3A/Arina F_2 crosses deviated significantly ($\chi^2 = 252.9$, P = 0.000) from the 1:3 Mendelian ratio by segregating into 72 resistant and 8 susceptible plants. This indicated clearly that the resistance gene in Red Chief and Arina against the race 1 *Ptr* isolate ASC1a is located on chromosome 3A.

Table 18. Segregation for seedling reaction to isolate ASC1a (race 1) in F_1 and F_2 populations from crosses of 21 Chinese Spring monosomics with common wheat cultivar Red Chief

Monosomic chromosome involved	ASC1a (race 1)				χ^2 (1:3) in the F_2	P value
	Observed segregation in F_1		Observed segregation in F_2			
	Resistant	Susceptible	Resistant	Susceptible		
1A	0	10	25	70	0.126	0.723
2A	0	9	35	90	0.60	0.438
3A	**8**	**3**	**98**	**9**	**252.9**	**0.000****
4A	0	7	24	60	0.57	0.450
5A	0	8	30	78	0.444	0.505
6A	0	10	32	80	0.76	0.383
7A	0	9	19	50	0.236	0.627
1B	0	7	22	54	0.630	0.427
2B	0	8	30	76	0.616	0.432
3B	0	10	40	95	1.53	0.216
4B	0	11	27	72	0.272	0.601
5B	0	12	19	48	0.402	0.526
6B	0	10	20	72	0.521	0.470
7B	0	8	29	81	0.109	0.741
1D	0	10	35	84	1.234	0.266
2D	0	8	23	62	0.192	0.661
3D	0	9	37	98	0.416	0.518
4D	0	8	42	96	2.17	0.140
5D	0	7	25	68	0.175	0.675
6D	0	9	26	71	0.168	0.682
7D	0	8	28	74	0.326	0.568
Disome	0	20	50	142	0.111	0.739

** Significant at P = 0.001.

Table 19. Segregation for seedling reaction to *Ptr* isolate ASC1a (race 1) in F_2 populations from crosses of 21 Chinese Spring monosomics with common wheat cultivar Arina.

Monosomic chromosome involved	ASC1a (race 1)				χ^2 (1:3) in the F_2	P value
	Observed segregation in F_1		Observed segregation in F_2			
	Resistant	Susceptible	Resistant	Susceptible		
1A	0	10	14	35	0.328	0.566
2A	0	9	10	27	0.08	0.772
3A	**9**	**4**	**72**	**8**	**180.26**	**0.000****
4A	0	8	12	28	0.533	0.465
5A	0	10	14	30	1.09	0.296
6A	0	9	12	27	0.692	0.405
7A	0	8	10	28	0.034	0.853
1B	0	10	16	36	0.922	0.336
2B	0	9	12	30	0.285	0.593
3B	0	8	13	32	0.362	0.547
4B	0	8	11	29	0.133	0.715
5B	0	10	13	34	0.176	0.674
6B	0	10	10	26	0.148	0.700
7B	0	9	12	29	0.397	0.528
1D	0	8	15	39	0.221	0.638
2D	0	9	16	38	0.616	0.432
3D	0	10	22	49	1.356	0.244
4D	0	9	17	40	0.706	0.401
5D	0	10	10	25	0.237	0.626
6D	0	10	14	37	0.162	0.687
7D	0	8	18	42	0.8	0.371
Disome	0	20	29	86	0.002	0.964

*** Significant at P = 0.001.

4.4.4 Chromosomal location of the resistance gene in two Ethiopian wheat cultivars

Monosomic analyses of the F_1 and F_2 plants of the hybrids between the 21 monosomic lines of wheat cultivar Chinese Spring and the resistant cultivars HAR604 and HAR2562 are summarised in Tables 20 and 21, respectively. Mono5A/HAR604 failed. The F_1 CS mono/HAR 604 and CS mono/HAR2562 crosses were not tested against DW-16 isolate due to seed limitation. For the race 1 ASC1a isolate, all the CS mono/HAR604 and CS mono/HAR2562 F_1 hybrids, except CS mono3B/HAR604 and CS mono3B/HAR2562, were susceptible. The CS mono3B/HAR604 F_1 hybrids segregated into 7 resistant and 3 susceptible plants (Table 20). Similarly, the Cs mono3B/HAR2562 F_1 crosses segregated into 12 resistant and 4 susceptible plants (Table 21).

The F_2 monosomic and disomic populations, except Cs mono3B /HAR604 and Cs mono3B /HAR2562, segregated into 1 resistant : 3 susceptible Mendelian ratio for both race 1 (ASC1a) and race 5 (DW-16) *Ptr* isolates indicating that resistance in both cultivars, HAR604 and HAR2562, is controlled by a single recessive gene. The F_2 population of the CS mono3B/HAR604 segregated into 70 resistant and 9 susceptible plants for the ASC1a isolate and 60 resistant and 8 susceptible plants for DW-16 isolate (Table 20). Similarly, the CS mono3B/HAR2562 F_2 population segregated into 62 resistant and 6 susceptible plants for the ASC1a isolate and 69 resistant and 7 susceptible plants for DW-16 isolate (Table 21), deviating significantly ($P < 0.001$) from the expected 1 resistant : 3 susceptible ratio. Both the F_1 data and the significant deviation of the F_2 CS mono3B/HAR604 and CS mono3B/HAR2562 populations from the expected 1:3 segregation ratio indicated that the resistance in these two Ethiopian commercial cultivars, HAR604 and HAR2562, is clearly controlled by a single recessive gene located on chromosome 3B. This new gene is tentatively designated as *tsn5*.

Table 20. Segregation for seedling reaction to *Ptr* isolate ASC1a (race 1) and DW-16 (race 5 in F_2 populations from crosses of 21 'Chinese Spring' monosomics with HAR604

Monosomic chromosome involved	ASC1a (race 1)				χ^2 (1:3) in the F_2	DW-16 (Race 5)		
	Observed segregation in F_1		Observed segregation in F_2			Observed segregation in F_2		χ^2 (1:3)
	R	S	R	S		R	S	
1A	0	7	12	30	0.285	15	42	0.052
2A	0	8	10	28	0.034	22	50	1.186
3A	0	12	13	29	0.793	11	30	0.072
4A	0	9	14	35	0.333	12	34	0.028
5A	-	-	-	-	-	-	-	-
6A	0	11	13	27	1.20	13	35	0.108
7A	0	11	7	25	0.165	10	37	0.347
1B	0	10	14	34	0.444	16	41	0.287
2B	0	12	15	35	0.667	20	49	0.584
3B	**7**	**3**	**70**	**9**	**170.45**	**60**	**8**	**145.01****
4B	0	9	10	36	0.26	9	28	0.01
5B	0	9	11	29	0.133	13	33	0.26
6B	0	8	13	33	0.26	17	47	0.082
7B	0	8	12	31	0.214	10	29	0.012
1D	0	10	10	28	0.036	14	43	0.01
2D	0	11	17	45	0.193	19	48	0.403
3D	0	12	20	48	0.705	12	36	0.529
4D	0	10	18	44	0.537	10	27	0.08
5D	0	8	9	22	0.268	14	37	0.162
6D	0	8	11	31	0.033	16	44	0.089
7D	0	9	13	38	0.01	10	28	0.667
Disome	0	20	30	84	0.104	35	93	0.374

** Significant at P = 0.001.

Table 21. Segregation for seedling reaction to *Ptr* isolate ASC1a (race 1) and DW-16 (race 5 in F_2 populations from crosses of 21 'Chinese Spring' monosomics with HAR2562

Monosomic Chromosom involved	ASC1a (race 1)				χ^2 (1:3) in the F_2	DW-16 (race 5)		
	Observed segregation in F_1		Observed segregation in F_2			Observed segregation in F_2		χ^2 (1:3)
	Resistant	Susceptible	Resistant	Susceptible		Resistant	Susceptible	
1A	0	10	18	42	0.80	11	30	0.072
2A	0	12	15	36	0.529	10	36	0.26
3A	0	8	10	42	0.922	14	34	0.319
4A	0	11	10	45	2.239	15	38	0.308
5A	0	9	8	30	0.314	10	28	0.034
6A	0	8	13	29	0.793	14	35	0.333
7A	0	8	9	26	0.01	12	33	2.917
1B	0	10	12	39	0.1	17	40	0.706
2B	0	11	16	40	0.38	19	45	0.749
3B	**12**	**4**	**62**	**6**	**158.8**	**69**	**7**	175.35**
4B	0	10	15	40	0.15	10	36	0.26
5B	0	8	14	34	3.19	13	29	0.793
6B	0	7	14	41	0.01	16	40	0.38
7B	0	10	17	39	0.856	11	29	0.133
1D	0	11	10	34	0.12	13	37	0.03
2D	0	10	12	32	0.11	18	40	1.12
3D	0	9	18	40	1.12	16	41	0.287
4D	0	10	14	36	0.24	11	29	0.133
5D	0	8	8	25	0.01	10	26	0.148
6D	0	8	10	38	0.444	13	36	0.06
7D	0	10	15	40	0.151	15	35	0.666
Disome	0	25	25	85	0.302	30	98	0.167

** Significant at P = 0.001.

4.5 Allelism tests among resistance genes

Allelism studies were carried out among three tan spot resistant synthetic lines and three Ethiopian common wheat cultivars in order to identify whether resistance in these cultivars is goverened by allelic or different genes. As indicated in Table 22, all F_1 and F_2 progenies of the crosses between the resistant synthetic lines in all possible combinations were resistant to the race 1 *Ptr* isolate ASC1b. The lack of segregation into susceptible plants both in the F_1 and F_2 crosses among the three resistant lines indicated that the three genes are allelic/ tightly linked. However, they can be differentiated from each other by their mode of inheritance and differential reactions to different isolates. Phenotypically, the recessive genes *tsn3a* and *tsn3c* are highly resistant and moderately resistant to *Ptr* race 1 isolate ASC1b, respectively. The dominant gene *Tsn3b* showed a highly resistance response.

Table 22. Response of F_1 and F_2 populations for resistance to *Ptr* isolate ASC1b in resistant/ resistant synthetic wheat crosses

Crosses	Number of F_1 plants		Number of F_2 plants	
	Resistant	Susceptible	Resistant	Susceptible
XX41/XX45	8	0	150	0
XX41/XX110	10	0	200	0
XX45/XX110	8	0	150	0

All F_1 and F_2 plants of the three possible R/R crosses among Ethiopian wheats (HAR604/ HAR2562, HAR604/Dashen, HAR2562/Dashen) were resistant (Table 23) to both ASC1a and DW-16 isolates suggesting that the resistant genes in all these three cultivars are allelic or tightly linked.

Table 23. Response of F_1 and F_2 populations for resistance to *Ptr* isolate ASC1a (race 1) and DW-16 (race 5) in resistant/resistant Ethiopian wheat crosses

R/R crosses	ASC1a				χ^2 for F_2	DW-16 (race 5)				χ^2 for F_2
	F_1		F_2			F_1 plants		F_2 plants		
	R	S	R	S	**7:9 ratio**	R	S	R	S	**7:9 ratio**
HAR 604/HAR 2562	12	0	180	0	231.8**	10	0	200	0	257.1**
HAR 604/Dashen	11	0	175	0	225.3**	10	0	190	0	281.3**
HAR 2562/Dashen	10	0	200	0	257.1**	10	0	185	0	238**

** Significant at P = 0.001.

4. 6. Molecular mapping of tan spot resistance genes

4.6.1 Molecular mapping of the *tsn3* genes in synthetic wheat lines

The *tsn3* genes from three synthetic wheat lines (XX41, XX45 and XX110) have been located on chromosome 3D using monosomic analysis (Chapter 4.4.1). In order to map these genes at molecular level, a total of 12 SSR markers which are mapped on chromosome 3D was screened for polymorphism and 6, 7 and 9 of these markers were found to be polymorphic for CS/XX41, CS/XX45 and CS/XX110 $F_{2:3}$ populations, respectively. Electropherogram showing the variation between the parents, and some selected homozygous and hetrozygous lines of CS/XX41, CS/XX45 and CS/XX110 populations for some selected markers are indicated in Fig 7A, B, & C, respectively.

A B C

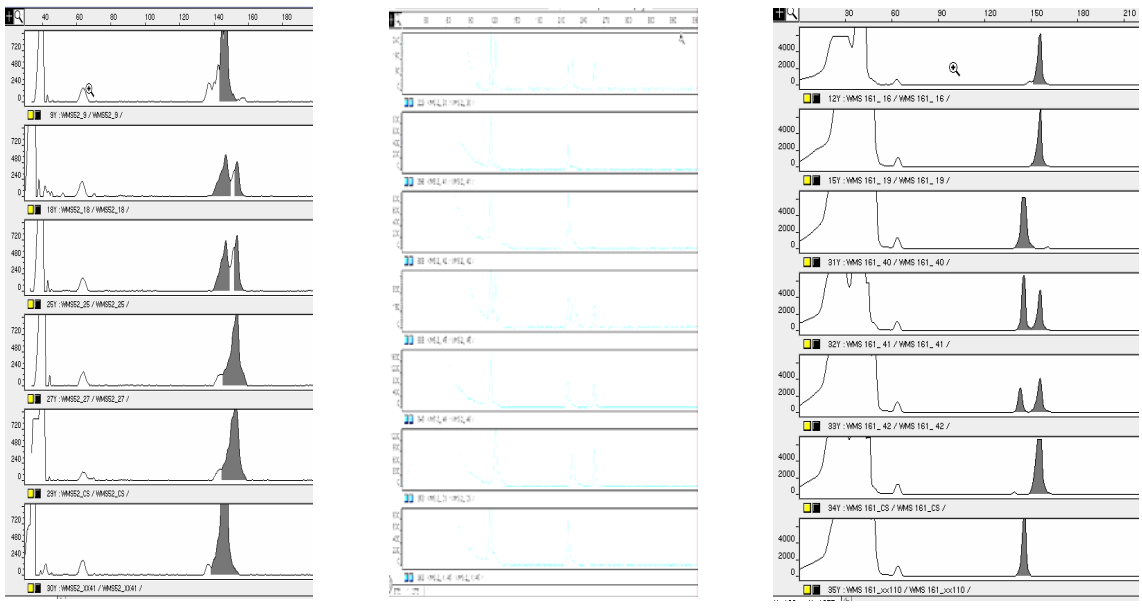


Fig 7. Electopherogram showing polymorphism in: CS, XX41 & some selected lines of CS/XX41 populations using *Xgwm52* (A); CS, XX45, & some selected lines of CS/XX45 population using *Xgwm2* (B); CS, XX110 & some selected lines of CS/XX110 population using *Xgwm161*

The SSR locus *Xgwm2* was classified into *Xgwm2a* and *Xgwm2b* since it showed two distinctly different alleles in CS and the three resistant synthetic lines. *Xgwm2a* has amplified 126 bp in CS, and 120 bp in XX41, XX45 and XX110 lines. *Xgwm2b*, on the other hand, has amplified 258 bp marker allele in CS, and 256 bp marker allele in XX41 and XX110, but was not amplified in XX45. *Xbarc1040*, *Xgwm2a*, *Xbarc42*, *Xgwm52*, *Xgwm341*, *Xgwm114* were

polymorphic in CS/XX41 population. All these markers plus *Xgwm2b* were also found to be polymorphic in CS/XX45 population. *Xbarc1040*, although it was polymorphic in all the three populations, it was linked only in CS/XX110 population. Markers which were polymorhic in CS/XX41 and CS/XX45, except *Xgwm114*, were also polymorphic and linked in CS/XX110.

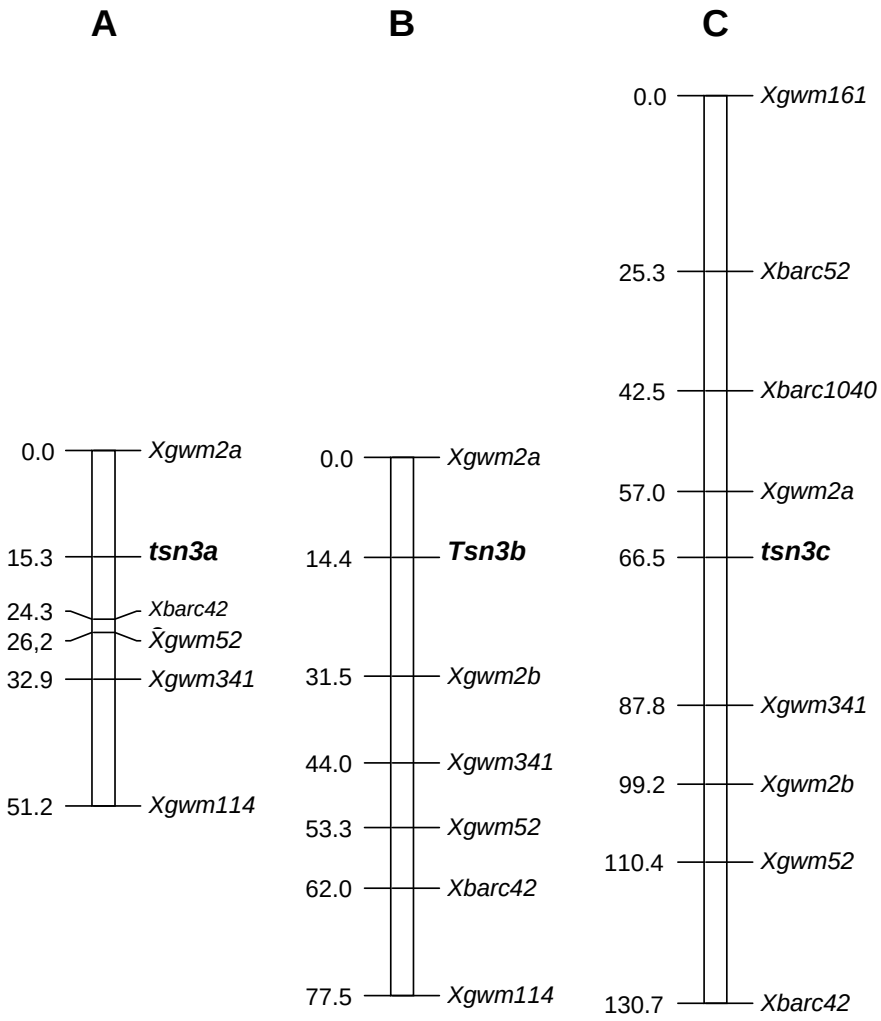


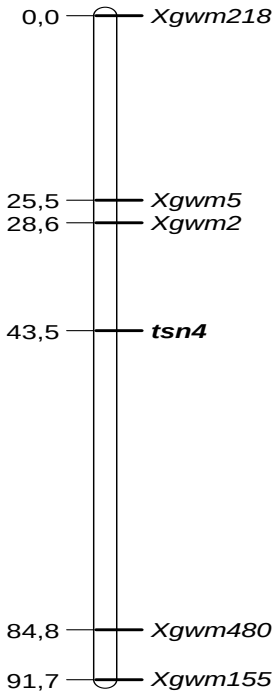
Fig 8. Microsatellite linkage maps showing *tsn3* genes on chromosome 3D in the populations: CS/XX41 (A), CS/XX45 (B) and CS/XX110 (C). Locus names and map distances (cM) are indicated on the right and left sides of the map, respectively

As shown in Fig 8, all the three genes: *tsn3a* in CS/XX41, *Tsn3b* in CS/XX45 and *tsn3c* in CS/XX110 were clustered in a region around *Xgwm2a,* which showed 120 bp marker allele in CS, and 126 bp in XX41, XX45 and XX110. *Xbarc42*, *Xgwm2b* and *Xgwm341* were the other flanking markers for *tsn3a*, *tsn3b* and *tsn3c,* respectively. *Xgwm2a* was the closest marker to *Tsn3b* and *tsn3c* at genetic distances of 14.4 and 9.5 cM, respectively. *Xbarc42* with a linkage distance of 11 cM was the closest marker to *tsn3a*.

Maps for CS/XX41 and CS/XX45 differed in the order of SSR loci *Xbarc42*, *Xgwm52* and *Xgwm341*. Furthermore, *Xgwm2b* was not polymorphic in CS/XX41. Maps for CS/XX45 and CS/XX110 varied in order of markers *Xgwm2b* and *Xgwm341*. *Xgwm2b* was dominant in CS/XX45 showing only the 258 bp from CS, but co-dominant in CS/XX110 amplifying marker alleles of 258 and 256 bp in CS and XX110, respectively. Furthermore, markers *Xgwm161, Xbarc52*, *Xbarc1040* were not linked in CS/XX45 and CS/XX41.

4.6.2 Molecular mapping of the *tsn4* gene in cultivar Red Chief

The *tsn4* gene has been first located on chromosome 3A in cultivar Salamouni through monosomic analysis. Monosomic anlysis of the 21 CS mono/Red Chief F_2 populations using *Ptr* race 1 isolate ASC1a has indicated that the resistance gene in cultivar Red Chief is also located on Chromosome 3A. Both Red Chief and Salamouni showed similar response to ASC1a indicating that they possess the same recessive gene, *tsn4*. A total of 140 CS/Red Chief F_2 lines was genotyped using 7 polymorphic SSR markers on chromosome 3A.



Markers *Xgwm218*, *Xgwm5*, *Xgwm2*, *Xgwm480* and *Xgwm155* were polymorphic and linked to *tsn4*. *Xgwm30* and *Xgwm369* were not linked even though they were polymorphic. *Xgwm218*, *Xgwm5*, *Xgwm2*, *Xgwm480*, *Xgwm155* and *Xgwm30* amplified fragments with sizes of 154, 167, 130, 173, 151 and 239 bps in Red Chief and 156, 180, 125, 193, 145 and 209 bps in Chinese Spring, respectively. The closest markers to *tsn4* were *Xgwm2* and *Xgwm5* with 14.9 and 18 cM, respectively. These markers were located on the short arm of chromosome 3A suggesting that *tsn4* is located on the short arm of chromosome 3A near the centromer region (Fig.9).

Fig 9. Microsatellite linkage map showing *tsn4* gene on chromosome 3A in CS/Red Chief F_2 population. Locus names and map distances (cM) are indicated on the right and left sides of the map, respectively.

4.7. Marker validation

A total of 34 wheat genotypes (28 resistant, and 6 susceptible) was screened using *Xgwm2a* in order to check the suitability of this marker for marker assisted selection since it was found to be the clossest marker to *tsn3* and *tsn4* genes amplifying 120 bp and 130 bp alleles in each genes, respectively. As indicated in Table 24, *Xgwm2a* has amplified a total of 7 fragments ranging from 118.6 to 230 bp. The 120 bp allele specific fragment was observed in 11 genotypes including the three synthetic lines XX41, XX45, and XX110. On the other hand, the 130 bp allele was amplified only in Red Chief and Dashen. The result suggests the SSR marker *Xgwm2a* can be utilized for marker assisted selection in wheat breeding programs to pyramidize tan spot resistance genes in a commonly grown and adaptable cultivar.

Table 24. Validation of *Xgwm2a* for MAS using 34 wheat geneotypes

Cultivar	resistance	Band size (Xgwm2a)
4B1149	R	120
Apollo	S	119
Arina	R	119
Bandit	S	119
CS	R	126
Dashen	R	130
Dream	S	119
Empire	R	119
Erik	R	120
Glenlea	S	119
HAR1775	R	129
HAR2562	R	119
HAR604	R	119
Hercule	R	119
Jenga	R	119
Karepwa	R	119
Lynx	S	122
Piko	R	118.6
Red Chief	R	130
Salamouni	R	119
Schwabenkorn	R	120
Septre	R	120
Solitär	R	119
Syn38	R	120
Syn44	R	119
Syn84	R	120
Vilmorin27	R	118.6
W7984	R	120
XX173	S	119
XX183	R	120
XX35	R	120
XX41	R	120
XX45	R	120
XX110	R	120

R = resistant; S = Susceptible

5. DISCUSSION

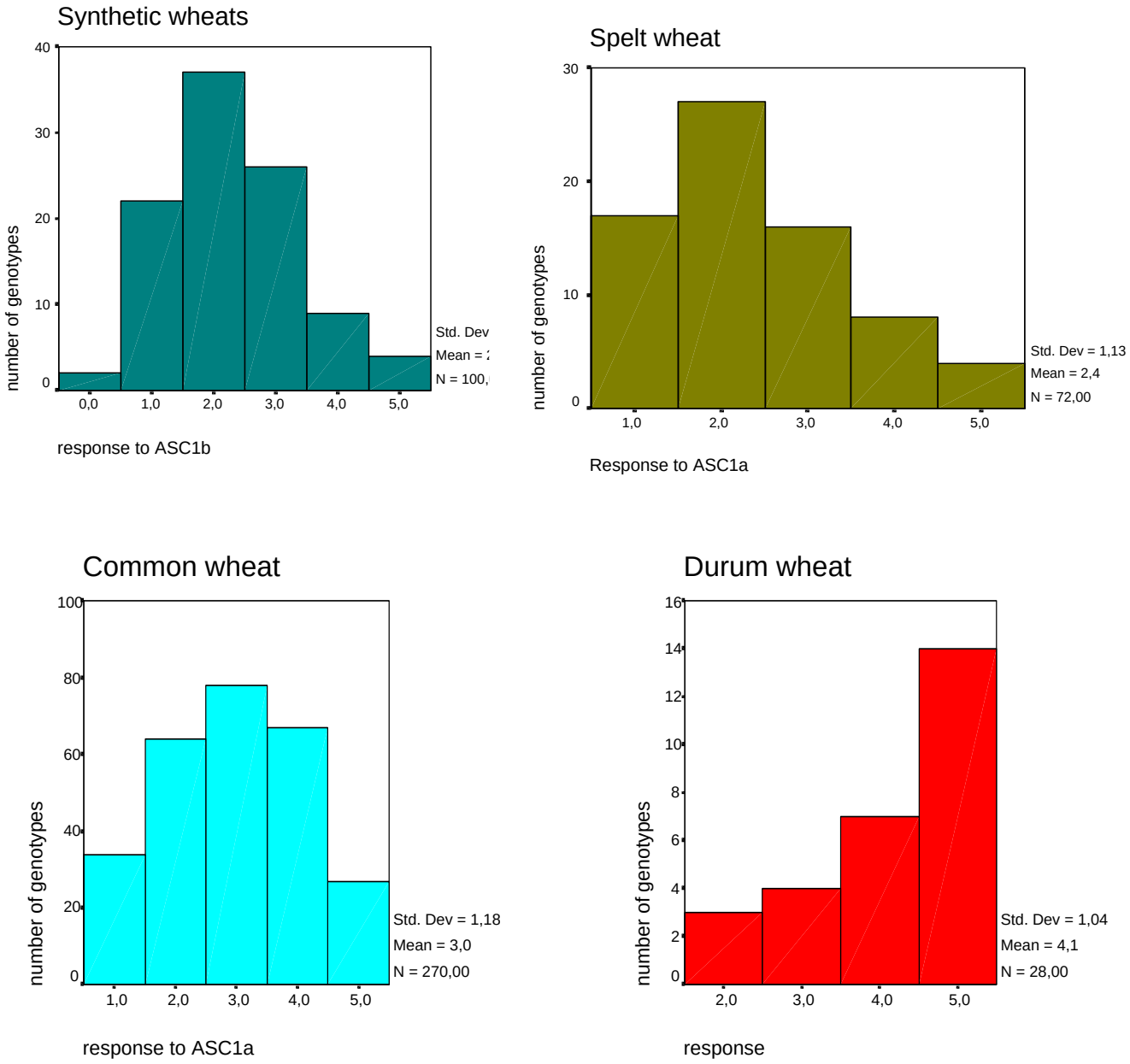
5.1 Genetic variation for tan spot resistance

The wheat primary gene pool comprises landraces of diploid, tetraploid and hexaploid groups, and wild tetraploid and diploid species. In the present study, about 98 synthetic wheat lines, 269 common wheat cultivars, 72 spelt wheat cultivars and 28 durum wheat genotypes were screened for tan spot resistance. About 62% of the synthetic wheat lines were found to be resistant to *Ptr* isolate ASC1b (race1) indicating that they are good sources of tan spot resistance (Fig 10).

The wild relatives of crop plants and germplasm from the center of diversity of the cultivated species are frequently utilized resources to identify new resistance genes. The wild grass *Aegilops tauschii* provides a large gene pool for new sources of resistance to major wheat pests. Furthermore, the fact that it is the D-genome donor of bread wheat allows for efficient and rapid transfer of genes into breeding populations.

Tan spot resistance sources have been identified previously by Siedler *et al*. (1994) and more recently by Xu *et al*. (2004) from synthetic wheats derived from *Aegilops tauschii* lines. Siedler (1991) had reported that the synthetic lines XX41, XX45 and XX110 to be highly resistant to mixtures of *Ptr* isolates. In the present study, XX41 and XX45 were higly resistant but XX110 was moderately resistant to the race 1 isolate ASC1b. As indicated in Fig 10, the majority of the synthetic wheat and spelt wheat genotypes are in the moderately resistance class (score 2). Most of the common wheat genotypes were moderately susceptible with a reasonable number of moderately resistant genotypes. On the other hand, most of the durum wheat genotypes showed highly susceptible (score 5) response (Fig 10).

Fig 10. Response of different wheat genotypes to *Ptr* race 1 isolates



Out of the total 269 common wheat cultivars screened for resistance, 37 cultivars showed highly resistant response (1 in 1-5 scale) to *Ptr* isolate ASC1a (race 1) suggesting the availability of good sources of tan spot resistance in common wheat cultivars as compared to the durum wheat genotypes. Similar results were reported by Lamari and Bernier (1989a) and Singh and Hughes (2005). Further more, most of the tan spot resistance genes reported to-date: *tsn1* on 5BL (Faris *et al*., 1996), *tsc*2 on 2BS (Friesen and Faris, 2004), *tsn3* on 3D, and *tsn4* on 3A are from hexaploid wheat. The major QTLs reported todate (Faris *et al*., 1997; Cheong *et al*., 2004; Faris and Frieson, 2005) were also from hexaploid wheats. More recently, however, Singh *et al*. (2006) have identified *tsn2* on the long arm of chromosome 3B using race 3 *Ptr* isolate in tetraploid wheat.

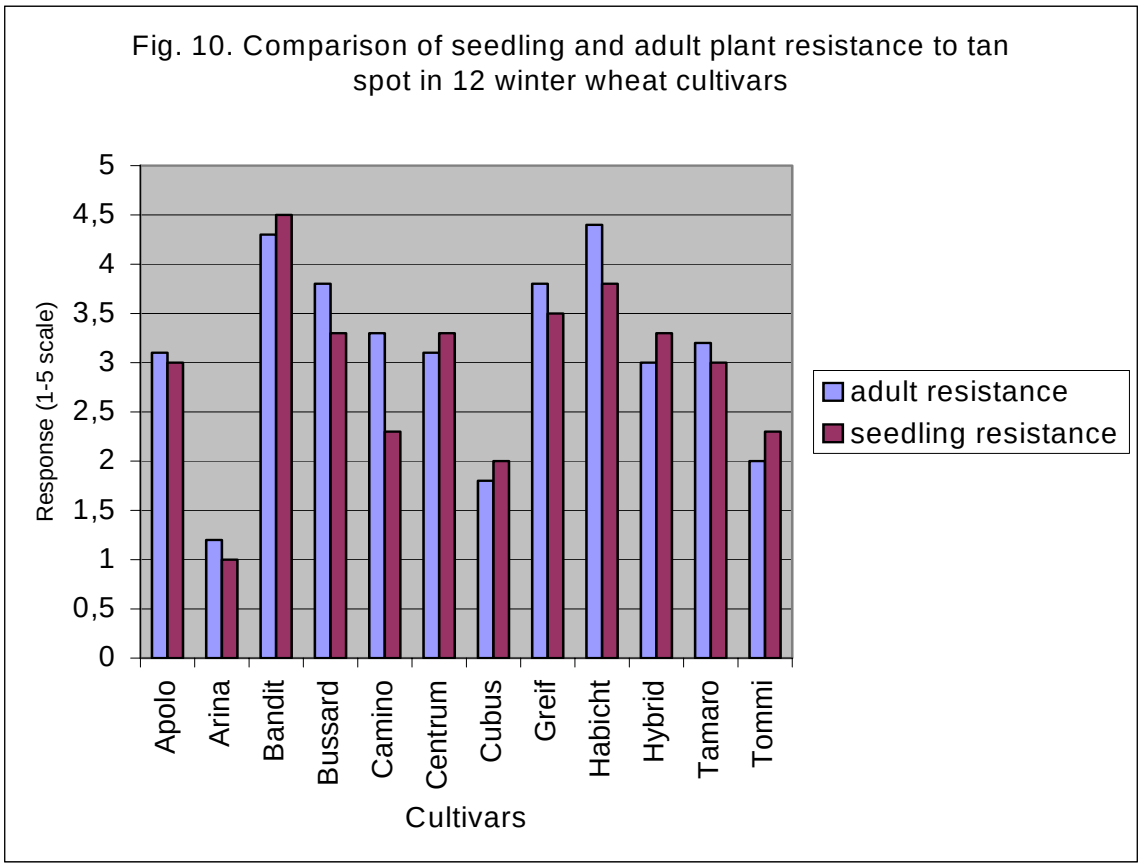
The narrow variability of tan spot resistance among the Ethiopian durum wheat genotypes (Fig 10) may be due to the low number of genotypes tested. Otherwise, much variation is expected to exist as the Ethiopian region is described as a center of diversity and origin of durum wheat (*Triticum turgidum* ssp *aethiopicum*) (Vavilov, 1951). Common wheat (*Triticum aestivum* L) is also found in great diversity though it is a recent introduction to Ethiopia. Owing to this diversity, the Ethiopian germplasms have been utilised world wide (Worede, 1991; Tesema, 1991), and many agronomically important genes have been found and incorporated into commercial cultivars (Tesema, 1991; Gebremariam, 1991; Negassa, 1986; Zeller and Hsam, 1998).

5.2 Association of seedling and adult plant resistance

It is often necessary to stay ahead of changing pathogens by searching for, understanding, and introgressing new sources of resistance in order to achieve sustainable resistance to diseases in major crops. It has been reported that there are resistances which are expressed at seedling growth stages and usually remain effective through out the life of the plant (seedling resistance), and resistances that are effective at adult plant growth stages (adult plant resistance). Some seedling resistances, however, may not be expressed at adult plant stage.

It is, therefore, highly important to study the association of such resistances both at seedling and adult plant growth stages. In the present study, association analysis between seedling resistance and adult plant resistance has shown a highly significant positive correlation ($r = 0.864$, $P = 0.000$) suggesting that large number of wheat germplasm can be screened effectively and rapidly against *P. tritici-repentis* at seedling stage in growth chambers. As indicated in Fig. 11, most of the cultivars showed slightly higher levels of susceptibility at adult stage under field conditions than at seedling stage in growth chamber conditions, even though there is a similar trend between the two resistance levels across cultivars. This may be due to the interaction of different *Ptr* isolates, the higher amount of inoculum build up resulted from monocropping of wheat year after year, and the presence of other pathogens like *Septoria nodorum* and *Septoria tritici* under field conditions, as opposed to the growth chamber condition where the test was carried out using a single monoconidial *Ptr* isolate in a controlled environment. The variability of these factors affects also the level of adult plant resistance across years.

Fig 11. Comparison of seedling and adult plant resistance to tan spot in 12 winter wheat cultivars



Similar positive correlations between seedling and adult plant resistances to tan spot of wheat were reported previously (Lamari and Bernier, 1989a; Raymond *et al*, 1985; Rees *et al*., 1988; Cox and Hosford, 1987).

5.3 Inheritance of tan spot resistance

The inheritance of tan spot was studied using F_1, F_2 and F_3 crosses of resistant and susceptible genotypes. The absence of segregation into resistant plants in the F_1 disomic crosses of the resistant cultivars HAR604, HAR2562, and Dashen with the susceptible cultivar Glenlea, and the segregation of the corresponding F_2 crosses into 1 resistant : 3 susceptible ratio indicated that resistance in these cultivars is controlled by a single recessive gene which inherits qualitatively. Segregation analysis of the $F_{2:3}$ (F_2 derived F_3 families) of CS/XX41, CS/XX45 and CS/XX110 have shown qualitative inheritance of tan spot resistance in these synthetic wheat lines. The monosomic and disomic F_1 and F_2 crosses of these resistant cultivars with the susceptible CS monosomic series and disomic CS cultivar showed the same result, which is in

line with previous reports by various authors (Lee and Gough, 1984; Lamari and Bernier, 1989b, 1991; Gamba and Lamari, 1998; Lamari *et al*., 2003, 2005; Singh and Hughes, 2005). On the other hand, quantitative inheritance of tan spot resistance was reported by Nagle *et al*. (1982); Elias *et al*. (1989), Faris *et al*. (1997), Effertz *et al*. (2002) and Friesen and Faris (2004). Comparison of these studies, however, is difficult due to the variations in the method of inoculation, rating scales, symptoms studied, isolates used, and the environmental conditions for disease development (Singh and Hughes, 2005).

5.4 Cytogenetic analysis

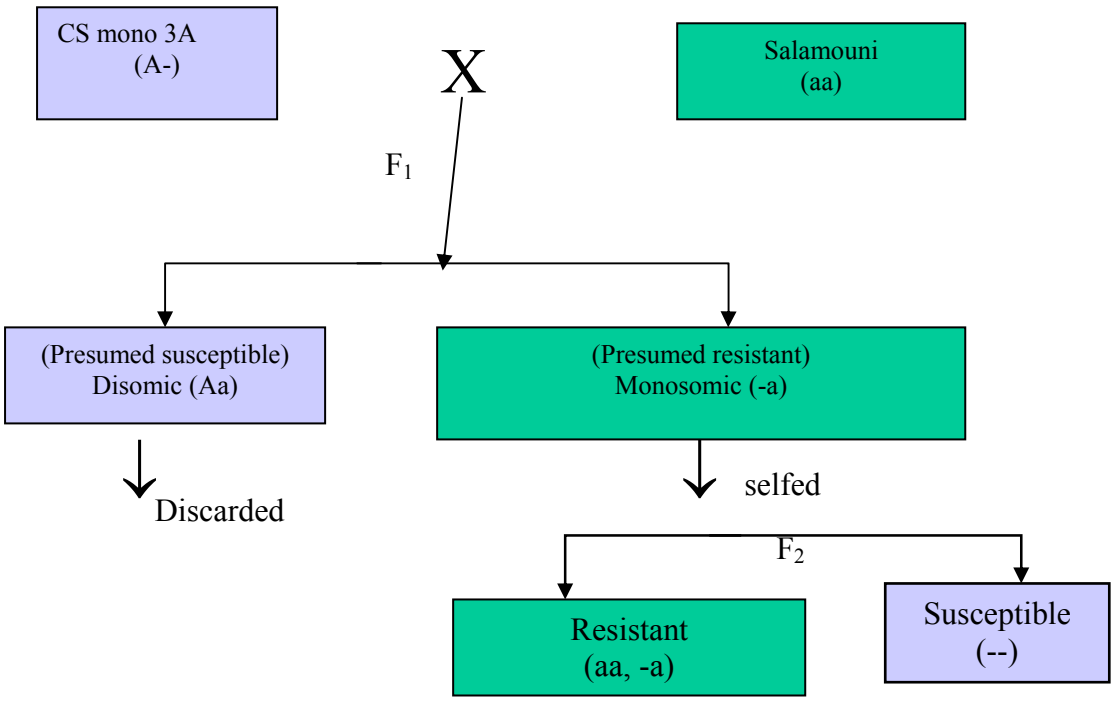
In monosomic analysis, when resistance is goverened by a single hemizygous recessive effective gene, all 20 non-critical monosomic crosses are expected to be heterozgous and susceptible, but the critical cross is expected to segregate into susceptible disomic and resistant monosomic plants at F_1. On the other hand, in the F_2, the 20 non-critical crosses segregate into a 1 susceptible : 3 resistant ratio, while the critical cross deviates significantly from this ratio. A genetic model for such type of recessive gene is indicated in Fig 12 using CS mono series/Salamouni population. When resistance is governed by a single dominant gene, all the monosomic F_1 crosses are expected to be resistant, and later all the F_2 monosomic crosses, except the critical cross, would segregate into 3 resistant : 1 susceptible ratio (Knott, 1989).

In the present investigation, monosomic anlysis of the F_2 crosses of CS-D genome monosomic series (1D-7D) with three resistant synthetic wheat lines (XX41, XX45 and XX110) have indicated that the resistance gene (*tsn3*) in these lines is located on chromosome 3D. Similarly, segregation analysis of the F_2 hybrids of the 21 CS monosomic series with the resistant cultivars Salamouni, Red Chief and Arina to *Ptr* isolate ASC1a (race 1) have clearly indicated that the resistance gene (*tsn4*) in these cultivars is located on chromosome 3A. The winter wheat cultivar Red Chief is derived from the cross early Red Clawson/Red Arcadian. Singh and Hughes (2005) have reported that Red Chief is highly resistant to *Ptr* isolates. In the present study, it was also found to be highly resistant to all the 9 *Ptr* isolates (Table 6). The winter wheat cultivar Arina is derived from the cross of Moisson/Zenith, and it has shown highly resistant response against tan spot both in greenhouse and field tests. Both Moisson and Zenith were screened for resistance, and Zenith showed resistance similar to Arina. Further pedigree analysis showed that Zenith is derived from Heines VII/Canada-382-

3663. Heines VII is resistant to both races 1 and 5 *Ptr* isolates (Table 8) indicating that the resistance source in Arina is derived from Heines VII.

In a similar way, monosomic analysis of the F_1 and F_2 crosses of CS monosomic series with two Ethiopian common wheat cultivars (HAR604, and HAR2562) indicated that only mono 3B/HAR604 and mono3B/HAR2562 segregated into resistant and susceptible plants at F_1, and their F_2 segeregation deviated significantly from the 1:3 (resistant : susceptible) ratio for both race 1 (ASC1a) and race 5 (DW-16) isolates, clearly indicating that the resistance gene in these cultivars is located on chromosome 3B. This is in agreement to the genetic model for a hemizygous effective recessive gene indicated below (Fig 12).

Fig 12. Schematic representation of the genetic model for a hemizygous recessive-effective gene, *tsn4* in the critical cross CS mono 3A/Salamouni



The location of the gene at the same locus for both race 1 and race 5 isolates suggests the presence of race non-specific resistance on these Ethiopian wheat cultivars. Similar race-non specific tan spot resistance using *Ptr* races 1-3 and 5 (Faris and Friesen, 2005) was also reported for a QTL on the chromosome arms of 1BS and 3BL in the Brazilian wheat cultivar BR34.

Lamari and Bernier (1991) have indicated the presence of differential development of tan necrosis and chlorosis by a given wheat genotype indicating that the necrosis and chlorosis components are controlled by different gene loci. Singh *et al*. (2006) have identified the resistance gene *tsn2* on chromosome 3B from the durum wheat line PI 352519 using race 3 *Ptr* isolate. The gene in Ethiopian wheats are not expected to be allelic to the *tsn2* gene since isolates of race 3, to which the *tsn2* gene is resistant, are virtually non existant in hexaploid wheats (Effertz *et al*., 2002). In the present study, both ASC1a (race1) and DW-16 (race 5) isolates have caused necrosis and chlorosis on susceptible cultivars, including the cultivar Glenlea. According to Lamari *et al*. (2003), however, race 5 *Ptr* isolates cause only chlorosis, and are avirulent to Glenlea. Because of this uncertainty in the race 5 isolate (DW-16), we named the resistance gene in Ethiopian wheats as *tsn5* by considering only the race 1 (ASC1a) isolate. The race 5 isolate (DW-16) needs further confirmation.

5.5 Allelism among resistance genes

Allelic crosses among the three resistant synthetic wheat lines (XX41/XX45, XX41/XX110 and XX45/XX110) and among Ethiopian common wheat cultivars (HAR604/HAR2562, HAR604/Dashen and HAR2562/Dashen) to study allelism. The lack of segregation in the F_1 and F_2 populations for the *Ptr* isolate ASC1b in the crosses between the resistant synthetic lines indicated that they all possess tightly linked/allelic genes. The recessive genes in XX41 and XX110 are named as *tsn3a* and *tsn3c*, respectively, while the dominant gene in XX45 is named *Tsn3b*. All the three genes (*tsn3a*, *Tsn3b* and *tsn3c*) belong to the same resistant gene cluster. The recessive genes *tsn3a* and *tsn3c* are either allelic or tightly linked genes while the dominant gene *Tsn3b* is a tightly linked gene.

Singh and Hughes (2005) have identified allelism among the tan spot resistant wheat cultivars Erik, Red Chief, Hadden, and 86ISMN 2137 using *Ptr* race 1 isolate. In the present study, wheat cultivars Salamouni, Red Chief and Arina showed similar resistance response (1 in 1-5 scale) against the race 1 *Ptr* isolate ASC1a indicating that they all possess the *tsn4* gene located on chromosome 3A. However, it is important to carry out allelism test among these cultivars. The lack of segregation among the F_2 crosses of HAR604/HAR2562, HAR604/Dashen and HAR2562/Dashen, and the significant deviation from the 7:9 ratio ($P < 0.001$) and other ratios for a two gene model such as 15R:1S, 1R:15S in the allelism study indicated that the three cultivars possess the same allelic/tightly linked genes, and are tentatively designated as *tsn5*. Resistance genes occurring as a single gene with one or more

alleles encoding different resistance specificities have been reported in many crops. A total of 10 different resistance specificities (*Pm3a* to *Pm3j*) against powdery mildew has been reported at the *Pm3* locus on the short arm of chromosome 1A (Zeller *et al*., 1993; Zeller and Hsam, 1998; Hsam *et al*, 1998). Allelic/linked genes have been also reported for powdery mildew at the *Pm5* locus (Zeller and Hsam, 1998), wheat leaf rust resistance (Singh *et al*., 2004), and resistance to Russian wheat aphid (Miller *et al*., 2001; Liu *et al*., 2005).

5.6 Molecular mapping of resistance genes

The development of molecular marker technologies has revolutionized plant breeding by enabling breeders to exercise indirect marker aided selection at the seedling stage in early generations. In the present study, molecular mapping of the *tsn3* (*tsn3a*, T*sn3b* and *tsn3c*) and *tsn4* genes was carried out using SSR markers on chromosome 3D and 3A, respectively. Linkage analysis of the CS/XX41, CS/XX45 and CS/XX110 $F_{2:3}$ populations has shown that the genes in all the three populations are located in the vicinity of *Xgwm2a* (Fig 8). Maps for CS/XX41 and CS/XX45 differed in the order of SSR loci *Xbarc42*, *Xgwm52* and *Xgwm341* which is probably due to a single inversion. Furthermore, *Xgwm2b* was not polymorphic in CS/XX41.

The difference in the order of *Xgwm2b* and *Xgwm341* for the CS/XX45 and CS/XX110 maps may be due to the variation in the informativeness of the marker in these two populations. The order of markers *Xbarc42*, *Xgwm52*, *Xgwm341* in the present genetic map for CS/XX41 was in line with the consensus map (Somers *et al*., 2004). However, their position was inverted in the maps for CS/XX45 and CS/XX110 populations. Such variations in the location of markers between the genetic and consensus maps were also reported recently by Wang *et al*. (2006). In general, this variation in the order of some markers among the maps in CS/XX41, CS/XX45 and CS/XX110 may be due to the low number of F_3 lines tested, the difference in populations, and the position of crossovers along chromosomes within the progeny lines (Somers *et al*., 2004). The relatively wide gap between some of the markers can also be associated to the low number of SSR markers available on the D genome as compared to the A and B genomes of wheat. This is the first report of mapping allelic/linked genes for tan spot resistance in the D genome of wheat using SSR markers. However, SSR markers were used to map allelic genes to powdery mildew of wheat indicating that they are ideal for comparative mapping of alleles at the same gene locus in different mapping populations (Singrün *et al*., 2004; Huang *et al*, 2004).

Linkage analysis of the CS/Red Chief F_2 population has revealed that *Xgwm2* to be the closest marker to the resistant gene *tsn4* suggesting homoeology of resistance with the *tsn3* genes. The order of markers *Xgwm218, Xgwm5*, *Xgwm2 Xgwm480 and Xgwm155* in the present genetic map for CS/Red Chief F_2 population was in line with the consensus map (Somers *et al*., 2004).

Table 25. List of localised/mapped tan spot resistance genes in wheat

Gene	Chromosome	Wheat cultivar	Reference
tsn1	5BL	Chinese Spring	Stock *et al*., 1996
tsn2	3BL	PI 352519 (durum)	Singh *et al*., 2006
tsc2	2BS	W-7984/Opata85	Friesen and Faris, 2004
tsn3a	3DS	XX41	Present study
Tsn3b	3DS	XX45	Present study
tsn3c	3DS	XX110	Present study
tsn4	3AS	Salamouni, Red Chief, Arina,	Present study
tsn5	3B	HAR2562, HAR604	Present study
QTsc.ndsu-1A	1A	W-7984/Opata85	Faris *et al*., 1997
Major QTL	3BL	BR34	Faris and Fiesen, 2005
Major QTL	1BS	BR34	Faris and Fiesen, 2005
Major QTL	5BL	Brookton	Cheong *et al*., 2004

Most of the tan spot resistance genes reported to-date (Table 25) are located in the B genome of hexaploid wheat. Faris *et al*. (1996) mapped the resistant locus *tsn1* on the long arm of 5B using restriction fragment length polymorphism (RFLP) markers. The *tsn1* gene was recently fine mapped and markers which are important for cloning of this gene were identified (Haen *et al*., 2004). A major QTL designated as *tsc*2, which is located on the short arm of chromosome 2B, was reported by Friesen and Faris (2004). Cheong *et al*. (2004) have also identified a major QTL on 5BL, which actually is expected to be the same as *tsn1*, in the Australian cultivar Brookton. More recently, Faris and Friesen (2005) have identified QTL on chromosome arms 1BS and 3BL in Brazilian cultivar BR34 using *Ptr* races 1-3 and 5 indicating presence of race- nonspecific tan spot resistance. Singh *et al*. (2006) have identified *tsn2* on the long arm of chromosome 3B using race 3 *Ptr* isolate in tetraploid wheat, which

actually may be the same gene reported as QTL on 3BL by Faris and Friesen (2005). The *tsn5* gene identified in the present study from the Ethiopian hexaploid wheats can be allelic to the major QTL on 3BL from the hexaploid wheat cultivar BR34, and hence they need to be tested for allelism. There are few reports of tan spot resistance in the A genome of wheat. A major QTL on the short arm of chromosome 1A (*QTsc.ndsu-1A*) and a minor QTL on the long arm of chromosome 4A were reported in W-7984/Opata85 population (Faris *et al*., 1997).

On wheat chromosome 3A, in addition to the tan spot resistance *tsn4* gene identified in the present study, other genes such as *Stb6* (Eriksen *et al*., 2003) for septoria resistance, and a QTL (*QFhs.inra-3A*) for fusarium resistance (Gervais *et al*., 2003) have been reported. The wheat cultivars Arina, Salamouni, and Red Chief possess resistance against Septoria and Fusarium. The existence of such a number of different genes for resistance provides the opportunity to pyramid resistance genes from different sources in order to enhance the level and durability of resistance.

The best way to utilize the genes found in the present study is to transfer them to adapted cultivars by backcrossing. However, since the genes are recessive, determination of the prescence or abscence of these genes in a backcross individual requires a phenotypic assay of progeny generated either by selfing or by crossing to the donor parent. Microsatellite markers are most suitable for marker assisted selection (Gupta *et al*., 1999; Huang *et al*., 2000), especially for pyramiding 2 or more resistance genes into a single adaptable cultivar. The SSR marker *Xgwm2a* which is mapped closer to the *tsn3* genes on chromosome 3D and *tsn4* gene on chromosome 3A in the present study, amplifies 120 bp allele in *tsn3* genes and 130 bp in the *tsn4* gene, and can be used to trace the presence of the target gene in successive backross generations and pyramiding of these genes into a commonly grown and adaptable cultivar. The *tsn5* gene from the Ethiopian wheat cultivars needs to be mapped in order to identify a closely linked SSR marker which will assist in marker assisted selection.

6. SUMMARY

Tan spot of wheat, caused by the ascomycete *Pyrenophora tritici-repentis* (Died) Drechs. (anamorph *Dreschslera tritici-repentis*, Died), is one of the major foliar diseases of wheat spreading world-wide at an increasing rate, and can cause yield losses of up to 50 % in susceptible wheat cultivars. Adoption of new farm management practices such as minimum or zero tillage, banning of stubble burning, and intensive wheat after wheat cultivation systems have contributed to the fast spread of the pathogen *Pyrenophora tritici-repentis*.

This study was carried out to evaluate and identify wheat genotypes for tan spot resistance, determine the association between seedling and adult plant resistance, study the inheritance of tan spot resistance, identify the chromosomal location of resistance genes through monosomic analysis and map the resistance genes using molecular markers.

Genetic resistance is the most effective, economical and environment friendly method of managing tan spot. Its successfulness, however, depends on the availability of broad genetic diversity and continuous search of novel resistance genes in order to cope with the rapidly changing pathogen population. In line with this, after developing differential wheat cultivars and *Ptr* isolates, a total of 467 wheat genotypes have been screened for their seedling resistance using the most virulent *Ptr* isolates, and some 75 genotypes with highly resistance response against *Ptr* race 1 isolate were identified.

Comparison of growth chamber and field studies showed a positive correlation (r= 0.864) between seedling resistance and adult plant resistance indicating that large number of wheat germplasm can be screened effectively and rapidly against *P. tritci-repentis* at seedling stage in growth chambers.

Segregation analysis of the phenotypic and molecular data in $F_{2:3}$ populations of CS/XX41, CS/XX45, and CS/XX110 has revealed a 1:2:1 segregation ratio indicating that resistance of tan spot in these synthetic lines is controlled by a single gene. The absence of segregation into resistant plants in the F_1 disomic crosses of the resistant cultivars HAR604, HAR2562, and Dashen with the susceptible cultivar Glenlea, and the segregation of the corresponding F_2 crosses into 1 resistant : 3 susceptible ratio indicated that resistance in these cultivars is controlled by a single recessive gene which inherits qualitatively. The monosomic and

disomic F_1 and F_2 crosses of these resistant cultivars with the susceptible Chinese Spring (CS) monosomic series and disomic CS cultivar showed the same result.

The chromosomal locations of new tan spot resistance genes: *tsn3* in synthetic wheat lines (XX41, XX45 and XX110), *tsn4* in Salamouni, Red Chief and Arina and *tsn5* in HAR2562 and HAR604 have been identified and located on chromosomes 3D, 3A and 3B, respectively through monosomic analyses. Allelism tests detected no segregation for susceptibility among F_1 and F_2 plants derived from intercrosses of the resistance lines XX41, XX45 and XX110 indicating that the genes are either allelic or tightly linked. Similarly, all F_1 and F_2 plants of the three possible R/R crosses (HAR604/HAR2562, HAR604/ Dashen, HAR2562/ Dashen) were resistant to both ASC1a and DW-16 isolates suggesting that the resistance genes in all these three cultivars are allelic or tightly linked. The *tsn5* gene identified in the present study from the Ethiopian hexaploid wheats can be allelic to the major QTL on 3BL from the hexaploid wheat cultivar BR34.

Linkage analysis using SSR markers showed that all the three genes: *tsn3a* in XX41, *Tsn3b* in XX45 and *tsn3c* in XX110 are clustered in the region around *Xgwm2a*, located on the short arm of chromosome 3D. The same *Xgwm2a* marker was found to be the closest marker to *tsn4* on chromosome 3A suggesting homoeology of resistance between the two genes.

In conclusion, the resistant cultivars identified in this study are recommended for use in breeding programmes to improve tan spot resistance in common wheat. Furthermore, the linked markers and genetic relationship of the genes identified will greatly facilitate their use in wheat breeding and deployment of tan spot resistant cultivars. As the currently available SSR markers in the D genome of wheat are limited, it is advisable to carry out fine mapping in the future when more markers are developed on wheat chromosome 3D in order to effectively delimit the genomic region containing the *tsn3* genes for cloning purpose. Further more, the *tsn5* genes from the Ethiopian wheat cultivars need to be mapped in order to identify a closely linked SSR marker which will assist in marker assisted selection.

7. ZUSAMMENFASSUNG

DTR- Weizenblattdürre, die von dem Askomyceten *Pyrenophora tritici-repentis* (Died) Drechs. (anamorph *Drechslera tritici-repentis,* Died) verursacht wird, zählt derzeit zu den gefährlichsten Blattkrankheiten des Saatweizens. Sie ist weltweit anzutreffen und breitet sich zunehmend aus. In anfälligen Weizensorten kann die Krankheit Ertragseinbußen bis zu 50% zur Folge haben. Die Einführung neuer Betriebsmaßnahmen wie minimale oder pfluglose Bodenbearbeitung, das Verbot des Abbrennens von Strohresten und eine intensive Fruchtfolge ‚Weizen nach Weizen' haben erheblich zur raschen Verbreitung des Erregers *Pyrenophora tritici-repentis* beigetragen.

Ziel der vorliegenden Arbeit war die Evaluierung und Identifizierung neuer Genotypen des Saatweizens (*Triticum aestivum* L.) für Resistenz gegenüber DTR-Weizenblattdürre. Darüber hinaus sollten Zusammenhänge zwischen Keimlimgs- und Feldresistenz, Untersuchungen zur Vererbung der Resistenz sowie der chromosomalen Lokalisierung der Resistenzgene mittels monosomer Linien und ihrer Kartierung mit Hilfe molekularer Marker durchgeführt werden.

Genetische Resistenz ist die wirksamste, wirtschaftlichste und umwelt-freundlichste Maßnahme, welche die Weizen-Blattdürre in Schach halten kann. Der Erfolg dieser Maßnahme ist jedoch wesentlich von einer breiten Diversität und einer kontinuierlichen Suche nach neuen Resistenzgenen abhängig, um den Kampf mit der sich rasch ändernden Erregerpopulation aufnehmen zu können. Nach der Erstellung von Weizen-Differenzial-Sortimenten und der Selektion von *Ptr*- Isolaten wurden insgesamt 467 Weizengenotypen auf ihre Keimlingsresistenz gegenüber den meisten virulenten *Ptr*- Isolaten gescreent und etwa 75 Genotypen mit hoher Resistenz gegenüber der *Ptr*- Rasse 1 identifiziert.

Vergleiche von Untersuchungen in der Klimakammer und auf dem Feld zeigten eine positive Korrelation (r = 0.864) zwischen Keimlings- und Erwachsenen- Resistenz, die darauf schließen lässt, dass Weizenzuchtstämme wirksam und schnell im Keimlingsstadium in der Klimakammer gegen *P. tritici-repentis* gescreent werden können.

Spaltungsanalysen der phänotypischen und molekularen Daten in $F_{2:3}$ Populationen der Kreuzungen CS/XX41, CS/XX45 und CS/XX110 ergaben ein 1:2:1 Spaltungsverhältnis, das darauf schließen lässt, dass die Resistenz gegen Weizen-Blattdürre in diesen synthetischen Linien von einem einzelnen Gen kontrolliert wird. Die Tatsache, dass in der F_1 disomer

Kreuzungen der resistenten Sorten HAR604, HAR2562 und Dashen mit der anfälligen Sorte Glenlea keine Spaltung auftritt, und die entsprechenden F_2- Kreuzungen im Verhältnis 1 resistent : 3 anfällig aufspalten, zeigt, dass die Resistenz in diesen Sorten von einem einzelnen rezessiven Gen vererbt wird. Die monosomen und disomen F_1 und bzw. F_2 Kreuzungen dieser resistenten Sorten mit den anfälligen Chinese Spring (CS) monosomen Linien und der disomen Sorte CS lassen auf das gleiche Ergebnis schließen.

Neue Blattdürre- Resistenzgene: *tsn3* in synthetischen Weizenlinien (XX41, XX45 und XX110), *tsn4* in Salamouni, Red Chief und Arina und *tsn5* in HAR 2562 und HAR 604 wurden identifiziert und auf den Chromosomen 3D, 3A bzw. 3B mit Hilfe der Monosomenanalyse lokalisiert. Untersuchungen auf Allelie haben keine Spaltung hinsichtlich Anfälligkeit der F_1- bzw. F_2- Pflanzen aus den Kreuzungen der resistenten Linien XX41, XX45 und XX110 gezeigt, was auf Allelie der Resistenzgene oder enge Kopplung schließen lässt. Ganz ähnlich waren alle F_1 und F_2- Pflanzen der drei möglichen R/R- Kreuzungen (HAR604/HAR2562, HAR604/Dashen, HAR2562/Dashen) gegenüber den Isolaten ASC1a und DW-16 resistent, was darauf schließen lässt, dass die Resistenzgene in allen drei Sorten entweder allel oder eng gekoppelt sind.

Kopplungsuntersuchungen mit Hilfe von SSR- Markern zeigten, dass alle drei Allele: *tsn3* in der Linie XX41, *Tsn3b* in der Linie XX45 und *tsn3c* in der Linie XX110 in einem Kluster in der Region rund um den Marker *Xgmw2a* auf dem Chromosom 3D vorliegen. Der gleiche Marker *Xgwm2a* war am engsten mit *tsn4* auf Chromosom 3A gekoppelt, was auf Homöologie der beiden Resistenzgene schließen lässt.

Die resistenten Sorten, die in der vorliegenden Untersuchung gefunden worden sind, werden zur Verwendung in Zuchtprogrammen zur Verbesserung der Resistenz gegenüber DTR-Weizenblattdürre empfohlen. Darüber hinaus können die gekoppelten Marker und die genetische Verwandtschaft der identifizierten Gene ihre Verwendung in in der Weizenzüchtung erleichtern. Da die zur Zeit zur Verfügung stehenden SSR- Marker auf dem Weizen-D-Genom sehr begrenzt sind, wird vorgeschlagen, ‚fine mapping' in der Zukunft dann durchzuführen, wenn weitere genetische Marker auf dem Weizenchromosom 3D vorhanden sind, um die Genomregion, in der die *tsn3*- Gene liegen, für Klonversuche besser abschätzen zu können. Darüber hinaus müssen die *tsn5*- Allele der äthiopischen Weizensorten kartiert werden, um die eng gekoppelten SSR- Marker zu identifizieren und in der Marker-gestützten Selektion zu nutzen.

8. REFERENCES

Akkaya, M.S., R.C. Shoemaker, J.E. Specht, A.A. Bhagwat, and P.B. Cregan. 1995. Integration of simple sequence repeat DNA markers into a soybean linkage map. Crop Sci. 35:1439-1445.

Ali, S., and L.J. Francl. 2003. Population race structure of *Pyrenophora tritici-repentis* prevalent on wheat and none cereal grasses in the Great plains. Plant Dis. 87:418-412.

Anderson, J.A., Y. Ogihara, M.E. Sorrells, and S.D. Tanksley. 1992. Development of a chromosomal-arm map for wheat based on RFLP markers. Theor. Appl. Genet. 83:1035-1043.

Bailey, K.L. 1996. Diseases under conservation tillage system. Can. J. Plant Sci. 76:635-639.

Bell, C.J., and J.R. Ecker. 1994. Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. Genomics 19:137-144.

Bennett, M.D., and J.B. Smith. 1976. Nuclear DNA amounts in angiosperms. Philos. Trans. R. Soc. Lond. Ser. B. Biol. Sci. 274:227-274.

Black, W. 1970. The nature of inheritance of field resistance to late blight (*Phytophthora infestans*) in potatoes. American Potato Journal 47:279-285.

Bockus, W.W., and M.M. Claasen. 1992. Effects of crop rotation and residue management practices on severity of tan spot of winter wheat. Plant Dis. 76:633-636.

Bostein, D., R.L. White, M. Skolnick, and R.W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32:314-331.

Castro, A.J., X. Chen, P.M. Hayes, and M. Johnston. 2003. Pyramiding Quantitative Trait Locus (QTL) alleles determining resistance to barley stripe rust: effects on resistance at the seedling stage. Crop Sci. 43:651-659.

Chalmers, K.J., R. Waugh, J.I. Sprent, A.J. Simons, and W. Powll. 1992. Detection of genetic variation between and within populations of *Gliricidia sepium* and *G. maculata* using RAPD markers. Heredity 69:465-472.

Chao, S.P., P.J. Sharp, A.J. Worland, E.J. Warham, R.M.D. Koebner, and M.D. Gale. 1989. RFLP-based genetic maps of wheat homoeologus group 7 chromosomes. Theor. Appl. Genet. 78:493-504.

Chen, X.M., Y.H. Luo, X.C. Xia, X. Chen, Z.L. Ren, Z.H. He, and J.Z. Jia. 2005. Chromosomal location of powdery mildew resistance gene *Pm16* in wheat using SSR marker analysis. Plant breed. 124:225-228.

Cheong, J., H. Wallwork, and K.J. Williams. 2004. Identification of a major QTL for yellow

leaf spot resistance in the wheat varieties Brookton and Cranbrook. Aust. J. Agric. Res. 55:315-319.

Cox, D.J., and R.M. Hosford Jr. 1987. Resistant winter wheats compared at differing growth stages and leaf positions for tan spot severity. Plant Dis. 71:883-886.

Daud, H.M., and J.P. Gustafson. 1996. Molecular evidence of *Triticum speltoides* as a B-genome progenitor of wheat (*Triticum aestivum*). Genome 39:543-548.

Devos, K.M., and M.D. Gale. 1992. The use of random amplified polymorphic DNA markers in wheat. Theor. Appl. Genet. 84:567-572

Devos, K.M., M.D. Atkinson, C.N. Chinoy, and M.D. Gale. 1992. RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye. Theor. Appl. Genet. 83:931-939.

Devos, K.M., and M.D. Gale. 1993. Extended genetic maps of the homoeologous group 3 chromosomes of wheat, rye and barley. Theor. Appl. Genet. 85:649-652.

Devos, K.M., T. Millan, and D.M. Gale. 1993. Comparative RFLP maps of the homoeologous group-2 chromosomes of wheat, rye and barley. Theor. Appl. Genet. 85:784-792.

Devos, K.M., J. Dubcovsky, J. Dvorak, C.N. Chinoy, and M.D. Gale. 1995. Structural evolution of wheat chromosomes 4A, 5A, and 7B and its impact on recombination. Theor. Appl. Genet. 91:282-288.

De Wolfe, E.D., R.J. Effertz, S. Ali, and L.J. Francl. 1998. Vistas of tan spot research. Can. J. Plant Pathol. 20:349-370.

Dong, C., R. Whitford, and P. Langridge. 2002. A DNA mismatch repair gene links to the *Ph2* locus in wheat. Genome 45:116-124.

Doyle, J.A., and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemistry Bulletin 19:11-15.

Dushnicky, L.G., G.M. Ballance, M.J. Sumner, and A.W. MacGregor. 1996. Penetration and infection of susceptible and resistant wheat cultivars by a necrosis toxin producing isolate of *Pyrenophora tritici-repentis*. Can. J. Plant Pathol. 18:392-402.

Duveiller, E., Y.R. Kandel, R.C. Sharma, and S.M. Shrestha. 2005. Epidemiology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas. Phytopathology 95:248-256.

Dvorak, J., and H.B. Zhang. 1990. Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. Proc. Natl. Acad. Sci. 87:9640-9644.

Dvorak, J., P. di Terlizzi, H.B. Zhang, and P. Resta. 1993. The evolution of polyploid wheats:

identification of the A genome donor species. Genome 36:21-31.

Dvorak, J., M.C. Luo, Z.L. Yang, and H.B. Zhang. 1998. The structure of the *Aegilops tauschii* gene pool and the evolution of hexaploid wheat. Theor. Appl. Genet. 97:657-670.

Effertz, R.J., S.W. Meinhardt, J.A. Anderson, J.G. Jordal, and L.J. Francl. 2002. Identification of a chlorosis inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitive locus in wheat. Phytopathology 92:527-533.

Elias, E., R.G. Cantrell, and R.M. Hosford Jr. 1989. Heritability of resistance to tan spot in durum wheat and its associations with other agronomic traits. Crop Sci. 29:299-304.

Eriksen, L., F. Borum, and A. Jahoor. 2003. Inheritance and localisation of resistance to *Mycosphaerella graminicola* causing *Septoria tritici* blotch and plant height in the wheat (*Triticum aestivum* L.) genome with DNA markers. Theor. Appl. Genet. 107:515-527.

FAO. 2006. FAOSTAT Agriculture Data. Agricultural production 2005. (http://faostat.fao.org).

Faris, J.D., J.A. Anderson, L.J. Francl, and J.G. Jordahl. 1996. Chromosomal location of a gene conditioning insensitivity in wheat to a necrotic inducing culture filtrate from *Pyrenophora tritici-repentis*. Phytopathology 86:459-463.

Faris, J.D., J.A. Anderson, L.J. Francl, and J.G. Jordahl. 1997. RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici-repentis* in wheat. Theor. Appl. Genet. 94:98-103.

Faris, J.D., and T.L. Friesen. 2005. Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. Theor. Appl. Genet. 111:386-392.

Feldmann, M., F.G.H. Lupton., and T.E. Miller. 1995. Wheats. P. 3-56. *In* Smart J., N.W. Simonds (eds) Evolution of crop plants, Longman Group Ltd., London.

Feldmann, M. 2001. Origin of cultivated wheat. P. 3-56. *In* A.P. Bonjean, and W.J. Angus (eds) The world wheat book. A history of wheat breeding, Lavoiser Publishing, France.

Feldman, M., and A.A. Levy. 2005. Allopolyploidy - a shaping force in the evolution of wheat genomes. Cytogenetic and Genome Research 109:250-258.

Fernandez, M.R., B.G. McConkey, and R.P. Zentner. 1998. Tillage and summer fallow effects on leaf spot diseases of wheat in the semi-arid Canadian Prairies. Can. J. Plant Pathol. 20:376-379.

Flor, H.H. 1955. Host-parasite interaction in flux rust – its genetics and other implications.

Phytopathology 45:680-685.

Friebe, B., M. Heun, N. Tuleen, F.J. Zeller, and B.S. Gill. 1994. Cytogenetically monitored transfer of powdery mildew resistance from rye into wheat. Crop Sci. 34:621-625.

Friesen, T.L., J.B. Rasmussen, C.Y. Kwon, S. Ali, L.J. Francl, and S.W. Meinhardt. 2002. Reaction of *Ptr* ToxA-insensitive wheat mutants to *Pyrenophora tritici-repentis* race 1. Phytopathology 92:38-42.

Friesen, T.L., and J.D. Faris. 2004. Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to *Ptr* ToxB in wheat. Theor. Appl. Genet. 109:464-471

Gale, M.D., M.D. Atkinson, C.N. Chinoy, R.L. Harcourt, J. Jia, Q.Y. Li, and K.M. Devos. 1995. Genetic maps of hexaploid wheat. P. 29-40. *In* S. Chen (ed.) Proceedings of the 8th international wheat genetics symposium, China Agricultural Scientech Press, Beijing, China.

Gamba, F.M., and L. Lamari. 1998. Mendelian inheritance of tan spot (*Pyrenophora tritici-repentis*) in selected genotypes of durum wheat (*Triticum durum*). Can. J. Plant Pathol. 20:408-414.

Gebremariam, H. 1991. Use of germplasm resources in breeding wheat for disease resistance. P. 298-302. *In* J.M.M. Engles, J.G. Hawkes, and M Worede (eds) Plant genetic resources of Ethiopia, Cambridge University Press, Cambridge.

Gervais, L., F. Dedryver, J.Y. Morlais,V. Bodusseau, S. Negre, M. Bilous, C. Groos, and M. Trottet. 2003. Mapping of quantitative trait loci for field resistance to Fusarium head blight in an European winter wheat. Theor. Appl. Genet. 106:961-970.

Gill, B.S., and B. Friebe. 2001. Cytogenetics, phylogeny and evolution of cultivated wheats. P. 71-88. *In* A.P. Bonjean, and W.J. Angus (eds) The world wheat book. A history of wheat breeding, Lavoiser Publishing, France.

Gill, K.S., E.L. Luberts, B.S. Gill, W.J. Raupp, and T.S. Cox. 1991. A genetic linkage map of *Triticum tauchii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome 34:362-374.

Gill, K.S., B.S. Gill, T.R. Endo, and Y. Mukai. 1993. Fine physical mapping of *Ph1*, a chromosome pairing regulator gene in polyploid wheat. Genetics 134:1231-1236.

Gupta, P.K., R.K.Varshney, P.C. Sharma, and B. Ramesh. 1999. Molecular markers and their application in wheat breeding. Plant Breed. 118:369-390.

Gupta, P.K., P.L. Kulwal, and S. Rustig. 2005. Wheat cytogenetics in the genomics era and its relevance to breeding. Cytogenetic and Genome Research 109:315-327.

Haen, K.M., H.J. Lu, T.L. Friesen, and J.D. Faris. 2004. Genomic targeting and high-

resolution mappingof the *tsn1* gene in wheat. Crop Sci. 44:951-962

Hammer, K., A.A. Filatenko, and V. Korzun. 2000. Microsatellite markers: a new tool for distinguishing diploid wheat species. Genet. Res. Crop Evol. 47:497-505.

Hartl, L., H. Weiss, F.J. Zeller, and A. Jahoor. 1993. Use of RFLP markers for the identification of alleles of the *Pm3* locus conferring powderymildew resistance in wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 86:959-963.

Hartl, L., V. Mohler, F.J. Zeller, S.L.K. Hsam, and G. Schweizer. 1999. Identification of AFLP markers closely linked to the powdery mildew resistance genes *Pm1c* and *Pm4a* in common wheat (*Triticum aestivum* L). Genome 42:322-329.

Heath, C.M. 1994. Current concepts of the determinants of plant-fungal specificity. P. 3-21. *In* K. Kohmoto, and O.C. Yoder (eds) Host-specific toxin biosynthesis, receptor and molecular biology, Tottori University, Tottory, Japan.

Helentjaris, T., G. King, M. Slocum, C. Sidenstrang, and S. Wegman. 1985. Restriction fragment length polymorphism as probes for plant diversity and their development as tools for applied plant breeding. Plant Mol. Biol. 5:109-118.

Helentjaris, T., M. Slocum, S. Wright, A. Shaefer, and J. Neinhuis. 1986. Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor. Appl. Genet. 72:761-769.

Heun, M., R. Schafer-Pregl, D. Klawan, R. Castagna, M. Accerbi, B. Borghi, and F. Salamini. 1997. Site of Einkorn wheat domestication identified by DNA fingerprinting. Science 278:1312-1314.

Hosford, R.M. Jr. 1971. A form of *Pyrenophora trichostoma* pathogenic to wheat and other grasses. Phytopathology 61:28-32.

Hosford, R.M. Jr., and R.H. Bush. 1974. Losses in wheat caused by *Pyrenophora trichostoma* and *Leptosphaeria avenaria* f. sp. *triticea*. Phytopathology 64:184-187.

Hosford, R.M. Jr. 1982. Tan spot. P. 1-24. *In* R.M. Hosford Jr. (ed.) Tan Spot of Wheat and Related Diseases, North Dakota State University, Fargo, ND.

Hsam, S.L.K., X.Q. Huang, F. Earnst, L. Hartl, and F.J. Zeller. 1998. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.). 5. Alleles at the *Pm1* locus. Theor. Appl. Genet. 96:1129-1134.

Hsam, S.L.K., X.Q. Huang, and F.J. Zeller. 2001. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.). 6. Alleles at the *Pm5* locus. Theor. Appl. Gent. 101:127-133.

Huang, X.Q., S.L.K. Hsam, F.J. Zeller, G. Wenzel, and V. Mohler. 2000. Molecular mapping

of the wheat powdery mildew resistance gene *Pm24* and marker validation for molecular breeding. Theor. Appl. Genet. 101:407- 414.

Huang X.Q., S.L.K. Hsam, V. Mohler, M.S. Röder, and F.J. Zeller. 2004. Genetic mapping of three alleles at the *Pm3* locus conferring powdery mildew resistance in common wheat (*Triticum aestivum* L.). Genome 47:1130-1136.

Hussien, S., J.J. Spies, Z.A. Pretorious, and M.T. Labuschagne. 2005. Chromosome location of leaf rust resistance genes in selected tetraploid wheats through substitution lines. Euphytica 141:209-216.

Jia, J., K.M. Devos. S. Chao, T.E. Miller, S.M. Röder, and M.D. Gale. 1996. RFLP-based maps of the homoeologus group-6 chromosomes of wheat and their application in the tagging of *Pm12,* a powdery mildew resistance gene transferred from *Aegilopes speltoides* to wheat. Theor. Appl. Genet. 92:559-565.

Jiang, J., and B.S. Gill. 1994. Different species-specific chromosome translocations in *Triticum timopheevii* and *T. turgidum* diphyletic origin of polyploid wheats. Chrom. Res. 2:59-64.

Johnson, B.L. 1975. Identification of the apparent B genome donor of wheat. Can. J. Genet. Cytol. 17:21-39.

Johnson, B.L., and H.S. Dhaliwal. 1976. Reproductive isolation of *Triticum boeoticum* and *Triticum urartu* and the origin of the tetraploid wheat. Am. J. Bot. 63:1088-1094.

Kam-Morgan, L.N.W., B.S. Gill, and S. Muthukrishnan. 1989. DNA restriction fragment length polymorphisms: a strategy for genetic mapping of D genome of wheat. Genome 32:724-732

Kihara, H. 1919. Über cytologische Studien bei einigen Getreidearten. 1. Spezies-Bastarde des Weizens und Weizenroggen-Bastard. Bot. Mag. 32:17-38.

Kihara, H. 1924. Cytologische und genetische Studien bei wichtigen Getreidearten mit besonderer Rücksicht auf das Verhalten der Chromosomen und die Sterilität in den Bastarden. Mem. Cell Sci. Kyoto Imp. Univ., Ser. B1:1-200.

Knott, D.R. 1989. The wheat rusts- breeding for resistance. Springer-Verlag, Berlin.

Koebner, R.M.D., and K.W. Shepherd. 1986. Controlled introgression to wheat of genes from rye chromosome arm 1RS by induction of allosyndesis. Theor. Appl. Genet. 73:197-208.

Kohli, M., Y. Mehta, and M. Ackerman. 1992. Spread of tan spot in southern cone region of south America. P. 86-90. *In* L. Francel, J. Krupinsky, and M. McMullen (eds) Advances in tan spot. Proceedings of the second international tan spot work shop,

North Dakota State University, Fargo, ND.

Kojima, T., T. Nagaoka, K. Noda, and Y. Ogihara. 1998. Genetic linkage map of ISSR and RAPD markers in einkorn wheat in relation to that of RFLP markers. Theor. Appl. Genet. 96:37-45.

Kosambi, D.D. 1944. The estimation of map distances from recombination values. Ann. Eugen. 12:172-175.

Krupinsky, J.M. 1982. Observations of the host ranges of isolates of *Pyrenophora trichostoma*. Can. J. Plant Pathol. 4:42-46.

Krupinsky, J.M. 1992. Grass hosts of *Pyrenophora tritici-repentis*. Plant Dis. 76:92-95.

Lagudah, E.S., R. Appels, A.H.D. Brown, and D. McNeil. 1991. The molecular-genetic linkage analysis of *Triticum tauschii*, the D-genome donor to hexaploid wheat. Genome 34:375-386.

Lamari, L., and C.C. Bernier. 1989a. Evaluation of wheat lines and cultivars to tan spot (*Pyrenophora tritici-repentis*) based on lesion type. Can. J. Plant Pathol. 11:49-56.

Lamari, L., and C.C. Bernier. 1989b. Toxin of *Pyrenophora tritici-repentis*: Host specificity, significance in disease and inheritance of host reaction. Phytopathology 79:740-744.

Lamari, L., and C.C. Bernier. 1991. Genetics of tan necrosis and extensive chlorosis in tan spot of wheat caused by *Pyrenophora tritici-repentis*. Phytopathology 81:1092-1095.

Lamari, L., R. Sayoud, M. Boulif, and C.C. Bernier. 1995. Identification of a new race in *Pyrenophora tritici-repentis*: implication for current pathotype classification system. Can. J. Plant Pathol. 17:312-318

Lamari, L., S.E. Strelkov, A. Yahyaoui, J. Orabi, and R.B. Smith. 2003. The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one-to-one relationship in tan spot of wheat: Phytopathology 93:391-396.

Lamari, L., B.D. McCallum, and R.M. dePauw. 2005. Forensic pathology of Canadian bread wheat: The case of tan spot. Phytopathology 95:144-152.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.

Landry, B.S., R.Q. Li, W.Y. Cheung, and R.L. Graner. 1994. Phylogeny analysis of 25 apple root stocks using RAPD markers and tactical gene tagging. Theor. Appl. Genet. 89:847-852.

Larez, C.R., R.M. Hosford Jr., and T.P. Freeman. 1986. Infection of wheat and oats by

Pyrenophora tritici-repentis and initial characterization of resistance. Phytopathology 76:931-938.

Lee, T.S., and F.J. Gough. 1984. Inheritance of Septoria leaf blotch (*S. tritici*) and *Pyrenophora* (*P. tritici-repentis*) resistance in *Triticum aestivum* cv. Carifen 12. Plant Dis. 68:848-851.

Lindhout, P. 2002. The perspectives of polygenic resistance in breeding for durable disease resistance. Euphytica 124:217-226.

Litt, M., and J.A. Luty. 1989. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac-muscle actin gene. Am. J. Hum. Genet. 44:397-401.

Liu, Y.G., and K. Tsunewaki. 1991. Restriction fragment length polymorphism analysis of wheat. II. Linkage maps of the RFLP sites in common wheat. Jpn. J. Genet. 66:617-633.

Liu, X.M., C.M. Smith, B.R. Friebe, and B.S. Gill. 2005. Molecular mapping and allelic relationships of Russian wheat aphid- resistance genes. Crop Sci. 45:2273-228.

Lutz, J., S.L.K. Hsam, E. Limpert, and F.J. Zeller. 1995. Chromosomal location of powdery mildew resistance genes in *Triticum aestivum* L. (common wheat). 2. Genes *Pm2* and *Pm19* from *Aegilops squarrosa* L. Heredity 74:152-156.

Luz, da W.C., and R.M. Hosford Jr. 1980. Twelve *Pyrenophora trichostoma* races for virulence to wheat in the Central Plains of North America. Phytopathology 70:1193-1196.

Ma, Z.Q., and N.L.V. Lapitan. 1998. A comparison of amplified and restriction fragment length polymorphism in wheat. Cereal Res. Commun. 26:7-13.

Mackill, D.J., Z. Zhang, E.D. Redona, and P.M. Colowit. 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. Genome 39:969-977.

Marais, G.F., B. McCallum, J.E. Snyman, Z.A. Pretorius, and A.S. Marais. 2005. Leaf rust and stripe rust resistance genes *Lr54* and *Yr37* transferred to wheat from *Aegilops kotschyi*. Plant breed. 124:538-541.

Marino, C.L., J.C. Nelson, Y.H. Lu, M.E. Sorrells, P. Leroy, N.A. Tuleen, C.R. Lopes, and G.E. Hart. 1996. Molecular genetic maps of the group 6 chromosomes of hexaploid wheat (*Triticum aestivum* L.Em. Thell.). Genome 39:359-366.

Matsuoka, Y., and S. Nasuda. 2004. Durum wheat as a candidate for the unknown female progenitor of bread wheat: an empirical study with a highly fertile F_1 hybrid with *Aegilops tauschii* Coss. Theor. Appl. Genet. 109:1710-1717.

McFadden, E.S., and E.R. Sears. 1946. The origin of *Triticum spelta* and its free-threshing hexaploid relatives. Heredity 37:81-89.

McIntosh, R.A. 1987. Gene location and gene mapping in hexaploid wheat. P. 269-287. *In* Hayne, E.G. (ed) Wheat and wheat improvement. Am Soc Agron, Madison.

McIntosh, R.A., B. Friebe, J. Jiang, D. The, and B.S. Gill. 1995. Chromosome location of a new gene for resistance to leaf rust in Japanese wheat-rye translocation line. Euphytica 82:141-147.

McIntosh, R.A., K.M. Devos, J. Dubcovsky, and W.J. Roggers. 2002. Cataloge of gene Symbols for wheat. http://wheat.pw.usda.gov/ggpages/wgc/2002upd.html.

McMullen, M. 2003. Tan spot and Septoria/Stagonospora diseases of wheat. www.ag.ndsu.nodak.edu

Mello-Sampayo, T. 1971. Genetic regulation of meiotic chromosome pairing by chromosome 3D of *Triticum aestivum*. Nature New Biol. 230:22-23.

Mentewab, A., H.N. Rezanoor, N. Gosman, A.J. Worland, and P. Nicholson. 2000. Chromosomal location of Fusarium head blight resistance genes and analysis of the relationship between resistance to head blight and brown foot rot. Plant Breed. 119:15-20.

Miller, T.E. 1987. Systematics and evolution. P. 1-30. *In* F.G.H. Lupton (ed) Wheat breeding its scientific bases, Chapman and Hall, London.

Miller, A.C., A. Altinkut, and N.L.V. Lapitan. 2001. A microsatellite marker for tagging *Dn2*, a wheat gene conferring resistance to the Russian wheat aphid. Crop Sci. 41:1584-1589

Mielke, H., and A. Reichelt. 1999. Studien zur Biologie des Erregers *Drechslera tritici-repentis* zur Anfälligkeit des Weizens und verschiedener Artverwandter sowie zur Bekämpfung der DTR-Weizenblattdürre, Parey Verlag, Berlin.

Mohler, V., F.J. Zeller, G. Wenzel, and S.L.K. Hsam. 2005. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L.em Thell). 9. Gene *MIZec1* from *Triticum dicoccoides*-derived wheat line Zecoi-1. Euphytica 142:161-167

Mujeeb-Kazi, A., and R.L. Villareal. 2002. Wheat. P. 43-96. *In* V.L. Chopra, and S. Prakash (eds) Evolution and adaptation of cereal crops. Science Publishers, Inc. Enfield USA.

Nagle, B.J., R.C. Frohberg, and R.M. Hosford Jr. 1982. Inheritance of resistance to tan spot of wheat. P. 40-45. *In* R.M. Hosford Jr. (ed.) Tan spot of wheat and related diseases, North Dakota State University, Fargo, ND.

Naranjo, T. 1990. Chromosome structure of durum wheat. Theor. Appl. Genet. 79:397-400.

Negasa, M. 1986. Estimates of phenotypic diversity and breeding potential of Ethiopian wheats. Hereditas 104:41-48.

Nesbit, K.A, B.M. Potts, R.E. Vaillancourt, A.K.West, and J.B. Reid. 1995. Partitioning and distribution of RAPD variation in a forest tree species, *Eucalyptus globulus* (Myrtaceae). Heredity 74:628-637.

Nybom, H. 1994. DNA fingerprinting – a useful tool in fruit breeding. Euphytica 77:59-64.

Ohtsuka, I. 1998. Origin of the central European spelt wheat. P. 303-305. *In* A.E. Slinkard (ed.) Proceedings of the ninth international wheat genetics symposium, University Extension Press, University of Saskatchewan, Canada.

Orololaza, N.P., L. Lamari, and G.M. Ballance. 1995. Evidence of a host specific chlorosis toxin from *Pyrenophora tritici-repentis*, the causal agent of tan spot of wheat. Phytopathology 85:1282-1287.

Parlevliet, J.E. 1989. Identification and evaluation of quantitative resistance. P. 215-248. *In* K.J. Leonard, and W.E. Fry (eds) Plant disease epidemiology, Vol. 2. McGraw-Hill, New York.

Perello, A., V. Moreno, M.R. Simon, and M. Sisterna. 2003. Tan spot of wheat infection at different stages of crop development and inoculum type. Crop Protection 22:157-169.

Petersen, G., O. Seberg, M.Yde, and K. Berthelsen. 2006. Phylogenetic relationships of *Triticum* and *Aegilops* and evidence for the origin of A, B, and D genomes of common wheat (*Triticum aestivum*). Molecular phylogenetics and Evolution 39:70-82.

Peusha, H., S.L.K. Hsam, and F.J. Zeller. 1996. Chromosomal location of powdery mildew resistance genes in common wheat (*Triticum aestivum* L. em. Thell.) 3. Gene *Pm22* in cultivar Virest. Euphytica 91:149-152.

Plaschke, J., M.W. Ganal, and M.S. Röder. 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor. Appl. Genet. 91:1001-1007.

Ragiba, M., K.V. Prabhu, and R.B. Singh. 2004. Monosomic analysis of Helminthosporium leaf blight resistance genes in wheat. Plant Breed. 123:405-409.

Raymond, P.J., W.W. Bockus, and B.L. Norman. 1985. Tan spot of winter wheat: procedures to determine host response. Phytopathology 75:686-690.

Rees, R.G., and G.J. Platz. 1979. The occurrence and control of yellow spot of wheat in north-eastern Australia. Aust. J. Exp. Agric. Anim. Husb. 19:369-372.

Rees, R.G. 1982. Yellow spot, an important problem in the north eastern wheat areas of Australia. P. 68-70. *In* R.M. Hosford Jr. (ed.) Tan spot of wheat and related

diseases, North Dakota State University. Fargo, ND.

Rees, R.G., G.J. Platz, and R.J. Mayer. 1988. Susceptibility of Australian wheats to *Pyrenophora tritici-repentis*. Aus. J. Agric. Res. 39:141-151.

Riede, C.R., L.J. Francl, J.A. Anderson, J.G. Jordahl, and S.W. Meinhardt. 1996. Additional sources of resistance to tan spot of wheat. Crop Sci. 36:771-777.

Reiter, R.S., J.G.K. Williams, K.A. Feldmann, J.A. Rafalski, S.V. Tingey, and P.A. Scolnik. 1992. Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc. Natl. Acd. Sci. USA. 89:1477-1481.

Riley, R. and V. Chapman. 1958. Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature 182:713-715.

Riley, R., J. Unrau, and V. Chapman. 1958. Evidence on the origin of the B genome of wheat. Heredity 49:91-98.

Röder M.S., J. Plaschke, S.U. König, A. Borner, M.E. Sorrells, S.D. Tanksley, and M.W. Ganal. 1995. Abundance, variability and chromosomal location of microsatellites in wheat. Mol. Gen. Genet. 246:327-333.

Röder M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy, and M.W. Ganal. 1998. A microsatilite map of wheat. Genetics 149:2007-2023.

Rubiales, D., S.M. Reader, and A. Martin. 2000. Chromosomal location of resistance to *Septoria tritici* in *Hordeum chilense* determined by the study of chromosomal addition and substitution lines in Chinese Spring wheat. Euphytica 115:221-224.

Saghai-Maroof, M.A., K.M. Soliman, R.A. Jorgenson, and R.W. Allard. 1984. Ribosomal spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA. 81:8014-8018.

Saghai Maroof, M.A., R.B. Biyashev, G.P.Yang , Q. Zhang, and R.W. Allard. 1994. Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. Proc Natl Acad Sci USA 91:5466-5470.

Sakamura, T. 1918. Kurze Mitteilung über die Chromosomenzahlen und die Verwandtschaftsverhältnisse der *Triticum*-Arten. Bot. Mag. Tokyo 32:151-154.

Sarkar, P., and G.L. Stebbins. 1956. Morphological evidence concerning the origin of the B genome in wheat. Am. J. Bot. 43:297-304.

SAS Institute. 2004. The SAS System for Windows. Release 9.1. SAS Institute Inc., Cary, NC, USA.

Sax, K. 1922. Sterility in wheat hybrids. II. Chromosome behaviour in partially sterile hybrids. Genetics 7:513-550.

Schmolke, M., G. Zimmermann, H. Buerstmayr, G. Schweizer, T. Miedaner, V. Korzun, E. Ebmeyer, and L. Hartl. 2005. Molecular mapping of fusarium head blight resistance in the winter wheat population Dream/Lynx. Theor. Appl. Genet. 111:747-756

Schultz, A. 1913. Die Geschichte der kultivierten Getreide. Nebert, Halle.

Sears, E.R. 1953. Nullisomic analysis in common wheat. Am. Nat. 87:245-252.

Sears, E.R. 1954. The aneuploids of common wheat. Mo. Agric. Exp. Sta. Res. Bull. 572:1-58.

Sears, E.R. 1966. Nullisomic-tetrasomic combinations in hexaploid wheat. P. 29-45. *In* R. Riley, and K.R. Lewis, (eds) Chromosome manipulations and plant genetics, London, Oliver and Boyd.

Sears, E.R. 1976. Genetic control of chromosome pairing in wheat. Ann. Rev. Genet. 10:31-51.

Sears, E.R. 1977. Induced mutant with homoeologous pairing in common wheat. Can. J. Genet. Cytol. 19:585-593.

Senior, M.L., and M. Heun. 1993. Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. Genome 36:884-889.

Siedler, H. 1991. Untersuchungen zur Evaluierung von Resistanz gegenüber *Pyrenophora tritici-repentis* in *Aegilops squarrosa* und synthetischen Amphiploiden (*Triticum turgidum* ssp. x *Aegilops squarrosa*. MSc thesis, Technical University of Munich, Freising-Weihenstephan.

Siedler, H., A. Obst, S.L.K. Hsam, and F.J. Zeller. 1994. Evaluation for resistance to *Pyrenophora tritici-repentis* in *Aegilops tauchii* Coss. and synthetic hexaploid wheat amphiploids. Genet. Res. Crop. Evol. 41:27-34.

Simon, M.R., A.J. Worland, C.A. Cordo, and P.C. Struik. 2001. Chromosome location to *Septoria tritici* in seedlings of a synthetic hexaploid wheat, *Triticum spelta* and two cultivars of *Triticum aestivum*. 2001. Euphytica 119:149-153.

Singh, S., C.D. Franks, L. Huang, G.L. Brown-Guedira, D.S. Marshal, and B.S. Gill. 2004. *Lr41*, *Lr39* and a leaf rust resistance gene from *Aegilops cylinderica* may be allelic and are located on wheat chromosome 2DS. Theor. Appl. Genet. 108:586-591

Singh, P.K., and G.R. Hughes. 2005. Genetic control of resistance to tan necrosis induced by *Pyrenophora tritici-repentis*, race 1 and race 2, in spring and winter wheat genotypes. Phytopathology 95:172-177

Singh, P.K., J.L. Gonzalez-Hernandez, M. Mergoum, S. Ali, T.B. Adhikari, S.F. Kianian, E.M. Elias, and G.R. Hughes. 2006. Identification and molecular mapping of a gene conferring resistance to *Pyrenophora tritici-repentis* race 3 in tetraploid wheat. Phytopathology 96:885-889

Singrün, C., S.L.K. Hsam, L. Hartl, F.J. Zeller and V. Mohler. 2003. Powdery mildew resistance gene *Pm22* in cultivar Virest is a member of the complex *Pm1* locus in common wheat (*Triticum aestivum* L. em Thell.). Theor. Appl. Genet. 106:1420-1424.

Singrün, C., S.L.K. Hsam, L. Hartl, F.J. Zeller and V. Mohler. 2004. Localization of a novel recessive powdery mildew resistance gene from common wheat line RD30 in the terminal region of chromosome 7AL. Theor. Appl. Genet. 109:210-214.

Snedecor, G.W., and W.G. Cochran. 1989. Statistical Methods, 8th Ed. Iowa State University Press. Ames.

Somers, D.J., P. Isaac, and K. Edwards. 2004. A high-density microsatelite consensus map for bread wheat (*Triticum aestivum* L.).Theor. Appl. Genet. 109:1105-1114.

Stephenson, P., G. Bryan, J. Kirby, A. Collins, K. Devos, C. Busso, and M. Gale. 1998. Fifty new microsatellite loci for the wheat genetic map. Theor. Appl. Genet. 97:946-949.

Stock, W.S. 1996. Chromosomal location and RAPD marker development for tan spot resistance in hexaploid wheat (*Triticum aestivum*). M.Sc. Thesis, University of Manitoba, Winnipeg, Canada.

Stock, W.S., A.L. Brule-Bable, and G.A. Penner. 1996. A gene for resistance to a necrosis-inducing isolate of *Pyrenophora tritici-repentis* located on 5BL of *Triticum aestivum* cv. Chinese Spring. Genome 39:598-604.

Stoskopf, N.C. 1985. Cereal grain crops. Reston Publishing Company, Inc., Reston, Virginia.

Stzrelkov, S.E., and L. Lamari. 2003. Host-parasite interaction in tan spot (*Pyrenophora tritici-repentis*) of wheat. Can. J. Plant Pathol. 25:339-349.

Sutton, J.C., and T.J. Vyn. 1990. Crop sequences and tillage practices in relation to diseases of winter wheat in Ontario. Can. J. Plant Pathol. 12:358-368.

Tanksley, S.D., N.D. Young, A.H. Patterson, and M.W. Bonierbale. 1989. RFLP mapping in plant breeding – new tools for an old science. Bio Technology 7:257-264.

Tekauz, A., E. Mueller, M. Beyene, M. Stulzer, and D. Schultz. 2004. Leaf spot diseases of winter wheat in Manitoba in 2003. Can. Plant Dis. Survey 83:73-74.

Tesema, T. 1991. Improvement of indigenous durum wheat land races in Ethiopia. P. 288-295. *In* J.M.M. Engles, J.G. Hawkes, and M. Worede (eds) Plant genetic resources of Ethiopia, Cambridge University Press, Cambridge.

The, T.T., R.A. McIntosh, and F.G.A. Bennett. 1979. Cytological studies in wheat. IX.

Monosomic analysis, telocentric mapping and linkage relationship of gene *Sr21*, *Pm4* and *Mle*. Aust. J. Biol. Sci. 32:115-125.

Upadhya, M.D., and M.S. Swaminathan. 1963. Genome analysis in *Triticum zhukovskyi*, a new hexaploid wheat. Chromosoma 14:589-600.

Van der Planck, J.E. 1963. Plant disease epidemics and control. Academic Press, New York.

Van der Planck, J.E. 1968. Disease resistance in plants. Academic Press, New York.

Vavilov, N.I. 1951. The origin, variation, immunity and breeding of cultivated plants. Chronica botanica 13:1-366.

Vega, J.M., and M. Feldman. 1998. Effect of the pairing gene *Ph1* on centromere misdivision in common wheat. Genetics 148:1285-1294.

Voorrips, R.E. 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. Heredity 93:77-78.

Vos, P., R. Hogers, M. Reijans, T. van de Lee, M. Hornes, A. Friters, J. Pot, J. Peleman, M. Kupier, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucl. Acids Res. 23:4407-4414.

Wang, G.Z., N.T. Miyashita, and K. Tsunewaki. 1997. Plasmon analysis of *Triticum* (wheat) and *Aegilops*: PCR-single-strand conformational polymorphism (PCR-SSCP) analyses of organellar DNAs. Proc. Natl. Acad. Sci. 94:14570-14577.

Wang, T., S.S., Xu, M.O. Harris, J. Hu, L. Liu, and X. Cai. 2006. Genetic characterization and molecular mapping of Hessian fly resistance genes derived from *Aegilops tauschii* in synthetic wheat. Theor. Appl. Genet. 113:611-618

Ward, R. 2003. Genetic and physical maps of wheat loci. (http://www.scabusa.org).

Watkins, J.E., M.G. Boosalis, and B.L. Doupnik Jr. 1982. Foliar diseases of Nebraska´s winter wheat. P. 53-61. *In* R.M. Hosford Jr. (ed) Tan spot of wheat and related diseases. North Dakota State University, Fargo. ND.

Weber, D., and T. Helentjaris. 1989. Mapping RFLP loci in maize using B-A translocations. Genetics 121:583-590.

Wiese, M. 1987. Compendium of wheat diseases. American Phytopathology Society, St. Paul, MN.

Williams, J.K.A., A.R.K. Kubelik, J.L. Livak, J.L. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by random primers are useful as genetic markers. Nucl. Acids Res. 18:6531-6535.

Wolf, P.F.J., and G.M. Hoffmann. 1993. Zur Biologie von *Drechslera tritici-repentis* (Died.)

Shoem. (telomorph *Pyrenophora tritici-repentis* (Died.) Drechsler), dem Erreger einer Blattfleckenkrankheit an Weizen. Pfl. Krankh. Pflschutz 100:33-48.

Worede, M. 1991. An Ethiopian perspective on conservation and utilization of plant genetic resources. P. 3-19. *In* J.M.M. Engles, J.G. Hawkes, and M. Worede (eds) Plant genetic resources of Ethiopia. Cambridge University Press, Cambridge.

Wu, K.S., and S.D. Tanksley. 1993. Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol. Gen. Genet. 241: 225-235.

Xu, S.S., T.L. Friesen, and A. Mujeeb-Kazi. 2004. Seedling resistance to tan spot and *Stagonospora nodorum* blotch in synthetic hexaploid wheats. Crop Sci. 44:2238-2245.

Yan, Y., S.L.K. Hsam, J.Z. Yu, Y. Jiang, I. Ohtsuka, and F.J. Zeller. 2003. HMW and LMW glutenin alleles among putative tetraploid and hexaploid European spelt wheat (*Triticum spelta* L.) progenitors. Theor. Appl. Genet. 107 1321-1330.

Zeller, F.J., J. Lutz, and U. Stephan. 1993. Chromosome location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L.) 1. *Mlk* and other alleles at the *Pm3* locus. Euphytica 68:223.229.

Zeller, F.J., and S.L.K. Hsam. 1996. Chromosomal location of a gene suppressing powdery mildew resistance genes *Pm8* and *Pm17* in common wheat (*T. aestivum* L. em. Thell.). Theor. Appl. Genet. 93:38-40.

Zeller, F.J., and, S.L.K. Hsam. 1998. Progress in breeding for resistance to powdery mildew in common wheat (*Triticum aestivum* L). P. 178-180. *In* A.E. Slinkard (ed.) Proceedings of the 9th international wheat genetics symposium, University Extension Press, University of Saskatchewan, Canada.

Zeller, F.J., L. Kong, L. Hartl, V. Mohler, and S.L.K. Hsam. 2002. Chromosomal location of genes for resistance to powdery mildew in common wheat (*T. aestivum* L. em. Thell.) 7. Gene *Pm29* in line Pova. Euphytica 123:187-194.

Zohary, D., and, M. Hopf. 1993. Domestication of plants in the old world, 2nd edition, Clarendon Press, Oxford.

Zohary, D. 1999. Monophyletic vs. polyphyletic origin of the crops on which agriculture was founded in the Near East. Gen. Res. Crop Evol. 46:133-142.

9. APPENDIX

9.1 List of chemicals

Agar	Acros, Belgium
Acetic acid	Merk, Darmsadt
Acrylamid/Bis acrylamid (19:1)	Roth, Karlsruhe
Agarose	Roth, Karlsruhe
APS (Ammonium per sulfate)	Amresco, Ohio
Bromophenol blue	Pharmacia Biotech, Freiburg
Bromnaphthalin	Merk, Darmsadt
Bouric acid	Sigma, St. Louis
Calcium Carbonate	Roth, Karlsruhe
Chloroform	Riedel-de-Haen, Seelze
CTAB (Cethyltrimethyl amonium bromide)	Sigma, St. Louis
EDTA (ethylenediaminetetraacetic acid)	USB, Cleveland
Ethanol	Merk, Darmsadt
Ethidium bromide	Roth, Karlsruhe
Formamid	Roth, Karlsruhe
Glycerin	Roth, Karlsruhe
Harnstoff	Amresco, Ohio
HCl	Roth, Karlsruhe
Isoamyl alkohol	Roth, Karlsruhe
Isopropanol	Roth, Karlsruhe
Long-Ranger gel solution	FMC Bio products, Rockland
ß-Mercaptoethanol	Roth, Karlsruhe
$MgCl_2$	Roth, Karlsruhe
NaCl	Merk, Darmstadt
Orcein	Merk, Darmstadt
10x PCR buffer	Eurogenetic, Belgium
PDA (Ptato Dextrose Agar)	Acros, Belgium
Scheff'sches Reagent	Merk, Darmstadt
TEMED (Tetramethylethylenediamine)	Amresco, Ohio
TRIS	ICN Biomedicals, Ohio
V8 juice	Campbell, USA
Enzymes	
RNaseA	Qiagen, Hilden
Taq DNA polymerase	PeqLab, Erlangen
DNA-long standards	
λ-DNA/HindIII	MBI Fermentas, St.Leon-Rot
Genscan-500 TAMRA	Applera, Weitersadt
Nucleotide	
dNTP-mix	Pharmacia Biotech, Freiburg

9.2 Curriculum Vitae

1. PERSONAL DATA
Name: Wuletaw Tadesse Degu
Nationality: Ethiopian
Date of birth: 5 October 1972, Gondar, Ethiopia
Marrital status: married, 1 son

2. ACADEMIC QUALIFICATION
- April 2003-todate: PhD student Technical University of Munich, Germany
- January 2003 to September 2003: PhD student at the University of Free State, South Africa
- September 1997-July 1999: M. Sc. in Applied Genetics, Addis Ababa University, Ethiopia
- September 1988-July 1991: B.Sc. degree in Plant Sciences from Alemaya University, Ethiopia

3. WORK EXPERIENCE IN AGRICULTURAL RESEARCH
- July 1999 to January 2003: Breeder and heead of Breeding/Genetics Department Adet Research Center, Ethiopia
- 1992 –1996**:** Research Team Leader and Breeder, highland pulses, Adet Research Center, Ethiopia

4. ON JOB TRAINING
- Application of modern technologies in crop breeding, 12-25 October, 2002, Beijing, China
- Grass Pea Breeding and Biotechnology 28April to 28 July 2000, ICARDA, Aleppo, Syria,
- Participatory technology development: 5-11April 2000, Addis Ababa, Ethiopia

5. PUBLICATIONS IN JORNALS
1 **Wuletaw Tadesse**, Schmolke M, Mohler V, Wenzel G, Hsam SLK, Zeller FJ (2007). Molecular mapping of resistance genes to tan spot (*Pyrenophora tritici repentis race 1*) in synthetic wheat lines. *Theor Appl Genet* 114:855-862
2 **Wuletaw Tadesse**, Hsam SLK, Wenzel G Zeller FJ (2006a) Identification and monosomic analysis of tan spot resistance genes in synthetic wheat lines (*Triticum turgidum* L. x *Aegilops tauschii* Coss.). *Crop Sci.* **46:**1212-1217
3 Wuletaw Tadesse, Hsam SLK, Zeller FJ (2006b) Evaluation of common wheat cultivars for tan spot resistance and chromosomal location of a resistance gene in the cultivar 'Salamouni'. *Plant Breeding* **125**:318-322
4. **Wuletaw Tadesse** and Endashaw Bekele. 2004. Isozymes, protein and ODAP variability of grass pea (*Lathyrus sativus* l.) in Ethiopia. *SINET Ethiopian Journal of Science* 27:153-160
5. **Wuletaw Tadesse** and Endashaw Bekele. 2003a. Phenotypic diversity of Ethiopian grass pea in relation to geographical regions and altitudinal range. *Genetic resources and crop Evolution* 50: 497-505, 2003.
6. **Wuletaw Tadesse** and Endashaw Bekele. 2003b. Variation and Association of morphological and biochemical characters in grass pea(*Lathyrus sativus L.*). *Euphytica* 130: 315-324, 2003.
7. **Wuletaw Tadesse** and Endashaw Bekele. 2002. Variation and association analyses in grass pea (*Lathyrus sativus* L.). *SINET Ethiopian Journal of Science* 25**:** 191-204
8. **Wuletaw Tadesse**. 2002. Association of neurotoxin (ODAP) content in grass pea (*Lathyrussativus* L.) with soil properties, seed yield and other agronomic traits. *Ethiopian Journal of Natural Resources Management* **2**: 25-31

9.3. Published manuscripts (I, II, III)

I

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Identification and Monosomic Analysis of Tan Spot Resistance Genes in Synthetic Wheat Lines (*Triticum turgidum* L. × *Aegilops tauschii* Coss.)

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ABSTRACT

Tan spot, caused by the fungus *Pyrenophora tritici-repentis* (Died.) Drechs. (*Ptr*), anamorph *Drechslera tritici-repentis* (Died.) Shoem., is becoming a major yield limiting leaf disease of both durum (*Triticum turgidum* L. var durum) and common wheat (*Triticum aestivum* L.) worldwide. In this study, differential isolates and varieties were developed, and using the most virulent isolate, ASC1b, we screened about 100 synthetic wheat genotypes against the disease. Two (2%) and 20 (20.4%) of the genotypes were found to be immune and highly resistant, respectively. Monosomic analyses of the F_2 hybrids (crosses of the highly resistant accessions (XX41, XX45) and the moderately resistant accession XX110 with the monosomic lines (D-genome) of the wheat cultivar Chinese Spring have revealed that the resistance genes are located on chromosome 3D. The gene in lines XX41 and XX110 showed a recessive monogenic inheritance, whereas the gene in line XX45 exhibited a dominant mode of inheritance. The recessive genes from XX41 and XX110 are tentatively named *tsn3* and *tsn-syn1*, respectively, and the dominant gene from XX45 is named as *Tsn-syn2*.

Tan spot is one of the major destructive foliar diseases of wheat occurring worldwide (Hosford, 1982; Tekauz, 1976). The pathogen can attack both durum and common wheat as well as numerous other grass species (Hosford and Bush, 1974; Ali and Francl, 2003). The incidence of tan spot and its economic importance has dramatically increasing since the 1970s all over the world (Hosford, 1982; Wiese, 1987). A yield loss of 3 to 50% in susceptible wheat varieties was reported in Canada and USA (Hosford, 1982; Riede et al., 1996). According to a recent report (Tekauz et al., 2004), tan spot was the most prevalent wheat leaf disease during the year 2003 in Canada. In Germany, reduction of grain yield due to this disease could range from 10 to 36% (Wolf and Hoffmann, 1993). Perello et al. (2003) have indicated the fast spreading and destructive nature of this disease in the southern Cone region of South America, including Argentina, Brazil, Chile, Paraguay, and Uruguay. Duveiller et al. (2005) reported an average reduction in yield of 30% in south Asia.

The fast spread of the pathogen *Pyrenophora tritici-repentis* is attributed to its stubble-borne nature, a shift toward soil conservation practices such as minimum and zero tillage, the trend away from stubble burning (Rees, 1982; Wolf and Hoffmann, 1993), and intensive wheat after wheat cultivation. These practices retain crop residues on the soil surface, resulting in an increase of inoculum, since the pathogen survives from one season to the next on wheat and grass stubble. Many of the semidwarf wheat cultivars introduced in Australia after 1960 have a high susceptibility to the disease (Rees et al., 1988), indicating that changes in genotypes and the narrow genetic base of the cultivated wheat lines may also play a role in the increased incidence of tan spot (Lamari et al., 2005).

Effective control of tan spot can be achieved with foliar fungicides such as propiconazole {*cis-trans*-1-[2-(2, 4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole} and tebuconazole [(*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol] (Watkins et al., 1982), but costs may be prohibitive in addition to the negative ecological impact. The use of resistant cultivars, on the other hand, is believed to be cost effective, socially feasible, and ecologically safe. Research results to date indicate that there are possibilities of identifying resistance genes by screening wide arrays of wheat germplasm (Rees et al., 1988; Lamari and Bernier, 1989a; Mielke and Reichelt, 1999). Siedler et al. (1994) and more recently Xu et al. (2004) have reported the presence of resistance in synthetic wheat genotypes, which are hybrids between tetraploid wheats (*T. turgidum*) and diploid wild wheat (*Aegilops tauschii* Coss.).

There are different reports regarding the inheritance of tan spot resistance in wheat. Some researchers (Nagle et al., 1982; Elias et al., 1989; Faris et al., 1997; Effertz et al., 2002) reported quantitative inheritance, while others (Lamari and Bernier, 1989b, 1991; Gamba and Lamari, 1998; Lee and Gough, 1984) have reported the inheritance of tan spot is qualitative, controlled by single major recessive genes. More recently, Lamari et al. (2003) have also found that the inheritance of tan spot is qualitative indicating that a gene-for-gene relationship exists in the *Triticum–P. tritici repentis* system. However, to date only very few sources of resistance against the disease have been identified and mapped (Faris et al., 1996, 1997; Friesen and Faris, 2004; Cheong et al., 2004). Therefore, it is essential to undertake a constant search for novel resistance genes to cope with the dynamic and rapidly evolving pathogen population. Hence, this study was performed with the objectives of screening available synthetic wheat genotypes to identify further sources of resistance and to determine the chromosomal location of the genes.

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Abbreviations: CIMMYT, International Wheat and Maize Improvement Center; CS, Chinese Spring; PDA, Potato Dextrose Agar; Ptr, *Pyrenophora tritici-repentis*; S.E., Standard Error; TUM, Technical University of Munich.

MATERIALS AND METHODS

Plant Materials

Wheat cultivars ($2n = 6x = 42$, AABBDD)—Salamouni, Glenlea, Katepwa, Red Chief, 6B365, Chinese Spring, and Kanzler—were used for selection of differential isolates. A total of 98 synthetic wheat genotypes ($2n = 6x = 42$, AABBDD), which are amphiploids developed from the hybrid between tetraploid wheat (*T. turgidum*, $2n = 4x = 28$, AABB) and *Ae. tauschii* ($2n = 2x = 14$, DD), were used for this study. Some of these lines were obtained from International Maize and Wheat Improvement Center (CIMMYT) and others were developed by the Division of Applied Genetics and Plant Breeding of the Technical University of Munich, Germany. Average of 10 seeds per genotype were planted in a pot (10-cm diameter) containing peat moss, at a temperature of approximately 20 to 23°C with 16 h of photoperiod in the greenhouse. Each genotype was replicated three times. Water was supplied by capillary action via holes in the base of the pots. Table 1 indicates the list and pedigrees of the synthetic wheat genotypes.

Inoculum Production

Three isolates, ASC1a, ASC1b, and 86–1241a, were kindly provided by Dr Lamari, University of Manitoba. Isolate Cz1–2 was obtained from Czech Republic. DTR 1/2000 and DTR 12/2000 were supplied from the Bavarian State Research Center for Agriculture (LFL), Germany, while the remaining two isolates, NunBr-1 and Rog5/04, were developed during the course of this study following the method described by Lamari and Bernier (1989a) from infected leaf samples collected in Nürnberg and Roggenstein areas of southern Germany, respectively. The isolates were screened for their effectiveness using standard varieties and cultivar Chinese Spring, and the most virulent isolate ASC1b (race 1) was selected and used both for the screening of synthetic genotypes and monosomic analysis.

Inoculum production followed the method of Lamari and Bernier (1989a) and Raymond et al. (1985). A single medium consisting of 150 mL V8 juice (Campbell Soup Company, Camden, NJ), 10 g Potato Dextrose Agar (PDA), 3 g $CaCO_3$, 10 g Bacto agar (Difco, Detroit, MI), and 850 mL distilled water was prepared and poured into Petri dishes. Small plugs with 0.5-cm diameter from a 7-d-old culture of *P. tritici-repentis* were transferred singly into the above mentioned plates. The cultures were then incubated in the dark for about 8 d, flooded with sterile distilled water, and the mycelia were flattened with a sterilized glass rod. Water was decanted from the plates and the cultures were transferred to a regime of 24 h of light at room temperature followed by 22 h of darkness at 15°C. The light period enables for the formation of conidiophores while the dark period induces the formation of conidia. After 22 h of darkness, conidia were harvested by flooding the plates in sterile distilled water and scraping the spores from the plates. The concentration was adjusted approximately to 3000 spores mL^{-1}.

Conidial Inoculation and Rating

Eight *Ptr* isolates—ASC1a, ASC1b, 86–124a, Nubr-1, Rog5/04, DTR 1–2000, and DTR 12–2000—were used for the development of differential varieties, while only the most virulent isolate (ASC1b) was used for the screening of synthetic lines and monosomic analysis. Plants were inoculated at the two leaf stage and were placed into a 2- × 1.5- × 1-m portable plastic tent inside the greenhouse. The tent was further covered by a black plastic sheet to ensure complete darkness. A relative humidity of 100% was maintained with a humidifier. After 24 h of leaf-wetness period in the dark as indicated above, the plants were transferred into a growth chamber at a temperature of 22°C and photoperiod of 12 h/day for about 7 d. The plants were evaluated for their resistance to tan spot 7 d after inoculation following the 0-to-5 rating scale with a little modification of the 0-to-5 rating scale developed by Lamari and Bernier (1989a): where 0 = immune, 1 = resistant, 2 = moderately resistant, 3 = moderately resistant to moderately susceptible, 4 = susceptible, 5 = highly susceptible.

Monosomic Analyses

Seven monosomic lines of the D genome of wheat cultivar Chinese Spring (CS), which is susceptible to tan spot, were crossed with three synthetic lines XX41, XX45, and XX110. CS monosomic lines and the three synthetic lines ($2n = 6x = 42$, AABBDD) were used as female and pollen parents, respectively. The synthetic lines XX41, XX45, and XX110 were previously tested in our laboratory and found to be highly resistant to mixtures of *Ptr* isolates (Siedler, 1991), and hence we used them further for monosomic crossing before screening of the 98 synthetic lines. The seven monosomic lines of Chinese Spring and the F_1 crosses were screened for monosomy ($2n = 41$) by chromosome counts from squashes of root-tip cells pretreated with mono-bromonapthalene and stained by the Feulgen method (Zeller et al., 2002). Only confirmed $2n = 41$ chromosome seedlings of the monosomic series of Chinese Spring and the F_1 hybrids were planted (three seedlings per pot) in 50-cm diameter pot and raised in the greenhouse following standard wheat agronomic practices. Crosses of disomic cultivar Chinese Spring with XX41, XX45, and XX110 were made as controls to study the segregation and inheritance of tan spot resistance. The monosomic families were screened in three sets of inoculations using *Ptr* isolate ASC1b. For each set of inoculation, 17-d-old seedlings were raised by planting F_2 seeds in three pots at a rate of 10 seeds per pot for each combination depending on the availability of seeds. Inoculum production, inoculation techniques and rating scales used for the screening of the synthetic lines were also applied here. Evaluation was made on single plant basis, and score values of 0, 1, and 2 were grouped as resistant while 3, 4, and 5 were grouped as susceptible. The number of resistant and susceptible plants in each set of inoculation was summed up to get the total frequency of susceptible and resistant F_2 plants per each combination. χ^2 analyses were performed using Agrobase 20 software (Agronomix Software Inc., 1990) to determine the goodness of fit either for a 3: 1 or 1:3 (resistant: susceptible) segregation.

RESULTS AND DISCUSSION

Differential Wheat Cultivars and *Ptr* Isolates

As indicated in Table 2, the cultivars responded differentially toward *Ptr* isolates possessing different virulence.

Salamouni, which was previously identified to be resistant, showed moderately susceptible response to isolates ASC1b, DTR1–2000, and DTR 12–2000. Glenlea, Katepwa, and Kanzler were susceptible to most of the isolates. The cultivar Red Chief showed resistant response across all the isolates. Cultivar Chinese Spring was susceptible to isolates ASC1a and ASC1b but moderately susceptible to the isolates Rog5/04, DTR1–2000, and DTR 12–2000.

Table 1. Evaluation of synthetic wheat accessions for tan spot resistance using isolate ASC1b in 0-to-5 scale.

Acc. number†	Pedigree‡	Response to Ptr ASC1b (M§ + S.E)
XX 41	Langdon durum/*Ae. tauschii* (CI 00017)	1.0 + 0.0
XX 45	Langdon durum/*Ae. tauschii* (RL 5565)	1.0 + 0.0
XX 50	Langdon durum/*Ae. tauschii* (268210)	2.3 + 0.3
XX 52	Longdon durum/*Ae. tauschii* (RL 5392)	3.0 + 0.0
XX 110	*T. dicoccum* (A 38)/*Ae. tauschii* (CI 33)	1.7 + 0.3
XX 111	*T. dicoccum* (119)/*Ae. tauschii* (33)	1.0 + 0.0
XX 175	*T. durum* (22909)/*Ae. tauchii* 202229-c	2.0 + 0.0
XX 183	*T. durum* (22912)/*Ae. tauschii* (CI 0221)	1.3 + 0.3
XX 186	*T. turgidum* (90)/*Ae. tauschii* (BGRC 1458)	3.3 + 0.3
XX 193	*T. turgidum* (88)/*Ae. tauschii*(BGRC 1457)	3.3 + 0.3
XX 194	*T. turgidum* (89)/*Ae. tauschii* (AE457/78)	2.7 + 0.3
XX 195	*T. turgidum* (88)/*Ae. tauschii* (BGRC 1458)	1.0 + 0.0
XX 196	*T. durum* (488)/*T. turgidum* (88)	3.0 + 0.0
XX 197	*T. durum* (488)/*T. turgidum* (89)	3.3 + 0.3
XX 199	*T. carthlicum*/*Ae. tauschii* (RL5320)	2.3 + 0.3
XX 199	*T. turgidum*(61)/*Ae. tauschii* (AE 724/82)	5.0 + 0.0
XX 200	*T. turgidum* (61)/*Ae. tauschii* (AE 432/80)	2.7 + 0.3
XX 201	*T. turgidum* (89)/*Ae. tauschii*(CI 33)	2.3 + 0.3
XX 202	*T. turgidum* (01)/*Ae. tauschii* (CI 18)	1.3 + 0.3
XX 203	*T. turgidum* (01)/*Ae. tauschii* (AE 432/80)	4.0 + 0.0
XX 205	*T. turgidum* (01)/*Ae. tauschii*(CI 33)	1.7 + 0.3
XX 206	*T. turgidum* (235)/*Ae. tauschii*(RL 5688)	1.7 + 0.3
XX 208	*T. turgidum* (01)/*Ae. tauschii*(CI 38)	2.0 + 0.0
XX 216	*T. turgidum* (61)/*Ae. tauschii* (CI 18)	2.0 + 0.0
XX 218	*T. turgidum* (61)/*Ae. tauschi* (AE 724/82))	2.0 + 0.0
XX 220	*T. turgidum* (61)/*Ae. tauschii* (CI 33)	2.3 + 0.3
XX 222	*T. turgidum* (235)/*Ae. tauschii* (RL 5686)	2.0 + 0.0
XX 224	*T. turgidum* (90)/*Ae. tauschii* (AE 141/78)	4.3 + 0.3
XX 227	*T. turgidum* (89)/*Ae. tauschii* (26)	1.0 + 0.0
XX 229	*T. turgidum* (89)/*Ae. tauschii* (RL 5670)	2.7 + 0.3
XX 231	*T. turgidum* (80)/*Ae. tauschii* (AE 431/83)	3.0 + 0.0
XX 233	*T. turgidum* (01)/*Ae. tauschii* (CI 33)	2.0 + 0.0
XX 234	*T. turgidum* (90)/*Ae. tauschii* (PI 210987)	2.3 + 0.3
XX 235	*T. turgidum* (90)/*Ae. tauschii* (26)	1.3 + 0.3
Syn 1	Altar 84/*Ae. tauschi*i (188)	1.0 + 0.0
Syn 2	Doy1/*Ae. tauschii* (188)	2.7 + 0.3
Syn 4	Altar 84/*Ae. tauschii* (193)	4.7 + 0.3
Syn 5	Altar 84/*Ae. tauschii* (198)	4.0 + 0.0
Syn 7	Altar 84/*Ae. tauschii* (205)	2.3 + 0.3
Syn 9	Altar 84/*Ae. tauschii* (211)	2.0 + 0.0
Syn 10	D67–2/P66.270//*Ae. tauschii* (211)	2.0 + 0.0
Syn 11	D67–2/P66.270//*Ae. tauschii* (213)	1.0 + 0.0
Syn 12	ROK/KML//*Ae. tauschii* (214)	2.7 + 0.3
Syn 14	YUK/*Ae. tauschii* (217)	2.0 + 0.0
Syn 23	D67–2/P66–270//*Ae. tauschii* (223)	2.7 + 0.3
Syn 25	Altar 84/*Ae. tauschii* (224)	2.7 + 0.3
Syn 26	Aco89/*Ae. tauschii* (309)	2.0 + 0.0
Syn 29	68–11/RGB-U//Ward/3/*Ae. tauschii* (326)	3.3 + 0.3
Syn 30	68112/Ward//*Ae. tauschii* (369)	1.0 + 0.0
Syn 31	68112/Ward//*Ae. tauschii* (369)	3.3 + 0.3
Syn 32	Doy1/*Ae. tauschii* (447)	1.3 + 0.3
Syn 33	Yav 3/sco//JO69/cra/3/Yav79/4/*Ae. tauschii*(498)	1.7 + 0.3
Syn 34	Doy1/*Ae. tauschii* (511)	2.0 + 0.0
Syn 35	68.111/RGB-U//Ward/3/*Ae. tauschii* (511)	2.3 + 0.3
Syn 37	68–11/RGB-U//Ward/3/FGO/4/Rabi/5/*Ae. tauschii* (629)	3.7 + 0.3
Syn 38	Fgo/USA2111//*Ae. tauschii* (658)	0.0 + 0.0
Syn 39	Croc 1/*Ae. tauschii* (725)	2.0 + 0.0
Syn 42	Yar/*Ae. tauschii* (783)	1.3 + 0.3
Syn 43	Yuk/*Ae. tauschii* (864)	2.3 + 0.3
Syn 44	68–11/RGB-U//Ward/3/FGO/4/RABI/5/*Ae. tauschii* (878)	0.0 + 0.0
Syn 45	68–11/RGB-U//Ward/3/FGO/4/RABI/5/*Ae. tauschii* (878)	1.0 +0.0
Syn 46	Croc 1/*Ae. tauschii* (879)	2.3 + 0.3
Syn 47	68–11/RGB-U//Ward/3/FGO/4/RABI/5/*Ae. tauschii* (882)	1.0 + 0.0
Syn 48	Sora/*Ae. tauschii* (884)	1.0 + 0.0
Syn 49	68–11/RGB-U//Ward/3/FGO/4/RABI/5/*Ae. tauschii* (890)	1.7 + 0.3
Syn 53	Yav 2/TEZ//*Ae. tauschii* (249)	1.7 + 0.3
Syn 54	Ceta/*Ae. tauschii* (895)	4.7 + 0.3
Syn 55	Gan/*Ae.tauschii* (180)	1.7 + 0.3
Syn 56	D67–2/P66–270/*Ae. tauschii* (257)	3.7 + 0.3
Syn 57	LCK59–61/*Ae. tauschii* (313)	3.0 + 0.0
Syn 59	SRN/*Ae. tauschii* (358)	3.0 + 0.0
Syn 60	Scoop 1/*Ae. tauschii* (358)	1.0 + 0.0
Syn 61	Gan/*Ae. tauschii* (408)	3.0 + 0.0
Syn 62	Sca/*Ae. tauschii* (518)	2.7 +0.3
Syn 63	Yar/*Ae. tauschii* (518)	2.0 + 0.0
Syn 64	Botno/*Ae. tauschii* (617)	4.0 + 0.0
Syn 65	Botno/*Ae. tauschii* (620)	4.0 + 0.0
Syn 67	Snipe/Yav79//Dack/Teal/3/*Ae. tauschii* (629)	2.7 + 0.0
Syn 69	D67–2/P66–270//*Ae. tauschii* (659)	2.0 + 0.0
Syn 70	Snipe/Yav79//Dack/Teal/3/*Ae. tauschii* (700)	1.7 + 0.3
Syn 72	Snipe/Yav79//Dack/Teal/3/*Ae. tauschii* (877)	2.0 + 0.0
Syn 73	Gan/*Ae. tauschii* (897)	1.0 + 0.0
Syn 74	YAV/TEZ//*Ae. tauschii* (895)	2.7 + 0.3
Syn 75	Arlin/*Ae. tauschii* (283)	2.0 + 0.0
Syn 76	Falcin/*Ae. tauschii* (312)	1.7 + 0.3
Syn 77	Rascon/*Ae. tauschii* (312)	3.0 + 0.0
Syn 82	68–11/RGB-U//Ward/3/*Ae. tauschii* (454)	2.7 + 0.3
Syn 84	Green/*Ae. tauschii* (458)	1.0 + 0.0
Syn 85	Ceta/*Ae. tauschii* (174)	2.3 + 0.3
Syn 86	Doy1/*Ae. tauschii* (372)	2.3 + 0.3
Syn 87	SCA/*Ae. tauschii* (409)	1.0 + 0.0
Syn 88	CPI/Gediz/3/GOO//JO69/CRA/4/*Ae. tauschii* (409)	2.0 + 0.0
Syn 89	STY-US/Celta//PALS/3/SRN_5/4/*Ae. tauschii* (502)	2.7 + 0.3
Syn 90	Altar 84/*Ae. tauschii* (502)	2.7 + 0.3
Syn 91	Croc 1/*Ae. tauschii* (517)	2.0 + 0.0
Syn 92	Ceta/*Ae. tauschii* (1024)	1.7 + 0.3
Syn 94	Ceta/*Ae. tauschii* (1027)	3.0 + 0.0
Syn 95	Doy1/*Ae. tauschii* (1030)	2.3 + 0.3
Red Chief	–	1.0 + 0.0
Glenlea	Pembina *2/Bage//CB-100	5.0 + 0.0
Mean		2.2 + 0.1

† XX: synthetic lines developed from the Institute of Plant Breeding, TUM, Weihenstephan; Syn: synthetic lines obtained from CIMMYT and maintained at TUM.

‡ Numbers in parenthesis indicate accession numbers of parental lines in the respective institutes.

§ M: mean response value of the three replications.

Screening Synthetic Wheat Genotypes for Tan Spot Resistance

A total of 98 synthetic wheat lines were screened with the most virulent *Ptr* isolate ASC1b for their seedling resistance against tan spot caused by *P.tritici-repentis*. The response of the genotypes to *Ptr* ASC1b ranged from 0 (immune) to 5 (highly susceptible) with a mean value of 2.2 in the 0-to-5 scale (Table 1). Two genotypes (syn 38 and syn 44) were found to be immune and twenty genotypes were highly resistant. The majority of the genotypes (40.8%) were moderately resistant. Siedler (1991) had reported that lines XX41, XX45, and XX110 showed highly resistant response to mixtures of *Ptr* isolates. In the present study XX41 and XX45 were confirmed to be highly resistant, while XX110 was moderately resistant to the most virulent monoconidial *Ptr* isolate, ASC1b.

This result indicated the presence of broad level of resistance against tan spot from synthetic wheats. Similar results were reported previously by Siedler et al. (1994) and more recently by Xu et al. (2004).

Chromosomal Location

Three resistant synthetic genotypes, XX41 [a hybrid between Langdon durum and *Ae. tauschii* (CI 00017)],

Table 2. Reaction of Chinese Spring and other standard varieties to different *Ptr* isolates.

Variety	Reaction to different isolates (1-to-5 scale)†							
	ASC 1a	ASC 1b	86–124a	Cz1–2	NuBr-1	Rog 5/04	DTR 1–2000	DTR 12–2000
Salamouni	1	3	0	1	1	1	4	3
Glenlea	5	5	3	3	2	3	5	4
Katepwa	5	5	3	3	3	3	5	4
Red Chief	1	1	0	0	0	0	1	0
6B365	2	4	1	2	0	0	2	2
Kanzler	4	5	1	3	3	4	5	4
Chinese Spring	4	4	2	2	1	3	3	3

† 0 = immune; 1 = small dark brown or black spots without chlorosis or tan necrosis, resistant; 2 = small dark brown or black spots with very little chlorosis or tan necrosis, moderately resistant; 3 = small dark brown or black spots with distinct chlorosis or necrotic ring (moderately resistant- moderately susceptible); 4 = small dark brown or black spots completely surrounded by chlorosis or necrosis (moderately susceptible- susceptible); 5 = The dark brown or black centers may or may not be distinguishable; most lessions consist of coalessing chlorotic or necrotic zones (highly susceptible).

XX45 (Langdon durum/*Ae. tauschii*, RL 5565), and XX110 [(*T. dicoccum* Schrank (A38)/*Ae. tauschii,* CI 33] were crossed as pollen parents to the D genome Chinese Spring monosomic lines (1D-7D). The F_1 hybrids were checked for monosomy and planted in the greenhouse to obtain F_2 seeds. The results of the F_2 monosomic analyses are indicated in Tables 3, 4, and 5.

As expected, the F_2 populations from the crosses between disomic Chinese Spring and the resistant parents XX41 and XX110 (Tables 3 and 5), segregated into 34 and 110 susceptible and 11 and 42 resistant plants, respectively, fitting a 1:3 (resistant: susceptible) Mendelian ratio, which indicated that the resistance in these two lines to tan spot was controlled by a single recessive gene. On the other hand, the F_2 populations from crosses between disomic Chinese Spring and XX45 segregated into 97 resistant and 41 susceptible plants (Table 4), indicating that the resistance gene is dominant. This qualitative inheritance of the resistance genes is in agreement with previous reports of Lee and Gough (1984), Lamari and Bernier (1989b, 1991), Gamba and Lamari (1998), Lamari et al. (2003), and Singh and Hughes (2005). Parental lines of XX41 (Langdon durum and *Aegilops tauchii*, CI 00017), XX45 (Langdon durum and *Aegilops tauschii*, RL 5565), and XX110 (*T. dicoccum*, A38 and *Aegilops tauschii*, CI 33) were evaluated using *Ptr* isolate ASC 1b so as to identify the source of resistance in the respective synthetic lines. The tetraploid parents Langdon durum and *T. dicoccum* (A38) were susceptible (4 in 0-to-5 scale), while the diploid *Ae. tauchii* parents (CI 00017 and RL 5565) were highly resistant (1) and CI 33 was moderately resistant (2), indicating that the source of resistance in the synthetic lines was from the diploid *Ae. tauschii* lines.

The χ^2 analyses of the segregation ratio from the three populations indicated that the combinations of mono-3D segregated differently and significantly ($p < 0.01$) from the expected 1:3 and 3:1 (resistant: susceptible) ratios indicating that these were the critical crosses. The segregation pattern in the critical crosses were 60: 5, 74: 4 and 70:7 resistant: susceptible plants in CS/XX41, CS/XX45 and CS/XX110 F_2 populations, respectively. The results clearly indicated that the resistance genes are located on chromosome 3D. The recessive genes from XX41 and XX110 are tentatively designated *tsn*3 and *tsn-syn1*, respectively, and the dominant gene from XX45 is named *T*sn-*syn2.* However, further studies of allelism tests and analyses of molecular markers are necessary to confirm whether the genes are allelic or at different loci. Resistance genes for tan spot have not been located on D-genome chromosomes in hexaploid wheat (Xu et al., 2004). Most of the tan spot resistant genes reported to date were located on the B-genome of hexaploid wheat. Faris et al. (1996) mapped the resistance locus *tsn-1* on the long arm of 5B using restriction fragment length polymorphism (RFLP) markers. Friesen and Faris (2004) identified a QTL on the short arm of chromosome 2B and designated *tsc*2 using molecular analysis. A major QTL on 5BL, which actually is expected to be the same as *tsn-1*, was also reported from the Australian variety Brookton (Cheong et al., 2004). More recently, Faris and Friesen (2005) have identified QTL on chromosome arms 1BS and 3BL in cultivar BR34 using *Ptr* races 1–3 and 5 indicating presence of race-nonspecific tan spot resistance.

As compared with bread wheat, synthetic lines showed a large degree of genetic variation for resistance to different wheat diseases (Xu et al., 2004). In the present

Table 3. Frequencies of resistant and susceptible seedlings in crosses of Chinese Spring monosomics and XX41 tested with *Ptr* isolate ASC1b.

Crosses	Number of plants in the F_2 populations			
	Resistant	Susceptible	χ^2 Ratio	*P* for 1:3
mono-1D/XX41	26	55	2.321	0.1276
mono-2D/XX41	17	53	0.018	0.8912
mono-3D/XX41	60	5	157.048	<0.0001**
mono-4D/XX41	27	60	1.869	0.1937
mono-5D/XX41	20	52	0.296	0.5864
mono-6D/XX41	24	58	0.796	0.3723
mono-7D/XX41	15	38	0.308	0.5789
Disomic/XX41	11	34	0.008	0.9287

** significantly different at 1% level of significance.

Table 4. Frequencies of resistant and susceptible seedlings in crosses of Chinese Spring monosomics and XX45 tested with *Ptr* isolate ASC1b.

Crosses	Number of plants in the F_2 populations			
	Resistant	Susceptible	χ^2 Ratio	*P* for 3:1
mono-1D/XX45	–	–	–	–
mono-2D/XX45	–	–	–	–
mono-3D/XX45	74	4	16.4268	0.000**
mono-4D/XX45	56	17	0.1141	0.7355
mono-5D/XX45	54	21	0.360	0.5485
mono-6D/XX45	52	20	0.2962	0.5862
mono-7D/XX45	56	25	1.4853	0.2229
Disomic/XX45	97	41	1.6330	0.2012

** significantly different at 1% level of significance.

Table 5. Frequencies of resistant and susceptible seedlings in crosses of Chinese Spring monosomics and XX110 tested with *Ptr* isolate ASC1b.

	Number of plants in the F_2 populations			
Crosses	**Resistant**	**Susceptible**	**χ^2 Ratio**	***P* for 1:3**
mono-1D/XX110	**15**	**40**	**2.321**	**0.1514**
mono-2D/XX110	**25**	**50**	**2.777**	**0.0956**
mono-3D/XX110	**70**	**7**	**136.380**	**0.000****
mono-4D/XX110	**23**	**52**	**1.824**	**0.1768**
mono-5D/XX110	**21**	**49**	**0.933**	**0.3340**
mono-6D/XX110	**17**	**55**	**0.074**	**0.7855**
mono-7D/XX110	**24**	**58**	**0.796**	**0.3722**
Disomic/XX10	**42**	**110**	**2.771**	**0.0960**

** **significantly different at 1% level of significance.**

study, about 100 synthetic lines were screened for tan spot resistance. Two genotypes were found immune and 20 genotypes were highly resistant. Furthermore the chromosomal location of the resistance gene from the previously identified resistance lines (XX41, XX45, and XX110) was identified to be on chromosome 3D, which according to our knowledge, is the first report to locate tan spot resistance gene on D-genome of wheat. Allelism test of the immune/resistant lines such as syn 11, syn 38, syn 44, syn 84, syn 87, XX111, and XX195 with XX41, XX45, and XX110 to test the identity of the gene in one of these lines with the genes *tsn-3*, *tsn-syn1*, and *Tsn-syn2* should be undertaken to confirm that they are different. The source of resistance in the synthetic lines XX41, XX45, and XX10 was identified to be the *A. tauschii* parent. Similarly, it is important to identify the source of resistance for the other synthetic lines. The immune and highly resistant lines identified in the present study including the lines used for the monosomic crosses (XX41, XX45, and XX110) are recommended to be used as parental lines for development of tan spot resistant wheat cultivars and mapping populations.

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REFERENCES

Agronomix Software Inc. 1990. Agrobase 20. Winnipeg, Canada.

Ali, S., and L.J. Francl. 2003. Population race structure of *Pyrenophora tritici-repentis* prevalent on wheat and none cereal grasses in the Great plains. Plant Dis. 87:418–422.

Cheong, J., H. Wallwork, and K.J. Williams. 2004. Identification of a major QTL for yellow leaf spot resistance in the wheat varieties Brookton and Cranbrook. Aust. J. Agric. Res. 55:315–319.

Duveiller, E., Y.R. Kandel, R.C. Sharma, and S.M. Shrestha. 2005. Epidemiology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas. Phytopathology 95:248–256.

Effertz, R.J., S.W. Meinhardt, J.A. Anderson, J.G. Jordal, and L.J. Francl. 2002. Identification of a chlorosis inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitive locus in wheat. Phytopathology 92:527–533.

Elias, E., R.G. Cantrell, and R.M. Hosford, Jr. 1989. Heritability of resistance to tan spot in durum wheat and its associations with other agronomic traits. Crop Sci. 29:299–304.

Faris, J.D., J.A. Anderson, L.J. Francl, and J.G. Jordahl. 1996. Chromosomal location of a gene conditioning insensitivity in wheat to a necrotic inducing culture filtrate from *Pyrenophora tritici-repentis*. Phytopathology 86:459–463.

Faris, J.D., J.A. Anderson, L.J. Francl, and J.G. Jordahl. 1997. RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici-repentis* in wheat. Theor. Appl. Genet. 94:98–103.

Faris, J.D., and T.L. Friesen. 2005. Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. Theor. Appl. Genet. 111:386–392.

Friesen, T.L., and J.D. Faris. 2004. Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. Theor. Appl. Genet. 109:464–471.

Gamba, F.M., and L. Lamari. 1998. Mendelian inheritance of tan spot (*Pyrenophora tritici-repentis*) in selected genotypes of durum wheat (*Triticum durum*). Can. J. Plant Pathol. 20:408–414.

Hosford, R.M., Jr. 1982. Tan spot. p. 1–24. *In* R.M Hosford, Jr. (ed.) Tan spot of wheat and related diseases. North Dakota State University, Fargo.

Hosford, R.M., Jr., and R.H. Bush. 1974. Losses in wheat caused by *Pyrenophora trichostoma* and *Leptosphaeria avenaria* f. sp. *triticea*. Phytopathology 64:184–187.

Lamari, L., and C.C. Bernier. 1989a. Evaluation of wheat lines and cultivars to tan spot (*Pyrenophora tritici-repentis*) based on lesion type. Can. J. Plant Pathol. 11:49–56.

Lamari, L., and C.C. Bernier. 1989b. Toxin of *Pyrenophora tritici-repentis*: Host specificity, significance in disease and inheritance of host reaction. Phytopathology 79:740–744.

Lamari, L., and C.C. Bernier. 1991. Genetics of tan necrosis and extensive chlorosis in tan spot of wheat caused by *Pyrenophora tritici-repentis*. Phytopathology 81:1092–1095.

Lamari, L., S.E. Strelkov, A. Yahyaoui, J. Orabi, and R.B. Smith. 2003. The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one-to-one relationship in tan spot of wheat. Phytopathology 93:391–396.

Lamari, L., B.D. McCallum, and R.M. dePauw. 2005. Forensic pathology of Canadian bread wheat: The case of tan spot. Phytopathology 95:144–152.

Lee, T.S., and F.J. Gough. 1984. Inheritance of Septoria leaf blotch (*S. tritici*) and Pyrenophora (*P. tritici-repentis*) resistance in *Triticum aestivum* cv. Carifen 12. Plant Dis. 68:848–851.

Mielke, H., and A. Reichelt. 1999. Studien zur Biologie des Erregers *Drechslera tritici-repentis* zur Anfälligkeit des Weizens und verschiedener Artverwandter sowie zur Bekämpfung der DTR-Weizenblattdürre. Parey Verlag, Berlin.

Nagle, B.J., R.C. Frohberg, and R.M. Hosford, Jr. 1982. Inheritance of resistance to tan spot of wheat. p. 40–45. *In* R.M Hosford, Jr. (ed.) Tan spot of wheat and related diseases. North Dakota State University, Fargo.

Perello, A., V. Moreno, M.R. Simon, and M. Sisterna. 2003. Tan spot of wheat infection at different stages of crop development and inoculum type. Crop Prot. 22:157–169.

Raymond, P.J., W.W. Bockus, and B.L. Norman. 1985. Tan spot of winter wheat: Procedures to determine host response. Phytopathology 75:686–690.

Rees, R.G. 1982. Yellow spot, an important problem in the north eastern wheat areas of Australia. p. 68–70. *In* R.M Hosford, Jr. (ed.) Tan spot of wheat and related diseases. North Dakota State University, Fargo.

Rees, R.G., G.J. Platz, and R.J. Mayer. 1988. Susceptibility of Australian wheats to *Pyrenophora tritici-repentis*. J. Agric. Res. 39: 141–151.

Riede, C.R., L.J. Francl, J.A. Anderson, J.G. Jordahl, and S.W. Meinhardt. 1996. Additional sources of resistance to tan spot of wheat. Crop Sci. 36:771–777.

Siedler, H. 1991. Untersuchungen zur Evaluierung von Resistanz gegenüber *Pyrenophora tritici-repentis* in *Aegilops squarrosa* und synthetischen Amphiploiden (*Triticum turgidum* ssp. × *Aegilops squarrosa*. MSc thesis, Technical University of Munich, Freising-Weihenstephan.

Siedler, H., A. Obst, S.L.K. Hsam, and F.J. Zeller. 1994. Evaluation for resistance to *Pyrenophora tritici-repentis* in *Aegilops tauchii* Coss. and synthetic hexaploid wheat amphiploids. Genet. Res. Crop. Evol. 41:27–34.

Singh, P.K., and G.R. Hughes. 2005. Genetic control of resistance to tan necrosis induced by *Pyrenophora tritici-repentis*, race 1 and race 2, in spring and winter wheat genotypes. Phytopathology 95: 172–177.

Tekauz, A. 1976. Distribution, severity, and relative importance of leaf

spot diseases of wheat in western Canada in 1974. Can. Plant Dis. Surv. 56:36–40.

Tekauz, A., E. Mueller, M. Beyene, M. Stulzer, and D. Schultz. 2004. Leaf spot diseases of winter wheat in Manitoba in 2003. Can. Plant Dis. Surv. 83:73–74.

Watkins, J.E., M.G. Boosalis, and B.L. Doupnik, Jr. 1982. Foliar diseases of Nebraska's winter wheat. p. 53–61. *In* R.M Hosford, Jr. (ed.) Tan spot of wheat and related diseases. North Dakota State University, Fargo.

Wiese, M. 1987. Compendium of wheat diseases. American Phytopathology Society, St. Paul, MN.

Wolf, P.F.J., and G.M. Hoffmann. 1993. Zur Biologie von *Drechslera tritici-repentis* (Died.) Shoem. (telomorph *Pyrenophora tritici-repentis* (Died.) Drechsler), dem Erreger einer Blattfleckenkrankheit an Weizen. Z. Pfl.krankh. Pflschutz 100:33–48.

Xu, S.S., T.L. Friesen, and A. Mujeeb-Kazi. 2004. Seedling resistance to tan spot and Stagonospora nodorum blotch in synthetic hexaploid wheats. Crop Sci. 44:2238–2245.

Zeller, F.J., L. Kong, L. Hartl, V. Mohler, and S.L.K. Hsam. 2002. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.) 7. Gene *Pm29* in line Pova. Euphytica 123:187–194.

II

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Evaluation of common wheat cultivars for tan spot resistance and chromosomal location of a resistance gene in the cultivar 'Salamouni'

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With 2 figures and 3 tables

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Abstract

A total of 50 wheat (*Triticum aestivum* L.) cultivars were evaluated for resistance to tan spot, using *Pyrenophora tritici-repentis* race 1 and race 5 isolates. The cultivars 'Salamouni', 'Red Chief', 'Dashen', 'Empire' and 'Armada' were resistant to isolate ASC1a (race 1), whereas 76% of the cultivars were susceptible. Chi-squared analysis of the F_2 segregation data of hybrids between 20 monosomic lines of the wheat cultivar 'Chinese Spring' and the resistant cultivar 'Salamouni' revealed that tan spot resistance in 'Salamouni' was controlled by a single recessive gene located on chromosome 3A. This gene is designated *tsn4*. The resistant cultivars identified in this study are recommended for use in breeding programmes to improve tan spot resistance in common wheat.

Key words: *Triticum aestivum* — *Pyrenophora tritici-repentis* — disease resistance — *tsn4*

Tan spot of wheat, caused by the ascomycete *Pyrenophora tritici-repentis* (Died) Drechs. (anamorph *Dreschslera tritici-repentis*, Died), is one of the major wheat leaf diseases worldwide (Hosford 1982, Rees et al. 1988, Wolf and Hoffmann 1993, Perello et al. 2003, Tekauz et al. 2004, Duveiller et al. 2005). *Pyrenophora tritici-repentis (Ptr)* causes tan necrosis and/or extensive chlorosis on susceptible wheat cultivars, resulting in reduced grain yield and quality by lowering test weights and producing a high degree of kernel shrivelling (Cheong et al. 2004). Yield losses of up to 50%, in severe cases, have been reported (Hosford 1982, Rees et al. 1988). Recent increases in the incidence and severity of tan spot have been attributed to altered cultural practices, such as changing from conventional tillage and stubble burning to conservation tillage systems, shorter crop rotations, continuous wheat cultivation and the use of susceptible wheat cultivars (Fernandez et al. 1998, Lamari et al. 2005).

Friesen and Faris (2004) and Lamari and Bernier (1989a) indicated that the best approach for controlling tan spot is incorporation of genetic resistance into accepted cultivars. In this regard, evaluations for resistance have been carried out and sources of resistance have been reported from different ploidy groups of the genus *Triticum* and its relatives (Rees et al. 1988, Lamari and Bernier 1989a, Siedler et al. 1994, Mielke and Reichelt 1999, Xu et al. 2004). Reports regarding the inheritance of tan spot-resistance range from the quantitative (Nagle et al. 1982, Elias et al. 1989, Faris et al. 1997, Friesen and Faris 2004) to the qualitative (Lee and Gough 1984, Lamari and Bernier 1989b, 1991, Gamba and Lamari 1998, Lamari et al. 2003, Singh and Hughes 2005).

Resistance to tan necrosis and extensive chlorosis are conferred by independent loci (Lamari and Bernier 1991). A major quantitative trait loci (QTL), *QTsc.ndsu-1A*, which controls resistance to the chlorotic component of the disease was identified on the short arm of wheat chromosome 1A in a population of recombinant inbred lines derived from the synthetic hexaploid wheat W-7984 × 'Opata 85' using a race 1 (nec+, chl+) isolate (Faris et al. 1997). Friesen and Faris (2004) identified a major QTL, *tsc2*, on chromosome 2BS and a minor QTL on chromosome 4AL using *Ptr* ToxB extracted from race 5 (nec− chl+) isolates. The necrosis component of the disease was controlled by a single recessive gene, *tsn1*, located on the long arm of chromosome 5B (Faris et al. 1996). More recently, a recessive gene, *tsn3*, controlling resistance against race 1 isolate (ASC1b) was identified on chromosome 3D in the synthetic wheat line XX41 (a hybrid between 'Langdon' durum and *Aegilops tauschii* (CI 00017) and XX110 [*Triticum dicoccum* (A38)/*Ae. tauschii*, (CI 33)] using monosomic analysis (Tadesse et al. 2006). In this report, results from the evaluation of wheat cultivars using race 1 (ASC1a) and race 5 (DW-16) isolates and the chromosomal location of a resistance gene in the spring wheat cultivar 'Salamouni' are presented.

Materials and Methods

Plant materials: Including the standard differential cultivars, 50 wheat cultivars (*T. aestivum* L.) were used for evaluation against tan spot. The resistant cultivar 'Salamouni' was kindly provided by Dr L. Lamari, University of Manitoba, Canada and the 21 monosomic lines of the cultivar 'Chinese Spring' were obtained from the late Dr E.R. Sears, University of Missouri, USA.

Inoculum production: A total of nine isolates were used to determine the response of eight standard cultivars, including 'Salamouni' and 'Chinese Spring' (Table 1).

Three isolates (ASC1a, ASC1b and 86–1241a) were kindly provided by Dr L. Lamari, University of Manitoba, Canada. Isolates Cz1–2 and Dw-16 were obtained from Dr J. Palicova, Czech Republic and Dr S. Ali, North Dakota State University USA, respectively. DTR1/2000 and DTR 12/2000 were obtained from Dr B. Rodemann, Braunschweig, Germany, and the other two isolates, NunBr-1 and Rog5/04, were obtained from infected leaf samples during the course of this study following the method described by Lamari and Bernier (1989a). The infected leaf samples were collected in Nürnberg and Roggenstein, Southern Germany, respectively. Isolates ASC1a (race 1) and DW-16 (race 5) were used for screening, as they represent the most virulent races (1 and 5) that are common in wheat fields. Single spore

Table 1: Response of differential wheat cultivars to differential *Pyrenophora tritici-repentis* isolates

	Isolate								
Wheat cultivar	DW-16 (5)[1]	ASC 1a (1)	ASC 1b (1)	86–124a (2)	Cz1–2 (x)	NuBr-1(x)	Rog 5/04 (x)	DTR 1–2000 (x)	DTR 12–2000 (x)
'Salamouni'	MR[2]	R	MR	R	R	R	R	MS	MS
'Glenlea'	S	S	S	MS	MS	R	MS	S	S
'Katepwa'	S	S	S	MS	MS	MS	MS	S	S
'Red Chief'	R	R	R	R	R	R	R	R	R
6B365	S	R	S	R	MR	R	R	R	R
'Kanzler'	S	S	S	R	MS	MS	S	S	S
XX41	R	R	R	R	R	R	R	R	R
'Chinese Spring'	S	S	S	R	R	R	MS	MS	MS

[1]x = unknown race.
[2]S, susceptible (4–5 on a 1–5 scale); MS, moderately susceptible (3); R, resistant (1); MR, moderately resistant (2).

cultures were produced for each of the isolates and stored at 4°C for inoculum production.

The method of inoculum production was according to Lamari and Bernier (1989a). Mycelial plugs, 0.5 cm diameter, from the stock cultures were transferred to 10 cm petri plates containing V8 juice (150 ml), Difco, potato dextrose agar (PDA) (10 g), $CaCo_3$ (3 g), Bacto agar (10 g) and distilled water (850 ml). These cultures were incubated in the dark at 22°C for about 8 days. The plates were then flooded with sterile distilled water, the mycelia were flattened using sterilized glass rods and any excess water was poured off. The plates were incubated under continuous light at room temperature for 24 h to induce conidiophore production and then for about 22 h at 16°C to induce the production of conidia. Conidia were harvested by flooding the plates in sterile distilled water and gently brushing the mycelium with a camel-hair brush to dislodge the conidia from the conidiophores. Ten drops of Tween 20 (polyoxyethylene sorbitan monolaureate) per litre were added to the spore suspension, which was then adjusted to a concentration of approximately 3000 conidia per ml.

Disease screening: Including the standard differential cultivars, about 50 wheat cultivars (*T. aestivum* L.) were screened using two *Ptr* isolates. About 10 seeds per genotype were planted in a pot (10 cm diameter) containing peat moss and placed at a temperature of 20–23°C and a 16 h photoperiod in a greenhouse. Water was supplied by capillary action via holes in the base of the pot. Each cultivar was replicated three times. Seedlings were inoculated at the two-leaf stage and incubated for 24 h in a 2 m × 1.5 m × 1 m portable plastic tent, constructed inside the greenhouse. The tent was further covered by a black plastic sheet to ensure complete darkness. A relative humidity of 100% was maintained using a humidifier during the incubation period. Plants were then transferred into a growth chamber at a temperature of 22°C and a photoperiod of 12 h.

Seven days after inoculation, seedlings were rated for reaction to tan spot using the 1–5 lesion type rating scale developed by Lamari and Bernier (1989a) where, 1 = small dark brown to black spots with very little chlorosis or tan necrosis (resistant); 2 = small dark brown to black spots with very little chlorosis or tan necrosis (moderately resistant); 3 = small dark brown to black spots completely surrounded by a distinct chlorotic or tan necrotic ring; lesions generally not coalescing (moderately resistant to moderately susceptible); 4 = small dark brown or black spots completely surrounded with chlorotic or tan necrotic zones; some of the lesions coalescing (moderately susceptible) and 5 = the dark brown or black centres may or may not be distinguishable, most lesions consist of coalescing chlorotic or tan necrotic zones (susceptible).

Monosomic analysis: The resistance of the spring wheat cultivar 'Salamouni' to race 1 was earlier reported by Lamari and Bernier (1989a,b). However, the location of the resistance gene was not determined. In this study, a set of 21 monosomic lines of the wheat cultivar 'Chinese Spring', which is susceptible to isolate ASC1a, was crossed with 'Salamouni'. A hybrid of disomic 'Chinese Spring' with 'Salamouni' was also made as a control to study the segregation and inheritance of the resistance. Mitotic chromosome counts were made on squashes of root-tip cells pretreated with mono-bromonapthalene and stained by the Feulgen method. For each combination, 30 (10 seeds per pot) of the F_2 hybrids were planted per inoculation. The 17-day-old seedlings were inoculated with isolate ASC1a. A minimum of two inoculations were made, depending on the number of seeds available for each population. Evaluation was carried out 7 days after inoculation following the 1–5 rating scale. In this case, score values (reaction groups) of 1 and 2 were grouped as resistant, while the other reaction groups (3, 4 and 5) were grouped as susceptible. The total number of resistant and susceptible plants for each combination in each inoculation was summed and the frequencies of resistant and susceptible plants in each cross were subjected to chi-squared analysis.

Results

Evaluation of germplasm

As indicated in Table 1, the standard genotypes: 'Red Chief' and XX41 were resistant to all isolates. 'Salamouni' was resistant to most of the isolates, but moderately susceptible to isolates DTR1/2000 and DTR 12/2000. 'Chinese Spring' was susceptible to most isolates but was resistant to isolates 86–124a (race 2), NuBr-1 and Cz1–2. Fifty wheat cultivars, including the standards, were screened using two of the most virulent *Ptr* isolates, ASC1a (race 1) and DW-16 (race 5). The cultivars showed disease reactions ranging from 1 to 5 with mean values of 3.2 and 3.6 for race 1 (ASC1a) and race 5 (DW-16) *Ptr* isolates, respectively (Table 2).

Five cultivars: 'Red Chief' (USA), 'Salamouni' (Lebanon), 'Armada' (United Kingdom), 'Dashen' (Ethiopia) and 'Empire' (United Kingdom) were highly resistant to *Ptr* isolate ASC1a. Seven cultivars (14%) were moderately resistant against race 1 isolate ASC1a whereas only five (10%) were moderately resistant to *Ptr* isolate DW-16. About 76% and 84% of the cultivars were susceptible for isolates ASC1a and DW-16, respectively (Fig. 1).

The resistances of 'Salamouni', 'Red Chief' and 'Erik' to the race 1 isolate were previously reported by Lamari and Bernier (1989a). Singh and Hughes (2005) also confirmed the resistance of 'Red Chief' and 'Erik' using isolate *Ptr* 200 (race 1). In the present study, 'Erik' was moderately resistant to race 1 isolate ASC1a, whereas it was moderately susceptible to the race 5 DW-16 isolate. 'Glenlea', 'Kanzler', 'Lynx' and 'Vicam 71' were highly susceptible to both isolates.

Table 2: Responses of 50 common wheat cultivars to ASC1a (race 1) and DW-16 (race 5) *Pyrenophora tritici-repentis* isolates

	Response to	
Cultivar	ASC1a	DW-16
6B365	2	4
Abo	3	3
Akteur	2	4
Altos	4	4
Amazon	4	5
Armada	1	3
Bussard	3	5
Cardos	3	4
Caribo	3	5
Centauro	2	2
Centrum	4	5
Champtol	3	4
Chinese Spring	4	4
Complet	3	4
Creative	2	4
CWW 926	4	4
Dashen	1	1
Dragon	2	2
Dream	2	3
Empire	1	1
Enorm	4	5
Erik	2	4
Estrella	5	3
Etoile de Choisy	3	4
Euris	2	4
Except	2	4
Flair	4	5
G18/90	3	3
Gb16.92	2	4
Hussar	2	3
Idol	2	3
Kanzler	5	5
Katepwa	5	5
Karpos	4	5
Lynx	5	5
Piko	2	3
Qualibo	2	3
Red Chief	1	1
Redford	4	3
Romanus	3	5
Salamouni	1	2
Septre	4	5
SW Maxi	3	2
Tommi	3	3
Topper	2	4
Travix	2	2
Vicam 71	5	5
Volkom	5	4
Vuka	3	4
Xiayans	4	2
Mean	3.2	3.6
SE of the mean	0.16	0.17

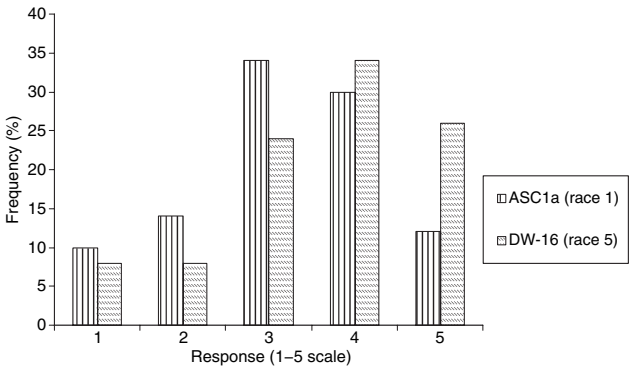

Fig. 1: Distribution of 50 common wheat cultivars to two *Pyrenophora tritici-repentis* isolates

Table 3: Frequencies of resistant and susceptible F_2 seedlings in crosses of 'Chinese Spring' monosomics and 'Salamouni' tested with *Pyrenophora tritici-repentis* isolate ASC1a

F_2 populations	Resistant	Susceptible	χ^2 (1 : 3)
1A/Salamouni	16	45	0.049
2A/Salamouni	18	42	0.800
3A/Salamouni	70	7	178.4*
4A/Salamouni	20	40	2.215
5A/Salamouni	–	–	–
6A/Salamouni	11	49	1.416
7A/Salamouni	16	45	0.049
1B/Salamouni	11	49	1.416
2B/Salamouni	13	47	0.356
3B/Salamouni	13	47	0.356
4B/Salamouni	11	51	1.741
5B/Salamouni	9	54	1.416
6B/Salamouni	15	50	0.128
7B/Salamouni	10	50	2.226
1D/Salamouni	16	44	0.089
2D/Salamouni	20	55	0.111
3D/Salamouni	17	50	0.004
4D/Salamouni	15	54	0.391
5D/Salamouni	15	43	0.023
6D/Salamouni	17	45	0.193
7D/Salamouni	11	63	1.910
Disomic	35	90	0.600
Total (excluding 3A)	309	1013	1.865

*Significant at $P = 0.001$.

Chromosomal location of the resistance gene

Evaluation of the F_2 plants of the hybrids between the 20 monosomic lines of wheat cultivar 'Chinese Spring' and the resistant cultivar 'Salamouni' was carried out using *Ptr* isolate ASC1a to determine the chromosomal location of the resistance gene. The cross with monosomic line 5A failed. The disomic 'Chinese Spring'/'Salamouni' F_2 population segregated in a 1 : 3 Mendelian ratio (Table 3) indicating that resistance in 'Salamouni' was controlled by a single recessive gene. Similar results were reported for 'Salamouni'/'Columbus' by Lamari and Bernier (1989b). Such qualitative inheritance of tan spot was also found previously in different spring and winter wheat cultivars (Lee and Gough 1984, Lamari and Bernier 1989b, 1991, Gamba and Lamari 1998, Lamari et al. 2003, Singh and Hughes 2005), and more recently, in three synthetic wheat lines (Tadesse et al. 2006). However, Elias et al. (1989), Faris et al. (1997), Friesen and Faris (2004) found quantitative inheritance of resistance to this disease in other cultivars.

As indicated in Table 3, the 3A/'Salamouni' F_2 population segregated 70 resistant and seven susceptible plants, deviating significantly ($P < 0.001$) from the expected one resistant to three susceptible ratio. This indicated that the resistance gene in 'Salamouni' (designated *tsn4*) is located on chromosome 3A. The significant segregation ratio also revealed that the gene is hemizygous effective (Knott 1989), which can be explained by the genetic model presented in Fig. 2. Tan spot resistance genes were reported to be non-active recessive genes which confer resistance because of the absence of recognition of the pathogen, as opposed to the gene-for-gene model, where absence of recognition between the pathogen and the host

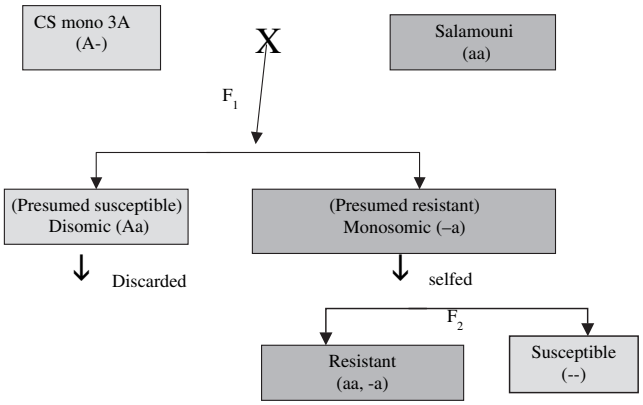

Fig. 2: Schematic representation of the genetic model for a hemizygous recessive-effective gene, *tsn4*, in the critical cross 'Chinese Spring' mono 3A/'Salamouni'

leads to compatibility (susceptibility) (Stzrelkov and Lamari 2003). This was confirmed by inoculating 'Chinese Spring' nulli-3A seedlings (total of ten seedlings) with *Ptr* isolate ASC1a. As expected, they were resistant because the susceptibility locus that recognizes this specific isolate was absent.

When resistance is governed by a single hemizygous recessive effective gene, all 20 non-critical monosomic crosses are heterozygous (Aa) and susceptible, but the critical cross, 'Chinese Spring' mono3A/'Salamouni' was expected to segregate into susceptible disomic (Aa) and resistant monosomic (a-) plants at F_1. Because of limited numbers of F_1 seeds, the F_1 plants were not tested. The F_1 seeds were germinated and any cytologically confirmed monosomic seedlings ($2n = 41$) were planted and selfed. As indicated in Fig. 2 and Table 3, the critical cross segregated in the F_2 generation into 77 resistant (aa, a-) and seven susceptible (–) plants. The seven susceptible plants appeared to be nullisomic (–) for chromosome 3A of 'Salamouni'.

Discussion

In the present study about 50 common wheat cultivars were evaluated for tan spot resistance and some sources of resistance to be used in wheat breeding programmes were identified. Further more, the resistance gene in cultivar 'Salamouni' was localized on chromosome 3A using monosomic analyses. This gene is designated as *tsn4*.

In an investigation of resistance to chlorosis induction produced by race 1 (nec+, chl+) in W-7984/'Opata 85' (International Triticeae Mapping Initiative) population, Faris et al. (1997) identified a QTL, with major effects, on the short arm of chromosome 1A (*QTsc.ndsu-1A*), a minor QTL on the long arm of chromosome 4A, and an epistatic interaction, which together accounted for 49% of the phenotypic variation. The same genes were reported to be responsible for resistance to chlorosis produced by race 3 (nec– chl+) isolates and *Ptr* toxin C (Effertz et al. 2002). Friesen and Faris (2004) have found a QTL on the short arm of chromosome 2B and they have designated it as *tsc*2. Quantitative trait loci's on the chromosome arms of 1BS and 3BL were also identified in the Brazilian wheat cultivar BR34 using *Ptr* races 1–3 and 5, indicating the presence of race-non-specific tan spot resistance (Faris and Friesen 2005). More recently, Tadesse et al. (2006) reported the chromosomal location of a single recessive resistance gene (*tsn*3) located on chromosome 3D of synthetic wheat lines. In addition, they found a dominant gene, located on the same chromosome, in another synthetic wheat line.

Singh and Hughes (2005) carried out allelism studies and found that resistance in the cultivars 'Erik', 'Red Chief', 6B-365 and 'Hadden' was controlled by a single recessive gene, which they suspected to be *tsn1*. They also hypothesized that resistance to necrosis caused by races 1 and 2 in both durum and common wheat was controlled by the same gene, indicating a narrow genetic base for resistance to the necrosis component of tan spot. However, the present result for 'Salamouni' indicated the availability of resistance genes other than *tsn1*. Allelism tests of the cultivars 'Erik', 'Red Chief', 6B-365 or 'Hadden' with 'Salamouni' to test the identity of the gene in one of these cultivars with the gene in 'Salamouni' (*tsn*4) should be undertaken to confirm that they are indeed different. Furthermore, the presently identified resistances in cultivars 'Empire', 'Dashen' and 'Armada' need to be compared with 'Salamouni'.

As shown in Table 1, 'Salamouni' was also moderately resistant to isolate *Ptr* DW-16 (race 5). However, monosomic analysis of the F_2 populations was not carried out using this isolate in the present study because of seed shortage. According to Lamari and Bernier (1989b), genes controlling resistance to the necrosis and chlorosis components are independent. Therefore, resistance in 'Salamouni' to the chlorosis-inducing *Ptr* isolate DW-16 (race 5) could be due to a gene that is different from the presently identified *tsn4*, which protects against the necrosis component of the disease induced by *Ptr* isolate ASC1a.

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References

Cheong, J., H. Wallworks and K. J. Williams, 2004: Identification of a major QTL for yellow leaf spot resistance in the wheat varieties Brookton and Cranbrook. Aust. J. Agric. Res. **55,** 315—319.

Duveiller, E., Y. R. Kandel, R. C. Sharma, and S. M. Shrestha, 2005: Epidemiology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas. Phytopathology **95,** 248—256.

Effertz, R. J., S. W. Meinhardt, J. A. Anderson, J. G. Jordal, and L. J. Francl, 2002: Identification of a chlorosis inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitive locus in wheat. Phytopathology **92,** 527—533.

Elias, E., R. G. Cantrell, and R. M. Jr Hosford, 1989: Heritability of resistance to tan spot in durum wheat and its associations with other agronomic traits. Crop Sci. **29,** 299—304.

Faris, J. D., and T. L. Friesen, 2005: Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. Theor. Appl. Genet. **111,** 386—392.

Faris, J. D., J. A. Anderson, L. J. Francl, and J. G. Jordahl, 1996: Chromosomal location of a gene conditioning insensitivity in wheat to a necrotic inducing culture filtrate from *Pyrenophora tritici-repentis*. Phytopathology **86,** 459—463.

Faris, J. D., J. A. Anderson, L. J. Francl, and J. G. Jordahl, 1997: RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici-repentis* in wheat. Theor. Appl. Genet. **94,** 98—103.

Fernandez, M. R., B. G. McConkey, and R. P. Zentner, 1998: Tillage and summer fallow effects on leaf spot diseases of wheat in the semiarid Canadian Prairies. Can. J. Plant Pathol. **20,** 376—379.

Friesen, T. L., and J. D. Faris, 2004: Molecular mapping of resistance to *Pyrenophora tritici- repentis* race 5 and sensitivity to *Ptr* ToxB in wheat. Theor. Appl. Genet. **109,** 464—471.

Friesen, T. L., J. B. Rasmussen, C. Y. Kwon, S. Ali, L. J. Francl, and S. W. Meinhardt, 2002: Reaction of *Ptr* ToxA-insensitive wheat mutants to *Pyrenophora tritici-repentis* race 1. Phytopathology **92,** 38—42.

Gamba, F. M., and L. Lamari, 1998: Mendelian inheritance of tan spot (*Pyrenophora tritici-repentis*) in selected genotypes of durum wheat (*Triticum durum*). Can. J. Plant Pathol. **20,** 408—414.

Hosford, R. M. Jr, 1982: Tan spot. In: R. M. Jr Hosford (ed.), Tan Spot of Wheat and Related Diseases. 1—24. North Dakota State University, Fargo.

Knott, D. R., 1989: The Wheat Rusts – Breeding for Resistance. Springer-Verlag, Berlin.

Lamari, L., and C. C. Bernier, 1989a: Evaluation of wheat lines and cultivars to tan spot (*Pyrenophora tritici-repentis*) based on lesion type. Can. J. Plant Pathol. **11,** 49—56.

Lamari, L., and C. C. Bernier, 1989b: Toxin of *Pyrenophora tritici-repentis*: host specificity, significance in disease and inheritance of host reaction. Phytopathology **79,** 740—744.

Lamari, L., and C. C. Bernier, 1991: Genetics of tan necrosis and extensive chlorosis in tan spot of wheat caused by *Pyrenophora tritici-repentis*. Phytopathology **81,** 1092—1095.

Lamari, L., S. E. Stzrelkov, A. Yahyaoui, J. Orabi, and R. B. Smith, 2003: The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one-to-one relationship in tan spot of wheat. Phytopathology **93,** 391—396.

Lamari, L., B. D. McCallum, and R. M. dePauw, 2005: Forensic pathology of Canadian bread wheat: the case of tan spot. Phytopathology **95,** 144—152.

Lee, T. S., and F. J. Gough, 1984: Inheritance of Septoria leaf blotch (*S. tritici*) and Pyrenophora (*P. tritici-repentis*) resistance in *Triticum aestivum* cv. Carifen 12. Plant Dis. **68,** 848—851.

Mielke, H., and A. Reichelt, 1999: Studien zur Biologie des Erregers *Drechslera tritici-repentis* zur Anfälligkeit des Weizens und verschiedener Artverwandter sowie zur Bekämpfung der DTR-Weizenblattdürre. Parey Verlag, Berlin.

Nagle, B. J., R. C. Frohberg, and R. M. Jr Hosford, 1982: Inheritance of resistance to tan spot of wheat. In: R. M. Jr Hosford (ed.), Tan Spot of Wheat and Related Diseases. 40—45. North Dakota State University, Fargo.

Perello, A., V. Moreno, M. R. Simon, and M. Sisterna, 2003: Tan spot of wheat infection at different stages of crop development and inoculum type. Crop Prot. **22,** 157—169.

Rees, R. G., G. J. Platz, and R. J. Mayer, 1988: Susceptibility of Australian wheats to *Pyrenophora tritici-repentis*. Aust. J. Agric. Res. **39,** 141—151.

Siedler, H., A. Obst, S. L. K. Hsam, and F. J. Zeller, 1994: Evaluation for resistance to *Pyrenophora tritici-repentis* in *Aegilops tauchii* Coss. and synthetic hexaploid wheat amphiploids. Genet. Resour. Crop. Evol. **41,** 27—34.

Singh, P. K., and G. R. Hughes, 2005: Genetic control of resistance to tan necrosis induced by *Pyrenophora tritici-repentis*, race 1 and race 2, in spring and winter wheat genotypes. Phytopathology **95,** 172—177.

Stzrelkov, S. E., and L. Lamari, 2003: Host-parasite interaction in tan spot (*Pyrenophora tritici-repentis*) of wheat. Can. J. Plant Pathol. **25,** 339—349.

Tadesse, W., S. L. K. Hsam, and F. J. Zeller, 2006: Identification and monosomic analysis of a tan spot resistance gene from synthetic wheat genotypes. Crop Sci. **46,** 1212—1217.

Tekauz, A., E. Mueller, M. Beyene, M. Stulzer, and D. Schultz, 2004: Leaf spot diseases of winter wheat in Manitoba in 2003. Can. Plant Dis. Survey **83,** 73—74.

Wolf, P. F. J., and G. M. Hoffmann, 1993: Zur Biologie von *Drechslera tritici-repentis* (Died.) Shoem. (telomorph *Pyrenophora tritici-repentis* (Died.) Drechsler), dem Erreger einer Blattfleckenkrankheit an Weizen. Z. Pfl.krankh. Pflschutz **100,** 33—48.

Xu, S. S., T. L. Friesen, and A. Mujeeb-Kazi, 2004: Seedling resistance to tan spot and Stagonospora nodorum blotch in synthetic hexaploid wheats. Crop Sci. **44,** 2238—2245.

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ORIGINAL PAPER

Molecular mapping of resistance genes to tan spot [*Pyrenophora tritici-repentis* race 1] in synthetic wheat lines

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Abstract Synthetic wheat lines ($2n = 6x = 42$, AABBDD), which are amphiploids developed from the hybrid between tetraploid wheat (*Triticum turgidum* L., $2n = 4x = 28$, AABB) and *Aegilops tauschii* Coss. ($2n = 2x = 14$, DD), are important sources of resistance against tan spot of wheat caused by *Pyrenophora tritici-repentis*. In the present study, inheritance, allelism and genetic linkage analysis in synthetic wheat lines have been carried out. Segregation analysis of the phenotypic and molecular data in $F_{2:3}$ populations of CS/XX41, CS/XX45, and CS/XX110 has revealed a 1:2:1 segregation ratio indicating that resistance of tan spot in these synthetic lines is controlled by a single gene. Allelism tests detected no segregation for susceptibility among F_1 and F_2 plants derived from intercrosses of the resistance lines XX41, XX45 and XX110 indicating that the genes are either allelic or tightly linked. Linkage analysis using SSR markers showed that all the three genes: *tsn3a* in XX41, *Tsn3b* in XX45 and *tsn3c* in XX110 are clustered in the region around *Xgwm2a*, located on the short arm of chromosome 3D. The linked markers and genetic relationship of these genes will greatly facilitate their use in wheat breeding and deployment of cultivars resistant to tan spot.

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Introduction

Tan spot, caused by the fungus *Pyrenophora tritici-repentis* (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoem, is one of the major foliar diseases of wheat spreading worldwide at an increasing rate, and can cause a yield loss of up to 50% in susceptible wheat cultivars (Hosford 1982; Rees et al. 1988; Riede et al. 1996). According to Duveiller et al. (2005), 20–30% yield loss of wheat was frequently recorded in farmers' fields. Adoption of new farm management practices such as minimum or zero tillage, banning of stubble burning, and intensive wheat after wheat cultivation systems have contributed to the fast spread of the pathogen *Pyrenophora tritici-repentis* (Wolf and Hoffmann 1993; Tekauz et al. 2004).

The development and use of resistant cultivars is regarded as the most cost effective, socially feasible and ecologically safe means of controlling tan spot. Because of the co-evolution of the host and pathogen, however, the deployment of individual resistance genes leads to the emergence of new virulent pathogen mutants. Hence, identification of new resistance sources and pyramiding of more resistance genes in a cultivar are of paramount importance for effective and better genetic control. However, selection of genotypes with such gene combinations via classical genetics and breeding methods is very time consuming and even may be impossible due to the lack of pathogen isolates with specific virulence genes.

On the other hand, the development of molecular markers that are closely associated with the respective resistance genes would enable to pyramidize genes of interest effectively and successfully (Gupta et al. 1999; Huang et al. 2000). Among the different molecular

markers developed to date, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified length polymorphisms (AFLPs) and micro satellites also called simple sequence repeats (SSRs) have been used for gene mapping in wheat. The use of RFLP (Chao et al. 1989; Kam-Morgan et al. 1989) and RAPD (Devos and Gale 1992) for wheat gene mapping, however, is very limited due to their very low level of frequency and polymorhism which may be associated with the polyploid nature, high proportion of repetitive DNA, large genome size and recent origin of hexaploid wheat (Gupta et al. 1999). On the other hand, AFLPs and SSRs have been used extensively for wheat gene mapping (Huang et al. 2000; Hartl et al. 1999; Singrün et al. 2004; Schmolke et al. 2005; Mohler et al. 2005). In particular, SSR loci are employed much more frequently than other markers due to their advantages of higher level of polymorphism, known map location, accuracy, repeatability and PCR-based amplification (Röder et al. 1998; Gupta et al. 1999; Huang et al. 2004).

To date, unlike powdery mildew and rust resistance genes of wheat, only very few sources of tan spot resistance genes are identified and mapped (Faris et al. 1996, 1997; Friesen and Faris 2004; Cheong et al. 2004). Recently, Tadesse et al. (2006a) through monosomic analysis have identified tan spot resistance genes on chromosome 3D in three synthetic wheat lines (XX41, XX45 and XX110) which are amphiploids ($2n = 6x = 42$, AABBDD) developed from the hybrid between tetraploid wheat (*Triticum turgidum* L., $2n = 4x = 28$, AABB) and *Aegilops tauschii* Coss. ($2n = 2x = 14$, DD), the progenitor of wheat D genome. The objectives of the present study were to study tan spot inheritance, allelism among the resistant synthetic wheat lines, and genetic linkage in order to identify molecular markers which could be useful for marker assisted selection (MAS).

Materials and methods

Plant material

Synthetic wheat genotypes: XX41 (a hybrid between *Langdon durum* and *Ae. tauschii*, CI 00017), XX45 (*Langdon durum*/*Ae. tauschii*, RL 5565) and XX110 (*T. dicoccum*, A38/*Ae. tauschii*, CI 33) were developed at the Institute of Plant Breeding, Technical University of Munich, Germany. About 60–66 F_2 derived F_3 families ($F_{2:3}$) originating from crosses between the susceptible cultivar Chinese Spring (CS) and each of the three synthetic lines, XX41, XX45 (highly resistant) and XX110 (moderately resistant) were used to study modes of inheritance and linkage to SSR markers. Crosses among the resistant synthetic lines were made in all possible combinations and their F_1 and F_2 lines were used to study allelism.

Tan spot evaluation

Individual lines of each $F_{2:3}$ families were planted along with the parents at a rate of about ten seeds per row in two rows per pot using pots of 13 cm diameter containing peat moss. The pots were arranged on a box (40 pots/box) and placed on a bench in the greenhouse at a temperature of 20–23°C with 16 h photoperiod. Water was supplied by capillary action via holes in the base of the pots. After 2 weeks, the second leaf from each plant was cut and bulked per family and used for DNA extraction, while the first leaf of each line was inoculated using the most virulent race 1 *Ptr* isolate ASC1b. Inoculum production followed the method of Lamari and Bernier (1989) in a medium prepared from 150 ml V8 juice, 10 g potato dextrose agar (PDA), 3 g $CaCo_3$, 10 g Bacto agar and 850 ml distilled water. Conidia were harvested and diluted approximately to 3,000 spores ml^{-1}. Plants were inoculated using a hand sprayer until runoff and placed into a 2 m × 1.5 m × 1 m portable plastic tent which was further covered by a black plastic sheet to ensure complete darkness for 24 h at a relative humidity of 100% as explained in Tadesse et al. (2006a). The plants were then transferred into a growth chamber at a temperature of 22°C and photoperiod of 12 h for about 7 days. Disease readings were taken on the 7th day post-inoculation using the 1–5 rating scale developed by Lamari and Bernier (1989).

Microsatellite analysis

Genomic DNA was extracted from the second leaf of 2 weeks old seedlings of each lines of the F_3 families and the parental lines using the cetyltrimethyl amonium bromide (CTAB) method as described by Saghai-Maroof et al. (1984). A total of 12 SSR markers from wheat chromosome 3D was screened for polymorphism (Table 1) following the procedure of Huang et al. (2000).

PCR reactions were performed in a PE 9600 thermal cycler in a total volume of 20 µl containing 2 µl of 10 × PCR buffer (50 mmol of KCl, 10 mmol of Tris–HCl, 1.5 mmol of $MgCl_2$, pH 8.3), 2.5 mM of each dNTPs, 2.5 mM of each labelled and unlabelled primer, 1 U Taq DNA polymerase (Qiagen) and 100 ng template DNA. The PCR was programmed at

Table 1 Description of SSR markers tested

Locus	Anealing temperature (°C)	SSR motif	Chromosome arm
Xgwm2	50	$(CA)_{18}$	3DS
Xgwm52	55	$(GT)_4AT$ $(AT)_{20}$	3DL
Xgwm161	60	$(CT)_{15}$	3DS
Xgwm314	55	$(CT)_{25}$	3DL
Xgwm3	55	$(CA)_{18}$	3DL
Xgwm497	55	$(GT)_{29imp}$	3DL
Xgwm645	55	$(GT)_{28}$	3DL
Xbarc1040	55	$(ATCT)_8$	3DS
Xwmc366	55	$(CA)_{12}$	3DL
Xbarc42	55	$(TTA)_{12}$	3DL
Xbarc52	55	$(ATCT)_5$	3DL
Xgwm114	55	$(GA)_{53}$	3DS

an initial denaturation step of 3 min at 95°C followed by 35 cycles of 1 min denaturation at 95°C, annealing at 50, 55 or 60°C (depending on the primer) for 1 min, initial extension at 72°C for 1 min and final extension at 72°C for 15 min. The PCR product was checked along with the molecular weight standard λ*Hind*III and a non-template control by running on 1.5% agrose gel containing 5 μg/μl of ethidium bromide for about 30 min at 5 v/cm. Depending on the intensity of the bands, PCR products were diluted with double distilled water at 1:3 or 1:4 ratio. The samples were mixed with 0.15 μl GenScan-500 TAMRA internal size standard (PE Biosystems) and 0.85 μl formamide dye (98% formamide, 0.01% dextran blue), denatured at 95°C for 2 min and chilled on ice.

Samples were loaded on 5% denaturing polyacrylamide gel (Long Ranger TM, FMC Bioproducts) in 1 × TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3). Electrophoresis was carried out in an ABI Prism™ 377 DNA sequencer (Applied Biosystems) at 1,200V for 1.5 h. ABI collection software version 1.1 was used for raw data collection. Microsatellite fragments were analysed using GENSCAN™ analysis software version 2.1.

Linkage analysis

Linkage between SSR markers and the *tsn3* loci was established with MAPMAKER/EXP, version 3.0b using a LOD value of 3.0 and a maximum distance of 50 cM (Lander et al. 1987). The Kosambi function was applied to convert recombination fractions into map distances (Kosambi 1944), and linkage maps were drawn using the Mapchart software (Voorrips 2002).

Results

Inheritance of tan spot resistance

According to Tadesse et al. (2006a), the F_2 populations of CS/XX41 and CS/XX110 crosses segregated into 1 resistant:3 susceptible (1:3) ratio *to Ptr* race 1 isolate ASC1b, indicating that resistance in XX41 and XX110 is controlled by a single recessive gene. The CS/XX45 F_2 population, on the other hand, segregated into 3 resistant:1 susceptible (3:1) ratio indicating that resistance is controlled by a single dominant gene. The heterozygous (Aa) F_2 plants are susceptible in CS/XX41 and CS/XX110 populations, but resistant in CS/XX45 population. In the present study, inoculation of $F_{2:3}$ seedlings (F_2 derived F_3 families) of each of the three populations (CS/XX41, CS/XX45 and CS/XX110) with the same *Ptr* race 1 isolate ASC1b has resulted in a segregation ratio of 1:2:1 indicating that tan spot resistance in these synthetic lines is controlled by a single gene. The recessive genes in XX41 and XX110 are named as *tsn3a* and *tsn3c*, respectively, while the dominant gene in XX45 is named *Tsn3b*. This is in line with the recommendation in Tadesse et al. (2006a) with a little modification in the naming of the genes

As indicated in Table 2, for the recessive gene *tsn3a*, the CS/XX41 population segregated into 14 AA (homozygous susceptible), 31Aa (heterozygous susceptible) and 17 aa (homozygous resistant), a satisfactory fit for segregation at a single locus ($\chi^2_{1:2:1} = 0.29$, $P = 0.865$ at 2 df). Similarly, CS/XX110 population segregated into 12 AA (homozygous susceptible), 37 Aa (heterozygous susceptible) and 11 aa (homozygous resistant) ($\chi^2_{1:2:1} = 1.71$, $P = 0.425$ at 2 df) for the recessive *tsn3c* gene. On the other hand, the dominant gene, *Tsn3b*, in CS/XX45 population segregated into 12 AA (homozygous resistant), 40 Aa (heterozygous resistant) and 14 aa (homozygous susceptible) ($\chi^2_{1:2:1} = 3.07$, $P = 0.215$ at 2 df). The respective flanking markers in each of the populations, except *Xgwm2b* in population CS/XX45, have also shown a satisfactory fit for a 1:2:1 segregation ratio (Table 2). *Xgwm2b* was dominant in this population, and showed a satisfactory fit for a 1:3 segeregation ratio ($\chi^2_{1:3} = 3.73$, $P = 0.053$ at 1 df).

Allelism studies

As indicated in Table 3, all F_1 and F_2 progenies of the crosses between the resistant synthetic lines in all possible combinations were resistant to the race 1 *Ptr* isolate ASC1b. The lack of segregation into susceptible plants both in the F_1 and F_2 crosses among the three resistant lines indicated that the three genes are very tightly

Table 2 Genotypes inferred from seedling reactions of $F_{2:3}$ families and the corresponding alleles at SSR loci for the CS/XX41, CS/XX45 and CS/XX110 populations

Population	R* gene/ flanking markers	F_2 genotypes					
CS/XX41		AA	Aa	aa	Total	χ^2 (1:2:1, df 2)	P
	Xbarc42	8	30	24	62	8.23	0.016
	tsn3a	14	31	17	62	0.29	0.865
	Xgwm2a	13	36	13	62	1.61	0.447
CS/XX45	*Xgwm2a*	13	40	12	65	3.49	0.175
	Tsn3b	12	40	14	66	3.07	0.215
	Xgwm2b	23	0	42	65	3.73 ($\chi^2$1:3, df 1)	0.053
CS/XX110	Xgwm2a	8	35	17	60	4.35	0.114
	tsn3c	12	37	11	60	1.71	0.425
	Xgwm341	20	22	18	60	4.39	0.111

**R* refers to resistance genes (*tsn3a*, *Tsn3b*, *tsn3c*); AA = homozygous susceptible in CS/XX41 & CS/XX110 populations but homozygous resistant in CS/XX45 population; Aa = segregating; aa = homozygous resistant in CS/XX41 & CS/XX110 populations but homozygous susceptible in CS/XX45 population; df = degrees of freedom

Table 3 Response of F_1 and F_2 populations for resistance to *Ptr* isolate ASC1b in resistant x resistant synthetic wheat crosses

Crosses	Number of F_1 plants		Number of F_2 plants	
	Resistant	Susceptible	Resistant	Susceptible
XX41/XX45	8	0	150	0
XX41/XX110	10	0	200	0
XX45/XX110	8	0	150	0

linked.. The recessive genes, *tsn3a* and *tsn3c*, can also be allelic genes. However, they can be differentiated from one another in their mode of inheritance and differential reactions to differential isolates (Tadesse et al. 2006a). Phenotypically, the recessive genes *tsn3a* (aa) and tsn3c (aa), are highly resistant and moderately resistant to *Ptr* race 1 isolate ASC1b, respectively. The dominant gene *Tsn3b* (AA) showed a highly resistant response.

Linkage analysis and genetic map

A total of 12 SSR markers located on chromosome 3D (Somers et al. 2004; Röder et al. 1998) was screened for polymorphism (Table 1), and 6, 7 and 9 of these markers were found to be polymorphic for CS/XX41, CS/XX45 and CS/XX110 populations, respectively. The SSR locus *Xgwm2* was classified into *Xgwm2a* and *Xgwm2b* since it showed two distinctly different bands in CS and the three resistant synthetic lines. *Xgwm2a* has amplified 126 bp in CS, and 120 bp in XX41, XX45 and XX110 lines. *Xgwm2b*, on the other hand, has amplified 258 bp marker allele in CS, and 256 bp marker allele in XX41 and XX110, but was not amplified in XX45. *Xbarc1040*, *Xgwm2a*, *Xbarc42*, *Xgwm52*, *Xgwm341*, *Xgwm114* were polyorphic in CS/XX41 population. All these markers plus *Xgwm2b* were also found to be polymorphic in CS/XX45 population. *Xbarc1040*, although it was polymorphic in all the three populations, it was linked only in CS/XX110 population. Markers which were polymorhic in CS/XX41 and CS/XX45, except *Xgwm114*, were also polymorphic and linked in CS/XX110. Electropherograms showing the variation between the parents, and some selected homozygous and heterozygous lines of CS/XX41, CS/XX45 and CS/XX110 populations for some selected markers are indicated in Fig 1a, b, and c, respectively.

As shown in Fig. 2, all the three genes: *tsn3a* in CS/XX41, *Tsn3b* in CS/XX45 and *tsn3c* in CS/XX110 were clustered in a region around *Xgwm2a*, which showed 120 bp marker allele in CS, and 126 bp in XX41, XX45 and XX110. *Xbarc42*, *Xgwm2b* and *Xgwm341* were the other flanking markers for *tsn3a*, *tsn3b* and *tsn3c*, respectively. *Xgwm2a* was the closest marker to *Tsn3b* and *tsn3c* at genetic distances of 14.4 and 9.5 cM, respectively. *Xbarc42* with a linkage distance of 11 cM was the closest marker to *tsn3a*.

Maps for CS/XX41 and CS/XX45 differed in the order of SSR loci *Xbarc42*, *Xgwm52* and *Xgwm341*. Furthermore, *Xgwm2b* was not polymorphic in CS/XX41. Maps for CS/XX45 and CS/XX110 varied in order of markers *Xgwm2b* and *Xgwm341*. *Xgwm2b* was dominant in CS/XX45 showing only the 258 bp from CS, but co-dominant in CS/XX110 amplifying marker alleles of 258 and 256 bp in CS and XX110, respectively. Furthermore, markers *Xgwm161*, *Xbarc52*, *Xbarc1040* were not linked in CS/XX45 and CS/XX41.

Discussion

Synthetic wheat genotypes ($2n = 6x = 42$, AABBDD) which are amphiploids developed from the hybrid

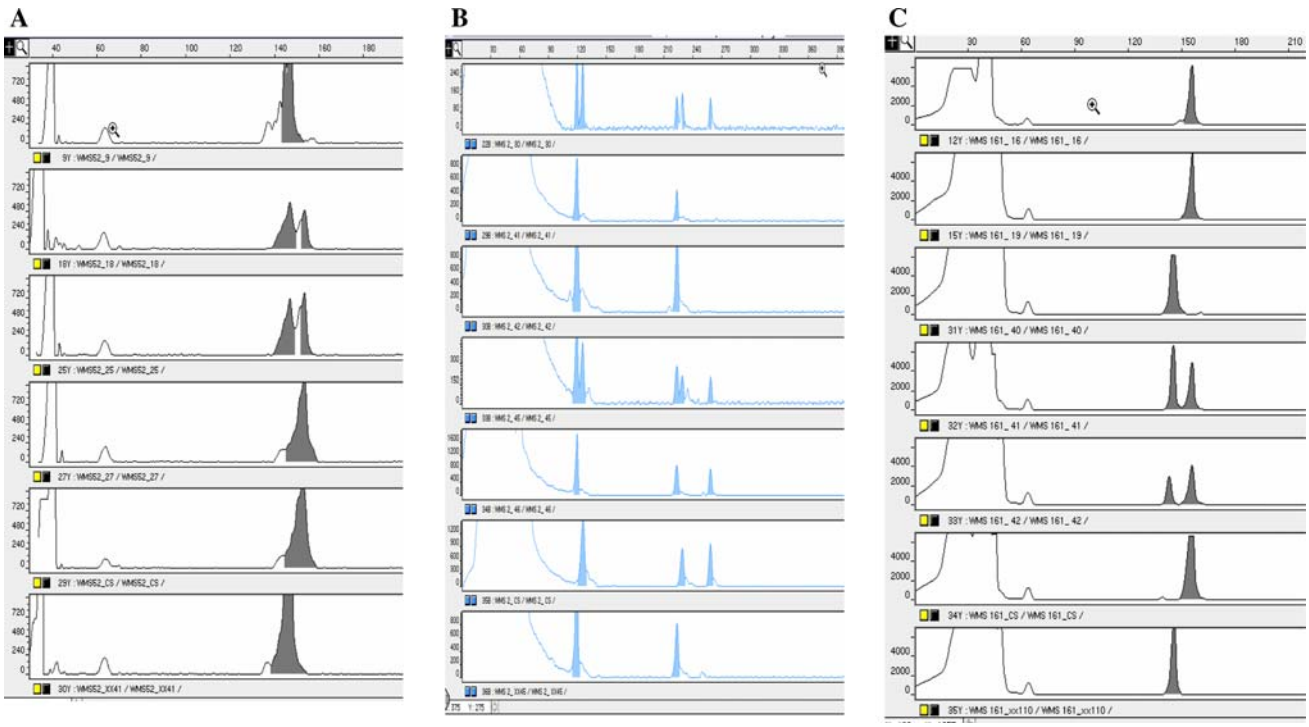

Fig. 1 Electropherograms showing polymorphism in: CS, XX41 and some selected lines of CS/XX41 population using *Xgwm52* (**a**); CS, XX45, and some selected lines of CS/XX45 population using *Xgwm2a* (**b**); CS, XX110 and some selected lines of CS/XX110 population using *Xgwm161* (**c**)

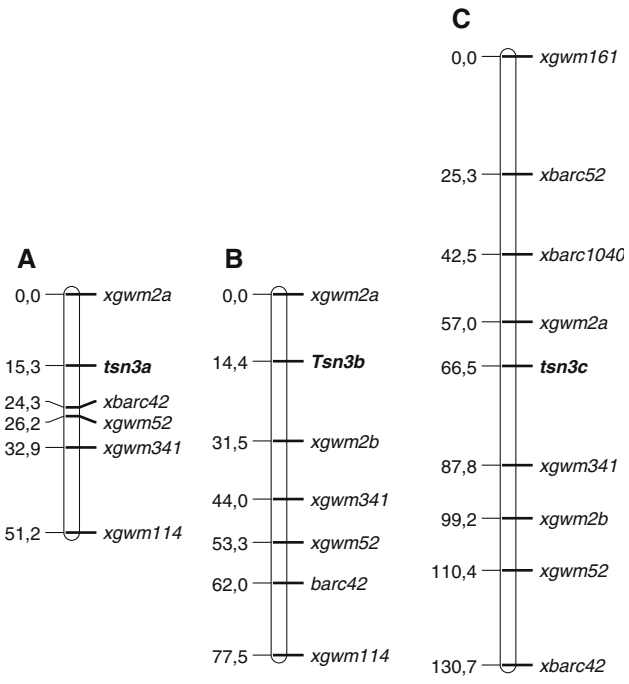

Fig. 2 Microsatellite linkage maps showing *tsn3* genes on chromosome 3D in the synthetic populations: CS/XX41 (**a**), CS/XX45 (**b**) and CS/XX110 (**c**). Locus names and map distances (*cM*) are indicated on the right and left sides of the maps, respectively

between tetraploid wheat (*Triticum turgidum* L., $2n = 4x = 28$, AABB) and *Aegilops tauschii* Coss. ($2n = 2x = 14$, DD) have a large degree of genetic variation for resistance to different wheat diseases in general, and to tan spot of wheat in particular (Siedler et al. 1994; Xu et al. 2004; Tadesse et al. 2006a). The source of resistance in the synthetic lines XX41, XX45 and XX10 was identified to be the *Aegilops tauschii* parents (Tadesse et al. 2006a).

In the present study, inheritance, allelism and genetic maps of tan spot resistance genes in synthetic wheat lines were investigated. Inheritance reports for tan spot resistance ranged from qualitative to quantitative. Some researchers (Nagle et al. 1982; Elias et al. 1989; Faris et al. 1997; Effertz et al. 2002) reported quantitative inheritance, while others (Lee and Gough 1984; Lamari and Bernier 1991; Gamba and Lamari 1998; Lamari et al. 2003; Singh and Hughes 2005) have reported the inheritance of tan spot to be qualitative, controlled by single major recessive genes. More recently, Tadesse et al. (2006a, b) have also reported qualitative inheritance from the F_2 segregation analyses of the disomic Chinese Spring/synthetic populations and CS/Salamouni crosses. The current study using the $F_{2:3}$ CS/synthetic lines has also confirmed qualitative inheritance of tan spot resistance in synthetic wheat lines.

The lack of segregation in the F_1 and F_2 populations for the *Ptr* isolate ASC1b in the crosses between the

resistant synthetic lines indicated all the three genes (*tsn3a*, *Tsn3b* and *tsn3c*) belong to the same resistant gene cluster. The recessive genes *tsn3a* and *tsn3c* can also be allelic while the dominant gene *Tsn3b* is a tightly linked gene. Singh and Hughes (2005) have also reported allelism among winter wheat cultivars for tan spot resistance using *Ptr* race 1isolate. Resistance genes occurring as a single gene with one or more alleles encoding different resistance specificities have been reported in many crops. In wheat, a total of ten different resistance specificities (*Pm3a* to *Pm3j*) against powdery mildew has been reported at the *Pm3* locus on the short arm of chromosome 1A (Zeller and Hsam 1998; Hsam et al. 1998). Allelic/linked genes have been also reported for wheat leaf rust resistance (Singh et al. 2004), resistance to Russian wheat aphid (Miller et al. 2001; Liu et al. 2005) and resistance to flax rust (Ellis et al. 1997).

Linkage analysis has also shown that the genes in all the three populations are located in the vicinity of *Xgwm2a* (Fig. 2). Maps for CS/XX41 and CS/XX45 differed in the order of SSR loci *Xbarc42*, *Xgwm52* and *Xgwm341* which is probably due to a single inversion. Furthermore, *Xgwm2b* was not polymorphic in CS/XX41. More number of markers were polymorphic and linked in CS/XX110 than in CS/XX41 and CS/XX45 populations. The difference in the order of *Xgwm2b* and *Xgwm341* for the CS/XX45 and CS/XX110 maps may be due to the variation in the informativeness of the marker in these two populations. The order of markers *Xbarc42*, *Xgwm52*, *Xgwm341* in the present genetic map for CS/XX41 was in line with the consensus map (Somers et al. 2004). However, their position was inverted in the maps for CS/XX45 and CS/XX110 populations. Such variations in the location of markers between the genetic and consensus maps were also reported recently by Wang et al. (2006).

In general, this variation in the order of some markers among the maps in CS/XX41, CS/XX45 and CS/XX110 may be due to the low number of F_3 lines tested, the difference in populations, and the position of crossovers along chromosomes within the progeny lines (Somers et al. 2004). The relatively wide gap between some of the markers can also be associated to the low number of SSR markers available on the D genome as compared to the A and B genomes of wheat.

This is the first report of mapping allelic/linked genes for tan spot resistance in the D genome of wheat using SSR markers. However, SSR markers were used to map allelic genes to powdery mildew of wheat indicating that they are ideal for comparative mapping of alleles at the same gene locus in different mapping populations (Singrün et al. 2004; Huang et al. 2004).

Most of the tan spot resistant genes reported to date were located in the B genome of hexaploid wheat. Faris et al. (1996) mapped the resistant locus *tsn-1* on the long arm of 5B using restriction fragment length polymorphism (RFLP) markers. The *tsn-1* gene is recently fine mapped and markers which are important for cloning of this gene are identified (Haen et al. 2004). A major QTL designated as *tsc*2, which is located on the short arm of chromosome 2B, was reported by Friesen and Faris (2004). Cheong et al. (2004) have also identifed a major QTL on 5BL, which actually is expected to be the same as *tsn-1*, in the Australian cultivar Brookton. More recently, Faris and Friesen (2005) have identified QTL on chromosome arms 1BS and 3BL in cultivar BR34 using *Ptr* races 1–3 and 5 indicating presence of race- nonspecific tan spot resistance. Recently, Singh et al. (2006) have identified *tsn2* on the long arm of chromosome 3B using race 3 *Ptr* isolate in tetraploid wheat, which actually may be the same gene reported as QTL on 3BL by Faris and Friesen (2005). There are few reports of tan spot resistance in the A genome of wheat. A major QTL on the short arm of chromosome 1A (*QTsc.ndsu-1A*), and a minor QTL on the long arm of chromosome 4A were reported in W-7984/Opata85 population (Faris et al. 1997). Recently, Tadesse et al. (2006b) have located the *tsn4* gene in the spring wheat cultivar Salamouni on chromosome 3A.

In conclusion, the absence of susceptible plants both in the F_1 and F_2 lines of the different intercrosses among the resistant parental lines in the allelism tests, and linkage analysis using SSR markers showed that all the three genes: *tsn3a* in XX41, *Tsn3b* in XX45 and *tsn3c* in XX110 are closely linked genes clustered in the vicinity of *Xgwm2a*, located on the short arm of chromosome 3D. The linked markers and genetic relationship of these genes will greatly facilitate their use in wheat breeding and deployment of tan spot resistant cultivars. As the currently available SSR markers in the D genome of wheat are limited, it is advisable to carry out fine mapping in the future when more markers are developed on wheat chromosome 3D in order to effectively delimit the genomic region containing the *tsn3* genes for cloning purpose.

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References

Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat

homeologous group 7 chromosomes. Theor Appl Genet 84:495–504
Cheong J, Wallwork H, Williams KJ (2004) Identification of a major QTL for yellow leaf spot resistance in the wheat varieties Brookton and Cranbrook. Aust J Agric Res 55:315–319
Devos KM, Gale MD (1992) The use of random amplified polymorphic DNA markers in wheat. Theor Appl Genet 84:567–572
Duveiller E, Kandel YR, Sharma RC, Shrestha SM (2005) Epidemiology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas. Phytopathology 95:248–256
Effertz RJ, Meinhardt SW, Anderson JA, Jordal JG, Francl LJ (2002) Identification of a chlorosis inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitive locus in wheat. Phytopathology 92:527–533
Elias E, Cantrell RG, Hosford Jr RM (1989) Heritability of resistance to tan spot in durum wheat and its associations with other agronomic traits. Crop Sci 29:299–304
Ellis JG, Lawrence G, Ayliffe M, Anderson P, Collins N, Finnegan J, Frost D, Luck J, Pryor T (1997) Advances in the molecular genetic analysis of the flax-flax rust interaction. Annu Rev Phytopathol 35:271–291
Faris JD, Friesen TL (2005) Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. Theor Appl Genet 111:386–392
Faris JD, Anderson JA, Francl LJ, Jordahl JG (1996) Chromosomal location of a gene conditioning insensitivity in wheat to a necrotic inducing culture filtrate from *Pyrenophora tritici-repentis*. Phytopathology 86:459–463
Faris JD, Anderson JA, Francl LJ, Jordahl JG (1997) RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici-repentis* in wheat. Theor Appl Genet 94:98–103
Friesen TL, Faris JD (2004) Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to *Ptr* ToxB in wheat. Theor Appl Genet 109:464–471
Gamba FM, Lamari L (1998) Mendelian inheritance of tan spot (*Pyrenophora tritici-repentis*) in selected genotypes of durum wheat (*Triticum durum*). Can J Plant Pathol 20:408–14
Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their application in wheat breeding. Plant Breed 118:369–390
Haen KM, Lu HJ, Friesen TL, Faris JD (2004) Genomic targeting and high-resolution mappingof the *tsn1* gene in wheat. Crop Sci 44:951–962
Hartl L, Mohler V, Zeller FJ, Hsam SLK, Schweizer G (1999) Identification of AFLP markers closely linked to the powdery mildew resistance genes *Pm1c* and *Pm4a* in common wheat (*Triticum aestivum* L). Genome 42:322–329
Hosford Jr RM (1982) Tan spot. Pages 1–24. In: RM Hosford Jr. (ed), Tan spot of wheat and related diseases. North Dakota State University. P 116
Hsam SLK, Huang XQ, Ernst F, Hartl L, Zeller FJ (1998) Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.). 5. Alleles at the *Pm1* locus. Theor Appl Genet 96:1129–1134
Huang XQ, Hsam SLK, Zeller FJ, Wenzel G, Mohler V (2000) Molecular mapping of the wheat powdery mildew resistance gene *Pm24* and marker validation for molecular breeding. Theor Appl Genet 101:407– 414
Huang XQ, Hsam SLK, Mohler V, Röder MS, Zeller FJ (2004) Genetic mapping of three alleles at the *Pm3* locus conferring powdery mildew resistance in common wheat (*Triticum aestivum* L.). Genome 47:1130–1136
Kam-Morgan LNW, Gill BS, Muthukrishnan S (1989) DNA restriction fragment length polymorphisms: a strategy for genetic mapping of D genome of wheat. Genome 32:724–732
Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175
Lamari L, Bernier CC (1989) Evaluation of wheat lines and cultivars to tan spot (*Pyrenophora tritici-repentis*) based on lesion type. Can J Plant Pathol 11:49–56
Lamari L, Bernier CC (1991) Genetics of tan necrosis and extensive chlorosis in tan spot of wheat caused by *Pyrenophora tritici-repentis*. Phytopathology 81:1092–1095
Lamari L, Strelkov SE, Yahyaoui A, Orabi J, Smith RB (2003) The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one-to-one relationship in tan spot of wheat. Phytopathology 93:391–396
Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181
Lee TS, Gough FJ (1984) Inheritance of Septoria leaf blotch (*S. tritici*) and Pyrenophora (*P. tritici-repentis*) resistance in *Triticum aestivum* cv. Carifen 12. Plant Dis 68:848–851
Liu XM, Smith CM, Friebe BR, Gill BS (2005) Molecular mapping and allelic relationships of Russian wheat aphid-resistance genes. Crop Sci 45:2273–2280
Miller AC, Altinkut A, Lapitan NLV (2001) A microsatellite marker for tagging *Dn2*, a wheat gene conferring resistance to the Russian wheat aphid. Crop Sci 41:1584–1589
Mohler V, Zeller FJ, Wenzel G, Hsam SLK (2005) Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L.em Thell). 9. Gene *MIZec1* from *Triticum dicoccoides*-derived wheat line Zecoi-1. Euphytica 142:161–167
Nagle BJ, Frohberg RC, Hosford Jr RM (1982) Inheritance of resistance to tan spot of wheat. In: Hosford RM Jr (eds) Tan spot of wheat and related diseases. State University, North Dakota, pp 40–45
Rees RG, Platz GJ, Mayer RJ (1988) Susceptibility of Australian wheats to *Pyrenophora tritici-repentis*. Aust J Agric Res 39:141–151
Riede CR, Francl LJ, Anderson JA, Jordahl JG, Meinhardt SW (1996) Additional sources of resistance to tan spot of wheat. Crop Sci 36:771–777
Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatilite map of wheat. Genetics 149:2007–2023
Saghai-Maroof MA, Soliman KM, Jorgenson RA, Allard RW (1984) Ribosomal spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8014–8018
Schmolke M, Zimmermann G, Buerstmayr H, Schweizer G, Miedaner T, Korzun V, Ebmeyer E, Hartl L (2005) Molecular mapping of Fusarium head blight resistance in the winter wheat population Dream/Lynx. Theor Appl Genet 111:747–756
Siedler H, Obst A, Hsam SLK, Zeller FJ (1994). Evaluation for resistance to *Pyrenophora tritici-repentis* in *Aegilops tauchii* Coss. and synthetic hexaploid wheat amphiploids. Genet Resour Crop Evol 41:27–34
Singh PK, Hughes GR (2005) Genetic control of resistance to tan necrosis induced by *Pyrenophora tritici-repentis*, race 1 and race 2, in spring and winter wheat genotypes. Phytopathology 95:172–177
Singh S, Franks CD, Huang L, Brown-Guedira GL, Marshal DS, Gill BS (2004) Lr41, Lr39 and a leaf rust resistance gene from *Aegilops cylinderica* may be allelic and are located on wheat chromosome 2DS. Theor Appl Genet 108:586–591
Singh PK, Gonzalez-Hernandez JL, Mergoum M, Ali S, Adhikari TB, Kianian SF, Elias EM, Hughes GR (2006) Identification

and molecular mapping of a gene confering resistance to *Pyrenophora tritici-repentis* race 3 in tetraploid wheat. Phytopathology 96:885–889

Singrün C, Hsam SLK, Hartl L, Zeller FJ, Mohler V (2004) Localization of a novel recessive powdery mildew resistance gene from common wheat line RD30 in the terminal region of chromosome 7AL. Theor Appl Genet 109:210–214

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Tadesse W, Hsam SLK, Wenzel G, Zeller FJ (2006a) Identification and monosomic analysis of tan spot resistance genes in synthetic wheat lines (*Triticum turgidum* L. x *Aegilops tauschii* Coss.). Crop Sci 46:1212–1217

Tadesse W, Hsam SLK, Zeller FJ (2006b) Evaluation of common wheat cultivars for tan spot resistance and chromosomal location of a resistance gene in the cultivar 'Salamouni'. Plant Breed 125:318–322

Tekauz A, Mueller E, Beyene M, Stulzer M, Schultz D (2004) Leaf spot diseases of winter wheat in Manitoba in 2003. Can Plant Dis Survey 83:73–74

Voorrips RE (2002) MapChart:Software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Wang T, Xu SS, Harris MO, Hu J, Liu L, Cai X (2006) Genetic characterization and molecular mapping of Hessian fly resistance genes derived from *Aegilops tauschii* in synthetic wheat. Theor Appl Genet 113:611–618

Wolf PFJ, Hoffmann GM (1993) Zur Biologie von *Drechslera tritici-repentis* (Died.) Shoem. (telomorph *Pyrenophora tritici-repentis* (Died.) Drechsler), dem Erreger einer Blattfleckenkrankheit an Weizen. Z Pfl Krankh Pflschutz 100:33–48

Xu SS, Friesen TL, Mujeeb-Kazi A (2004) Seedling resistance to tan spot and *Stagonospora nodorum* blotch in synthetic hexaploid wheats. Crop Sci 44:2238–2245

Zeller FJ, Hsam SLK (1998) Progress in breeding for resistance to powdery mildew in common wheat (*Triticum aestivum* L). In AE Slinkard (ed) Proceedings of the 9th international wheat genetics symposium, vol 1. University extension press, University of Saskatchewan, Saskatoon, pp 178–180