Belayneh Admassu Yimer

Genetic and virulence diversity of *Puccinia graminis* f. sp. *tritici* populations in Ethiopia and stem rust resistance genes in wheat

Cuvillier Verlag Göttingen
Internationaler wissenschaftlicher Fachverlag

Institute of Crop Science & Plant Breeding I
Head: Prof. Dr. Dr. h.c. W. Friedt
and
Julius Kuehn-Institute (JKI), Federal Research Centre for Cultivated Plants,
Institute for Resistance Research and Stress Tolerance

Genetic and virulence diversity of *Puccinia graminis* f. sp. *tritici* populations in Ethiopia and stem rust resistance genes in wheat

Inaugural Dissertation for the Achievement of the Degree
'Doctor of Agricultural Sciences'
At the Faculty of Agricultural and Nutritional Sciences, Home Economics and Environmental Management

Justus-Liebig-University Gießen

Submitted by
Belayneh Admassu Yimer
Born in Zigem, Gojjam, Ethiopia

Gießen, March 2010

Bibliografische Information der Deutschen Nationalbibliothek
Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über http://dnb.d-nb.de abrufbar.
1. Aufl. - Göttingen : Cuvillier, 2010
Zugl.: Gießen, Univ., Diss., 2010

978-3-86955-293-4

Board of Examiners

Chairman of the Committee	Prof. Dr. Steffen Hoy
1. Referee	Prof. Dr. Dr. h.c. Wolfgang Friedt
2. Referee	PD. Dr. Frank Ordon
Examiner	Prof. Dr. Sylvia Schnell
Examiner	Prof. Dr. Bernd Honermeier

Date of oral examination: 19.03.2010

Nonnenstieg 8, 37075 Göttingen
Telefon: 0551-54724-0
Telefax: 0551-54724-21
www.cuvillier.de

1. Auflage, 2010
Gedruckt auf säurefreiem Papier

978-3-86955-293-4

Berichte aus der Agrarwissenschaft

Belayneh Admassu

Genetic and virulence diversity of *Puccinia graminis* f. sp. *tritici* populations in Ethiopia and stem rust resistance genes in wheat

D26 (Dis. University of Giessen)

Cuvillier Verlag

Goettingen 2010

Dedication

To

Michael S. Admassu, Bisrat Workneh, Kidist Workneh

Nobel A. Shebeshe and Beza Belayneh

– I hope this will inspire you for a better work!

and

Admassu Yimer Hailu (1921 – 1991)

Table of Contents

Chapter 1

General Introduction

Wheat production in Ethiopia

The cultivation of wheat (*Triticum aestivum* L.) dates back about 8,000 years (Gill and Friebe 2001). It originated in the western part of Asia, gradually spreading to nearly all other regions of the world. Wheat is one of the principal cereal grains together with maize and rice that are most important and common food staples in the world. Global wheat production in 2008 reached over 600 million tons (FAO 2008). Of this, around 20% is traded on international markets. Developing countries account for about 80% of the imports. This is mainly because, despite a relatively large wheat output in the developing world, overall consumption outpaces production. China and the United States are the largest wheat producing and exporting countries in the world, respectively.

Ethiopia is the second largest wheat producer in sub-Saharan Africa next to South Africa. Wheat in Ethiopia is grown primarily as a rain-fed crop by subsistence farmers in mid- to highland areas that range from 1500 to 3000 m (White et al. 2001). The most suitable areas for production, however, fall between 1900 to 2700 m (Gebre Mariam 1991). It is an important food grain cultivated on ca. 1.4 million ha (CSA 2006) making it one of the major cereal crops grown in the country. The current acreage of wheat is very minimal compared to the total area classified as suitable for wheat production (Gebre Mariam 1991). However, in recent years wheat production is on the rise, and has drastically increased in the last couple of years to replace barley and sorghum at the top (CSA 1995; CSA 2006). The national agricultural extension package programme, which was introduced in the early 1990s, has played a significant role in expanding the area of wheat production and increasing its productivity through the use of improved agricultural technologies such as: selected seeds, fertilizers, pesticides and improved agronomic practices.

Wheat in Ethiopia is mainly represented by two species, bread wheat (*Triticum aestivum* L. em. Thell) and durum wheat (*T. turgidum* var. *durum* Defs.). Other wheat species like *T. dicoccum* (emmer wheat), *T. polonicum* and *T. aethiopicum* are also cultivated in small pocket

areas or in mixtures with other wheat species (Porceddu and Perinno 1973). Ethiopia is also the center of diversity for tetraploid wheat (*T. turgidum*), and an important source of landraces for quality traits like disease resistance (Klindworth et al. 2007; Bonman et al. 2007), yield (Teklu and Hammer 2009) and baking quality (Hailu and Merker 2008). Bread and durum wheat cover about half of the wheat production area each (Badebo 2002). Bread wheat production, nevertheless, is on the increase when it is compared to its share of 15% in 1967 (Gebre Mariam 1991). Bread wheat in Ethiopia is used to make bread and other traditional food items and drinks. The Ethiopian highlands also offer both climatic and geological conditions for the production of high quality durum wheat that is mainly suitable for making pasta, macaroni, and other manufactured products. In addition to the grain, the straw is an important source of animal feed and is used as a construction material for roofing of thatched houses in rural areas.

Ethiopia used to produce surplus wheat and to export excess grain till the early 1970s (EARO 2000). Since then, the growing demand for wheat due to significant changes in dietary habits in favor of wheat, and the imbalance between population growth and productivity made the country a net grain importer of wheat (Mohammed 1989). This trend has continued, where the supply is short by 30 to 50% of the annual wheat grain demand (White et al. 2001).

Although the productivity of wheat has increased in the last couple of years, it is still very low compared to other wheat producing countries. The national average productivity is estimated at 1.7 tons/ha (CSA 2006). The low productivity is attributed to a number of factors including: biotic (diseases, insect pests and weeds), abiotic (moisture, soil fertility, etc.) and low adoption of new agricultural technologies due to technical, socioeconomic, and institutional problems (Zegeye et al. 2001). Among these factors, diseases play a significant role in yield reduction.

Stem rust (*Puccinia graminis* f. sp. *tritici*) and other wheat diseases in Ethiopia

Wheat is susceptible to many diseases including the highly destructive ones like rusts (*Puccinia spp.*), powdery mildew (*Erysiphe graminis* f. sp. *tritici*), leaf blotches (*Septoria tritici*), Fusarium head blight (*Fusarium graminearum*), tan spot (*Pyrenophora tritici-repentis*) and smut (*Ustilago tritici*) (Prescott et al. 1986). As a result, most breeding programmes focus on improving existing varieties or to obtain new ones with such dominant characteristics as disease resistance and higher yield. Over 30 diseases have been reported on

wheat in Ethiopia (Bekele 1985). Of these, fungal diseases like rusts (stem, stripe and leaf rust), Fusarium head blight (FHB), Septoria blotch, *Helmenthosporium spp*., and tan spot are the dominant ones that were reported persistently over time (Yirgou 1967; Bekele 1985; Gebeyehu et al. 1990; Badebo 2002; CIMMYT 2005). Their importance however has changed from time to time. For example, yellow rust and FHB were the major diseases while stem rust was rarely found in the Arsi and Bale region in 1988 (Gebeyehu et al. 1990) (Fig. 1). In later years, the prevalence and severity of stem rust increased significantly to become a major threat to wheat production in Arsi, Bale and other wheat growing regions of Ethiopia (CIMMYT 2005; Admassu et al. 2009) while the importance of FHB decreased significantly to become a minor disease in Arsi and Bale. However, FHB is still causing some trouble in the northwest part of the country. In addition to fungal diseases, bacterial leaf streak and black chaff caused by *Xanthomonas translucens*, and viral diseases like barley yellow dwarf virus infect wheat at very low levels (PPRC 2000). Recently, unidentified root rot disease has emerged as a serious threat to wheat production in central and western parts of the country. It is also important to note that yellow rust has began to appear in mid-to low altitude areas at an alarming level. Traditionally, yellow rust was a problem only in high altitude (above 2400 m) wheat growing areas.

Wheat stem rust, also known as black rust, caused by the fungus *Puccinia graminis* f. sp. *tritici Ericks* and Henn (*Pgt*) is a serious disease affecting wheat, barley, rye and other grass weed species. It is the most prevalent of all the wheat rust diseases occurring in nearly all areas where wheat is grown (Roelfs et al. 1992). It had caused serious epidemics in North America in the 1950s causing yield losses of up to 50% (Roelfs 1978). Losses incurred due to rusts in Australia in 1999 were estimated at AUD $ 50 million (Park 2008). Yield loss due to stem rust in Ethiopia was estimated to reach up to 70% on susceptible wheat cultivars at times of disease epidemics (Bechere et al. 2000). According to Leppik (1970) Ethiopia is one of the hot spot areas of the wheat stem rust complex. Stem rust epidemics outbreaks in the early 1970s and 1993/4 in Ethiopia have put two resistant bread wheat varieties, Lacketch and Enkoy out of production, respectively (Shank 1994; Badebo 2000). The disease had since then subsided until the appearance of a new virulent race named Ug99 that overcomes the previously effective stem rust resistance gene *Sr31*. Although Ug99 in Ethiopia was first reported in 2005 in a few locations (Wanyera et al. 2006), it has now become the dominant race across all regions (Admassu et al. 2009).

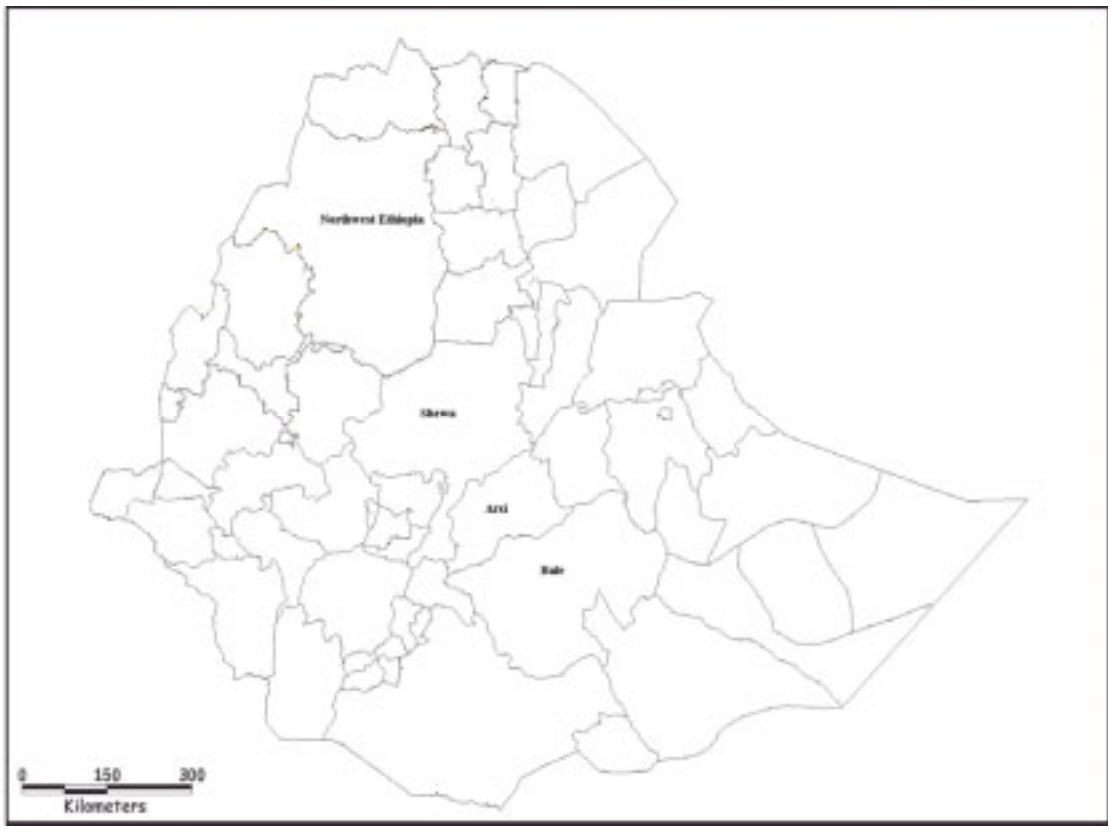

Fig. 1 Map of Ethiopia showing locations of the four major wheat producing regions (Bale, Arsi, Shewa and Northwest Ethiopia) from where stem rust samples were collected for the study

The origin and spread of Ug99

Historically stem rust used to be a major disease of wheat throughout the wheat growing regions of the world (Roelfs et al. 1992; CIMMYT 2005). The major epidemics that occurred in Australia in the 1940s and in the United States in the early-1950s are some to mention (Luig and Watson 1972; Roelfs 1986; Saari and Prescott 1985). Since then, the widespread use of resistant cultivars worldwide made stem rust to be a minor disease and reduced the importance of the disease as a significant factor in production. However, it continued to have local importance in South and North African countries, Far East and South American countries, and remained a major disease in East Africa (Roelf et al. 1992).The stem rust resistance gene *Sr31* in Kavkaz and similar wheats with the 1BL.1RS translocation remained effective to all known *Pgt* races until 1999 when the first virulence was detected in Uganda in a race known today as Ug99 (Pretorius et al. 2000). Later on several variants were identified within this race group with virulence on *Sr24*, *Sr36*, and other avirulence/virulence

combinations (Jin et al. 2008b; Jin et al. 2009). According to Jin et al (2008a) Ug99 and its variants in East Africa represent a distinct genetic lineage and that new variants may have arisen via mutation. This race is now widespread in the eastern African highlands and was also detected in Yemen and Iran (Singh et al. 2008; Nazari et al. 2009). At the moment genes that confer seedling and/or adult plant resistance to Ug99 include *Sr2, Sr13, Sr14, Sr22, Sr28, Sr29, Sr32, Sr33, Sr35, Sr37, Sr39, Sr40, Sr44 and SrTmp* (Singh et al. 2008).

Diversity of stem rust (*Puccinia graminis* f. sp. *tritici*)

The cereal rust pathogens, especially, the wheat rust pathogens (*Puccinia spp.*) are known for their high variability (Stakmann 1962; Leonard 2001). This variability encompasses morphological, virulence and molecular. Spore morphology variation has importance in the taxonomy of rust pathogens (Roelfs et al. 1992), whereas the virulence and molecular variations have practical implications in breeding for resistance. For example, a change in virulence in a pathotype can render a previously effective resistance gene ineffective as has been documented in many occasions (Stakmann 1957; Pretorius et al. 2000; Leonard 2001; Jin et al. 2009).

Differentiation of *Pgt* into races was first done in the early 1920s using a set of 12 different wheat species in the United States (Stakmann and Levine 1922). Since then, there have been a lot of efforts to understand the virulence and genetic structure of *Pgt* in many countries (Stakmann 1962; Roelfs and Martens 1988; Singh 1991; Jin 2005; Fetch 2005; Keiper et al. 2006; Admassu et al. 2009). The number and type of wheat lines used, and the race nomenclature system were modified throughout time (Stakmann et al. 1962; Roelf and Martens 1988; Fetch and Dunsmore 2004). Currently 20 near-isogenic lines are used to distinguish one race from another (Jin et al. 2008b). Many countries follow the nomenclature system developed by Stakmann and Levine (1922), and modified by Roelf and Martens (1988) and Jin et al. (2008b). However, Australia and South Africa have developed their own differential lines and nomenclature system to designated *Pgt* races (McIntosh et al. 1995).

Many studies showed the richness of *Pgt* in terms of virulence and genetic diversity. In the United States hundreds of *Pgt* races were recorded in the early 1920s that arose as a result of recombination on the alternate host (APS 2009). The average number of *Pgt* races found per year continuously declined in the next decades due to the eradication of the alternate host, and the pathogen population has stabilized at a limited number of races (Roelf 1982; Leonard

2001; Jin 2005). The same is true in Canada where in earlier times up to 63 races were identified (Green 1971). However, in recent years the average number of races found declined to about 10 races per year (Fetch and Dunsmore 2004; Fetch 2005). As a result, no major shift in the virulence structure was observed for decades. In Mexico where stem rust remains under control because of the use of resistant cultivars, race analysis studies in the 1980s showed the presence of six races that might have been the same as those prevalent in the 1950s (Singh, 1991). East Africa, consisting of Ethiopia, Kenya and Uganda, is the other important stem rust prone region. Recent studies showed that *Pgt* races are more diverse in this region than in other parts of the world (Wanyera et al. 2006; Singh et al. 2008; Admassu et al. 2009). These studies also showed that the region is the main source of newly evolving races like Ug99 and its variants (Pretorius et al. 2000; Jin et al. 2008b; Jin et al. 2009). This was consistent with Leppik (1970) and Saari and Prescott (1985) describing the region as a hot spot for the evolution and survival of new races of the stem rust complex. Hence, an extensive and detailed investigation in the whole of east Africa is needed to get a clear picture on the virulence structure of *Pgt* in the region.

Recent advances in DNA based techniques have allowed researchers to better understand the genetic diversity and phylogenetic relationships among *Puccinia spp.* isolates and races. Molecular markers like RAPDs, AFLPs, SNPs and SSRs were employed to this end (Steele et al. 2001; Kieper et al. 2006; Szabo 2007; Visser et al. 2008). Molecular markers have also the potential to trace the origin of new virulent races and track their dispersal pathway (Ridgway et al. 2005). This way, it is possible to alert wheat growers about a possible introduction of new races into their production system ahead so that they can devise control measures to the disease. Molecular markers can also be used to examine the background genotype of *Pgt* isolates, thereby facilitating a breeding programme for rust-resistant wheat by allowing breeders to produce and deploy the most effective combinations of resistance genes. Over all, a comprehensive collection of well characterized rust isolates, coupled with a basic understanding of the genetics of host-pathogen interactions are powerful tools to resolve the identities and relationships between resistance genes, and to assess the potential value of new resistance sources.

The genetic basis of diversity in *Puccinia graminis* f.sp. *tritici*

Mutation, recombination either through sexual reproduction or through a process of somatic hybridization, gene flow and selection are considered the main sources of diversity in *Pgt*

(Burdon and Roelfs 1985; Roelf et al. 1992; Burdon and Silk 1997). Historically, sexual reproduction used to be the main source of diversity especially in North America (Leonard 2001) and in local areas of Europe (Zadoks and Bouman 1985). The sexual life cycle of *Pgt*, in which nuclei of + and - mating types are reunited, encompasses five stages (uredospore, teliospore, basidiospore, pycniospore and aeciospore) and is completed on berberry (*Berberis vulgaris* L.). Berbery, the alternate host, serves as a breeding ground for new races. However, its role as a source of diversity had diminished since 1950s with the eradication of the alternate host in North America (Burdon and Silk 1997; Leonardo 1992). There are only few reports indicating the importance of *B. vulgaris* as alternate host to *Pgt* in other parts of the world (Zadoks and Bouman 1985).

Mutation is the other important source of diversity, which is believed to be the source of the majority of new races that have arisen in various clonal lineages (Burdon and Silk 1997). Variants of Ug99 detected recently were assumed to have arisen as a result of point mutations (Jin et al. 2008b; Jin et al. 2009). Usually new variants differ from pre-existing races by one or two virulence genes providing an indication that single step mutation in virulence to be the main process of evolutionary changes in *Pgt* populations (Green 1975; Burdon and Silk 1997). According to APS (2009) mutation in *Pgt* could occur spontaneously or as a result of selection imposed by resistant cultivars.

Host-induced selection is another source of variation in *Pgt*; but its effect on population diversity is unpredictable. In a uniform environment it favours certain genotypes thereby decreasing the diversity (Burdon and Silk 1997). However, there is also a case known in which host selection enhanced the diversity of another biotrophic pathogen population, *Erysiphe graminis* f. sp. *hordei* (Müller et al. 1996). This happened in a natural environment where important host variation existed that led to the maintenance of a more even distribution of a greater, and more dissimilar, range of pathotypes than in uniform host populations. Local or long distance gene flow leads to the founding of a new pathogen population in a new environment. This process was reported to be responsible for the development of over 100 *Pgt* races through a series of clonal lineages spread in Australia (Watson 1980).

Stem rust resistance genes in wheat and molecular markers

Around 60 stem rust resistance genes have been reported to confer resistance to stem rust in wheat. Of these 45 have been described and around 15 were temporarily designated pending

to confirmation of their distinctness to the already described genes (McIntosh et al. 1995). Each of these genes confers resistance to a range of stem rust races. Some of them have been incorporated into commercial wheat cultivars, and have been giving protection against stem rust in many countries. Good examples in this regard are the stem rust resistance genes *Sr2* and *Sr31*. The gene *Sr2* has provided durable and broad-spectrum adult plant resistance against wheat stem rust worldwide (Singh et al. 2006; McNeil et al. 2008) and is still one of the few effective genes against stem rust. *Sr31* was also one of the most effective resistance genes utilized extensively against stem rust until it succumbed to Ug99. This gene has been used in agriculture on the largest scale since 1980s in wheat breeding programs worldwide except Australia, and its use in CIMMYT wheat improvement resulted in the release of several popular cultivars worldwide carrying this gene (Singh et al. 2009). Likewise resistance genes like *Sr24* and *Sr26* have been widely deployed in Australia to protect wheat against stem rust (Mago et al. 2005).

The origin of the resistance genes is diverse including common wheat and wild relatives of wheat. The majority (twenty) of the designated resistance genes originated from common wheat. *T. turgidum* was a source for nine of the resistance genes. *T. monococcum*, *T. timopheevii* and *Thynopyrum ponticum* each was a source for three resistance genes, and *Secale cereale* and *T. speltoides* each for two resistance genes. The remaining three resistance genes originated from *T. tauschi*, *T. ventricosum* and *T. comosum* (McIntosh et al. 1995). According to these authors common wheat was also the main source of temporarily designated genes. The other sources for temporarily designated resistance genes include *T. turgidum*, *T. timopheevii*, triticale, and some unknown species.

The development of molecular markers has opened a new area of molecular plant breeding. In wheat a number of microsatellite markers have been developed and identified that have diagnostic values and can be used in marker assisted selection for stem rust resistance (Khan et al. 2005; Mago et al. 2005; Tsilo et al. 2007; Tsilo et al. 2008). Mago et al (2005) used high resolution mapping to separate the rust resistance genes *Sr31*, *Yr9* and *Lr26* that were believed to be completely linked. Overall, PCR based molecular markers have allowed the detection of resistance genes in crop cultivars and the precise localization of these genes on a chromosome. This increased the efficiency and speed of cultivar selection, and eased gene pyramiding and assessment of genetic diversity both in crops and pathogens.

Objectives and scope of the present study

Durable stem rust control in wheat requires detailed knowledge on the pathogen and the crop. In this regard, information on the virulence spectrum and the genetic diversity of *Pgt* are essential inputs towards achieving this goal. Molecular markers have proven to be easy to handle and efficient tools in variety development. However, prior to embarking on resistance breeding, it is essential to audit the resistance genes (if any) available in the cultivars grown in any given country, and decide which genes to deploy. Therefore, the objectives of this study were:

1. to determine the virulence spectrum of *P. graminis* f. sp. *tritici* populations in Ethiopia;
2. to describe the molecular genetic diversity of *P. graminis* f. sp *tritici* populations in Ethiopia;
3. to develop a genetic linkage map of *Sr13* using simple sequence repeat molecular markers;
4. to postulate stem rust resistance genes in Ethiopian wheat genotypes.

The above mentioned topics are treated in four separate chapters in this dissertation followed by a general summary.

References

Admassu B, Lind V, Friedt W and Ordon F. 2009. Virulence analysis of *Puccinia graminis* f. sp. *tritici* populations in Ethiopia with special consideration of Ug99. Plant Pathol 58:362-369

APS (American Phytopathological Society). 2009. Population Genetics of Plant Pathogens. APS*net* Education Center Advanced Topics. http://www.apsnet.org/Education/AdvancedPlantPath/Topics/PopGenetics/Pages/Interactions.htm. Accessed: 13 October 2009

Badebo A. 2002. Breeding Bread Wheat with Multiple Disease Resistance and High Yield for the Ethiopian Highlands: Broadening the Genetic Basis of Yellow Rust and Tan spot Resistance. Goettingen, Germany: Goettingen University, PhD thesis

Bechere E, Kebede H and Belay G. 2000. Durum wheat in Ethiopia: An old crop in an ancient land. Institute of Biodiversity Conservation and Research (IBCR), Addis Ababa, Ethiopia. 68 pp

Bekele E. 1985. A review of research on diseases of barley, tef and wheat in Ethiopia. In: Tsedeke Abate (Ed.), A Review of Crop Protection Research in Ethiopia. Institute of Agricultural Research (IAR), Ethiopia, pp. 79-107

Bonman JM, Bockelman HE, Jin Y, Hijmans RJ and Gironella AIN. 2007. Geographic distribution of stem rust resistance in wheat landraces. Crop Science 47: 1955 – 1963

Burdon JJ and Roelfs AP. 1985. The effect of sexual and asexual reproduction on the isozyme structure of populations of puccinia graminis. Phytopathology 75: 1068 – 1073

Burdon JJ and Silk J. 1997. Sources and Patterns of Diversity in Plant-Pathogenic Fungi. Phytopathology 87: 664 - 669

CIMMYT. 2005. Sounding the alarm on global stem rust: an assessment of race Ug99 in Kenya and Ethiopia and the potential for impact in neighbouring countries and beyond. Mexico city, Mexico

CSA (Central Statistics Authority). 1995. Report on area and crop production forecast for major grain crops. Addis Ababa, Ethiopia: Statistical bulletin

CSA 2006. Report on area and crop production forecast for major grain crops. Addis Ababa, Ethiopia: Statistical bulletin

EARO (Ethiopain Agricultural Research Organization) 2000. National Crop Research Strategy. Addis Ababa, Ethiopia: EARO. Unpublished

FAO (Food and Agriculture Organization) 2008. Crop prospects and food situation. No. 2, April 2008. http://www.fao.org/docrep/010/ai465e/ai465e04.htm. Accessed: November 30, 2009

Fetch TG. 2005. Races of *Puccinia graminis* on wheat, barley, and oat in Canada, in 2002 and 2003. Canadian Journal of Plant Pathology 27: 572 – 580

Fetch TG and Dunsmore KM. 2004. Physiologic specialization of *Puccinia graminis* on wheat, barley, and oat in Canada in 2001. Can. J. Plant Pathol. 26: 148–155

Gebeyehu G, van Ginkel M, Kebede T, Haregewoin M, Desta R, Bainbridge A, Hulluka M, Andnew Y, Tadesse D, Gorfu A and Badebo A. 1990. Wheat disease survey in Ethiopia in 1988. 6th Regional Wheat Workshop for Eastern, Central and Southern Africa, Addis Ababa, 2-6 Oct 1989, CIMMYT, Mexico City, DF (Mexico)

Gebre-Mariam H. 1991. Wheat production and research in Ethiopia. In: Hailu Gebre-Mariam, D. G. Tanner and M. Hulluka (eds.). Wheat Research in Ethiopia: A Historical Perspective. Addis Ababa: IAR/CIMMYT, pp. 1 – 16

Gill BS and Friebe B. 2001. Cytogenetics, phylogeny and evolution of cultivated wheats.

In: A.P. Bonjean and W.J. Angus (eds.) The world wheat book. A history of wheat breeding. Lavoiser Publishing, France, pp. 71-88

Green GJ. 1971. Physiologic races of wheat stem rust in Canada from 1919 to 1969. Can. J. Bot. 49: 1575 – 1588

Green GJ. 1975. Virulence changes in *Puccinia graminis* f. sp. *tritici* in Canada. Canadian Journal of Botany 53: 1377 – 1386

Hailu F and Merker A. 2008. Variation in gluten strength and yellow pigment in Ethiopian tetraploid wheat germplasm. Genet Resour Crop Evol 55:277–285

Jin Y. 2005. Races of *Puccinia graminis* identified in the United States in 2003. Plant Disease 89: 1125 – 1127

Jin Y, Szabo LJ, Pretorius Z. 2008a. Virulence variation within the Ug99 lineage. Wheat genetics international symposium proceedings. Available: http://hdl.handle.net/2123/3435.

Jin Y, Szabo LJ, Pretorius ZA, Singh RP, Ward R and Fetch TJ. 2008b. Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici.* Plant Dis. 92:923-926

Jin Y, Szabo LJ, Rouse MN, Fetch TJ, Pretorius ZA, Wanyera R and Njau P. 2009. Detection of virulence to resistance gene *Sr36* within the TTKS race lineage of *Puccinia graminis* f. sp. *tritici*. Plant Dis. 93:367-370

Keiper FJ, Haque MS, Hayden MJ and Park RF. (2006) Genetic diversity in Australian populations of *Puccinia graminis* f. sp. *avenae*. Phytopathol 96:96-104

Khan RR, Bariana HS, Dholakia BB, Naik SV, Lagu MD, Rathjen AJ, Bhavani S and Gupta VS. 2005. Molecular mapping of stem and leaf rust resistance in wheat. Theor Appl Genet 111: 846–850

Klindworth D., Miller J, Jin Y andXu SS. 2007. Chromosomal locations of genes for stem rust resistance in monogenic lines derived from tetraploid wheat accession ST464. Crop science. 47:1441-1450

Leonard KJ. 2001. Black stem rust biology and threat to wheat growers. Presentation to the central plant board meeting, February 5-8, 2001, Lexington, KY. http://www.ars.usda.gov/Main/docs.htm?docid=10755 Accessed: 13 October 2009.

Leppik EE. 1970. Gene centres of plants as sources of disease resistance. Annual Review of Phytopathology 8: 323 – 344

Mago R, Miah H, Lawrence GJ, Wellings CR, Spielmeyer W, Bariana HS, McIntosh RA, Pryor AJ and Ellis JG. 2005. High-resolution mapping and mutation analysis separate

the rust resistance genes Sr31, Lr26 and Yr9 on the short arm of rye chromosome 1. Theor Appl Genet 112: 41–50

McIntosh RA, Wellings CR and Park RF. 1995. Wheat rusts: an atlas of resistance genes. Canberra, Australia: CSIRO

McNeil MD, Kota R, Paux E, Dunn D, McLean R, Feuillet C, Li D, Kong X, Lagudah E, Zhang JC, Jia JZ, Spielmeyer W, Bellgard M and Appels R. 2008. BAC-derived markers for assaying the stem rust resistance gene, Sr2, in wheat breeding programs. Mol Breeding 22:15–24

Mohammed J. 1989. Potentials and possibilities of double-cropping wheat after cotton under irrigation in Awash Valley, Ethiopia. Ph.D. Thesis, Tropical Institute of Agriculture, Giessen University, Giessen, Germany.

Müller K, McDermott JM, Wolfe MS and Limpert E. 1996. Analysis of diversity in populations of plant pathogens: The barley powdery mildew pathogen across Europe. Eur J Plant Pathol 102:385-395

Park RF. 2008. Breeding cereals for rust resistance in Australia. Plant Pathol 57: 591– 602

Porceddu E and Perrino P. 1973. Wheat in Ethiopia: Preliminary report of a collection mission. Plant Genetic Resources Newsletter 30:33-36

PPRC (Plant Protection Research Center). 2000. Progress report for the year 2000. Department of plant pathology, Plant protection Research Center, Ambo, Ethiopia

Prescott JM, Burnett PA, Saari EE, Ranson J, Bowman J, Milliano W de, Singh RP and Bekele G. 1986. Wheat Diseases and Pests: a guide for field identification. CIMMYT, Mexico City, D.F., Mexico. 135 pp

Ridgway HJ, Steyaert JM, Pottinger BM, Carpenter M, Nicol D and Stewart A. 2005. Development of an isolate-specific marker for tracking *Phaeomoniella chlamydospora* infection in grapevines. Mycologia 97: 1093-1101

Roelfs AP. 1978. Estimated losses caused by rust in small grain cereals in the United States. Miscellaneous publication 1363. USDA, Washington DC

Roelfs AP. 1982. Effects of barberry eradication on stem rust in the United States. Plant disease 66:177-181

Roelfs AP and Martens JW. 1988. An international system of nomenclature for *Puccinia graminis* f. sp. *tritici*. Phytopathol 78:526-533

Roelfs AP, Singh RP and Saari EE. 1992. Rust diseases of wheat: Concepts and methods of disease management. CIMMYT, Mexico City, Mexico, pp. 81

Shank R. 1994. Wheat stem rust and drought effects on Bale agricultural production and future prospects. Report on February 17–28 assessment, United Nations emergency unit for Ethiopia. http://www.africa.upenn.edu/eue_web/Bale_mar.txt. Accessed: September 11, 2009

Singh RP. 1991. Pathogenicity variation of *Puccinia recondita* f. sp. *tritici and P. graminis* f. sp. *tritici* in wheat-growing areas of Mexico during 1988 and 1989. Plant Disease 75: 790-794

Singh RP, Hodson DP, Jin Y, Huerta-Espino J, Kinyua MG, Wanyera R, Njau P and Ward RW. 2006. Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 1: 1 – 13

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Njau P, Wanyera R, Herrera-Foessel SA and Ward WR. 2008. Will Stem Rust Destroy the World's Wheat Crop? Advances in Agronomy 98: 271 – 309

Stakman EC. 1957. Problems in preventing plant disease epidemics. American Journal of Botany 44:259-267

Stakman EC and Levine MN. 1922. The determination of biologic forms of *Puccinia graminis* on Triticum spp. Minn. Agric. Exp. Stn. Tech. Bull. 8:1-10

Stakman EC, Stewart DM and Loegering WQ. 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici.* U.S. Dep. Agric.–Agric. Res. Serv. Publ. E617

Steele KA, Humphreys E, Wellings CR and Dickinson MJ. 2001. Support for a stepwise mutation model for pathogen evolution in Australasian *Puccinia striiformis* f. sp. *tritici* by use of molecular markers. Plant Pathol 50:174 - 180

Szabo LJ. 2007. Development of simple sequence repeat markers for the plant pathogenic rust fungus, *Puccinia graminis*. Mol Ecol Notes 7:92-94

Teklu Y and Hammer K. 2009. Diversity of Ethiopian tetraploid wheat germplasm: breeding opportunities for improving grain yield potential and quality traits. Plant Genetic Resources 7: 1 – 8

Tsilo TJ, Jin Y, James A and Anderson JA. 2007. Microsatellite Markers Linked to Stem Rust Resistance Allele *Sr9a* in Wheat. Crop Sci 47: 2013 – 2020

Tsilo TJ, Jin Y and Anderson JA. 2008. Diagnostic Microsatellite Markers for the Detection of Stem Rust Resistance Gene *Sr36* in Diverse Genetic Backgrounds of Wheat. Crop Sci 48: 253 - 261

Visser B, Herselman L and Pretorius ZA. 2009. Genetic comparison of Ug99 with selected South African races of *Puccinia graminis* f.sp. *tritici*. Mol Plant Pathol 10:213-222

Wanyera R, Kinyua MG, Jin Y and Singh RP. 2006. The spread of stem rust caused by *Puccinia graminis* f. sp. *tritici*, with virulence on *Sr31* in wheat in Eastern Africa. Plant Dis 90: 113

Watson IA. 1980. Wheat and its rust parasites in Australia. In: L. T. Evans and W. J. Peacock (eds.). Wheat sciences-today and tomorrow. Cambridge University Press, Cambridge, pp.129-147

White JW, Tanner DG and Corbett JD. 2001. An agro-climatological characterization of bread wheat production areas in Ethiopia. CIMMYT, NRG-GIS Series 01-01. Mexico City, Mexico

Yirgou D. 1967. Plant diseases of economic importance in Ethiopia. Haile Selassie I University, College of Agriculture, Experimental station bulletin no.50, Addis Ababa, Ethiopia. 30 pp

Zadoks JC and Bouman JJ. 1985. Epidemiology in Europe. In: A. P. Roelfs & W. R. Bushnell (eds.), The cereal rusts, Volume II. Academic press, Orlando, pp. 329 - 369

Zegeye T, Taye G, Tanner D, Verkuijl H, Agidie A and Mwangi W. 2001. Adoption of improved bread wheat varieties and inorganic fertilizer by small-scale farmers in Yelmana Densa and Farta districts of Northwestern Ethiopia. EARO and CIMMYT. Mexico City, Mexico

Chapter 2

Virulence analysis of *Puccinia graminis* f. sp. *tritici* populations from Ethiopia

B. Admassu[ab], V. Lind[b], W. Friedt[c] and F. Ordon[b]

[a] Plant Protection Research Center, Ethiopian Institute of Agricultural Research, P. O. Box 37, Ambo, Ethiopia; [b] Julius Kuehn-Institute, Federal Research Institute for cultivated plants (JKI), Institute for Resistance Research and Stress Tolerance, Erwin-Baur-Str. 27, 06484 Quedlinburg, Germany; [c] IPZ, Justus-Liebig-University Giessen, Insitute of Crop Science and Plant Breeding I, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany

Abstract

As a result of a recent spread of a new and highly virulent race of *Puccinia graminis* f. sp. *tritici* called Ug99, stem rust is becoming a serious threat to wheat production in Ethiopia and other east African countries. Therefore, there is an urgent need to analyse the virulence spectrum of *Pgt* populations in Ethiopia. Wheat stem rust samples were collected in 2006 and 2007 in Arsi, Bale, Shewa and the Northwest regions of Ethiopia to determine the virulence diversity and race distribution in *P. graminis* f. sp. *tritici* populations. Stem rust incidence was high in Arsi, Bale and East Shewa. In Northwest Ethiopia, and North and West Shewa, stem rust was prevalent at low levels. A total of 152 isolates was analyzed and 22 races were identified. Races TTKSR (Ug99), TTHSR and RRTTR were predominant with frequencies of 26.6, 17.7 and 11.1%, respectively. These races were also detected in all regions. It turned out that the highly virulent race designated as Ug99 was present throughout the country and dominated in all regions except Northwest Ethiopia. A variant of Ug99, which is virulent against *Sr24* was not detected in this study. Four stem rust resistance genes (*Sr13, Sr30, Sr36 and SrTm*p) were found to confer resistance to most of the races prevalent in Ethiopia. With the exception of *Sr30*, which is not effective to Ug99, the other three genes can be used in breeding for resistance to stem rust in Ethiopia.

Keywords: Ethiopia, *Puccinia graminis*, Sr resistance genes, stem rust, virulence analysis, wheat

This article is published in the journal Plant Pathology

Introduction

Wheat (*Triticum aestivum* L.) is one of the major crops cultivated in Ethiopia. During the last 10 years the area covered by wheat has increased from 0.77 million ha in 1997 to 1.44 million ha in 2005, and it now ranks second among the crops next to Tef, *Eragrostis tef* Syn. (CSA, 1998; 2006). Wheat in Ethiopia is represented by hexaploid (bread wheat) and tetraploid (durum and emmer wheats). Though the production and productivity of wheat has increased in the last decade, stem rust, caused by the fungus *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*), remains a major threat to wheat production. The biggest risk to wheat production is the defeat of the resistance gene *Sr31* by a *Pgt* race designated as Ug99. This virulent race is spreading across regions since its first discovery in 1999 in Uganda by Pretorius *et al.* (2000). Though Ug99 is known to be present in Ethiopia (CIMMYT, 2005), the extent of its distribution and frequency of occurrence is not well understood (Singh *et al.*, 2006). This study examined the frequency and distribution of Ug99 and other races in the country.

Earlier surveys of *Pgt* in Ethiopia documented a number of races apart from Ug99 (van Ginkel *et al.*, 1989). A very recent preliminary survey showed the presence of races which were highly virulent, widespread, and varied in type and frequency across regions and over time (Admassu & Fekadu, 2005). This study suggested that continuous and exhaustive surveys have to be carried out to depict a clear picture of the virulence pattern of *Pgt* in Ethiopia.

Race surveys help to generate information regarding the virulence of races, their frequency and distribution pattern across regions and over time. This is of paramount importance to develop wheat varieties with durable stem rust resistance. In addition, virulence surveys are important for studying the evolution of new races and forecast the virulence shifts in a population. This information helps breeders to develop a breeding strategy and to respond to sudden changes in virulence thereby preventing a resistance break down in wheat cultivars.

Materials and Methods

Collection of rust samples

P. graminis f. sp. *tritici* samples were collected in wheat fields across the major wheat growing regions of Ethiopia: Shewa, Arsi, Bale and Northwest Ethiopia (Fig. 1). The time of collection was adjusted to coincide with the wheat growing periods of each region. In Shewa, Arsi and Northwest Ethiopia, the surveys of wheat fields were conducted in October 2006, whereas in Bale samples were collected in December 2006. In addition, samples were collected in March 2007 from off-season nursery sites in Debre Zeit (Shewa) and Kulumsa (Arsi). The surveys were carried out following main and feeder roads on pre-selected routes in areas where wheat is important and stem rust is known to be present. Stem tissue of wheat bearing uredia of *Pgt* were collected from commercial fields every 10 km or at the first field thereafter. Data on the geographic information (latitude, longitude and altitude) of each site were recorded using a GPS (eTrex Legend GPS System, Garmin). The data were later used to plot sample collection sites on a map using the computer programme ArcView 3.0 (ESRI). The samples were air dried, and kept in a refrigerator at 4^{o}C until used for the virulence analysis. Stem rust samples were collected from 152 locations representing 54, 39, 33 and 26 locations from Shewa, Arsi, Bale and Northwest Ethiopia, respectively.

Production of single pustule isolates and their multiplication

Bulked urediospores from each field were suspended in lightweight mineral oil, and sprayed for multiplication onto seven day old seedlings of the cultivar 'Morocco', which does not carry known stem rust resistance genes (Roelfs *et al.*, 1992). Two weeks later, leaves containing single pustules from each location were pruned, and multiplied on Morocco separately. Pots containing inoculated seedlings were covered with cellophane bags (145 x 235 mm) and tied up at the base with a rubber band to avoid cross contamination (Fetch & Dunsmore, 2004). A sufficient amount of spores was produced to inoculate the set of differential hosts (Table 3). The urediospores descending from one pustule made up a single pustule isolate. One isolate was developed from each location and used for the final race analysis.

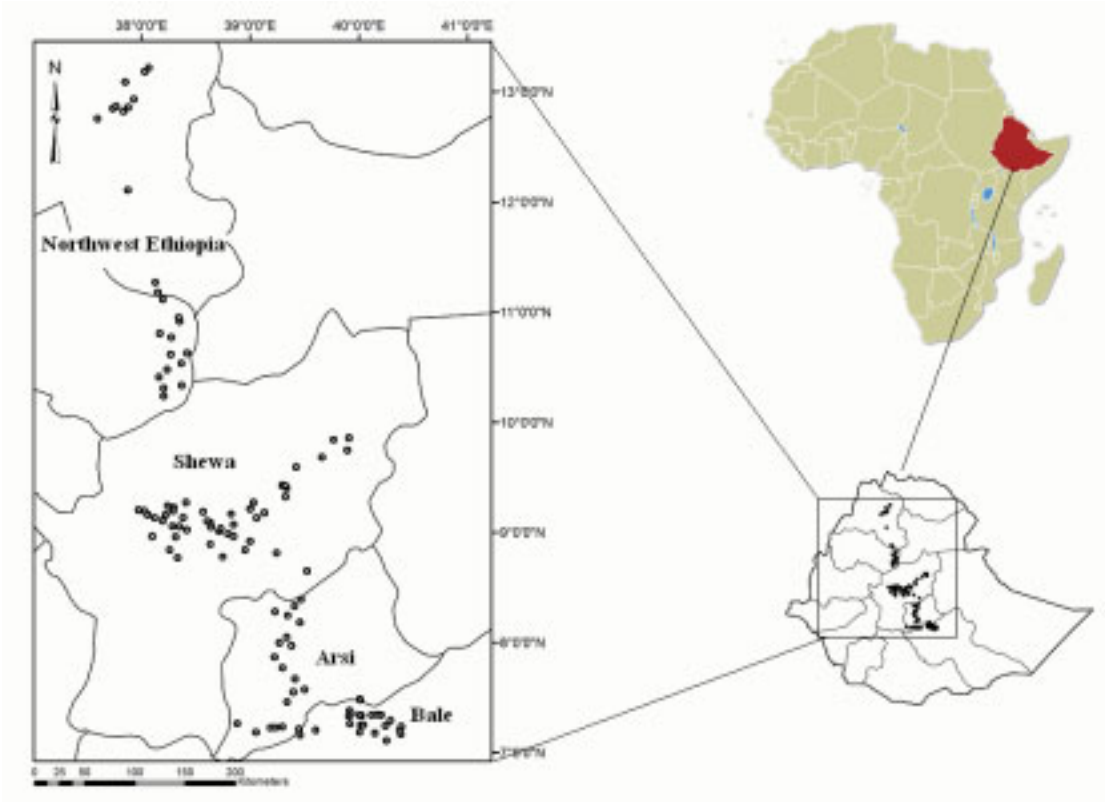

Fig. 1 Wheat stem rust collection sites in Arsi, Bale, Shewa and Northwest Ethiopia

Determination of races

Races were determined by inoculating urediospores on 20 wheat differential lines (Fetch & Dunsmore, 2004). The differential wheat lines possessed resistance genes *Sr5, Sr6, Sr7a, Sr7b, Sr8a, Sr8b, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr13, Sr17, Sr21, Sr30, Sr36, SrTmp* and *SrMcN* (Table 3). In addition, to confirm the identity of Ug99 unequivocally, all isolates were tested on differential lines carrying the resistance genes *Sr24, Sr31* and *Sr38*.

Single pustule isolates were inoculated onto seven day old seedlings of the differential lines as well as onto seedlings of the susceptible control cultivar 'Morocco'. Immediately after inoculation, seedlings were incubated in the dark for 18 hours at 18^{o}C and high relative humidity in a humid chamber. After 18 hours of darkness, the seedlings were exposed to fluorescent light for three hours. Then they were transferred to a growth chamber and grown constantly at 22^{o}C, a light intensity of 10,000 lx and a photoperiod of 16 hours.

Infection types were scored 14 days after inoculation using the scale of Stakman *et al.* (1962). Infection types 0 to 2+ were considered as resistant and 3- to 4+ as susceptible. The

experiment was repeated twice, and only differential hosts that produced similar infection types in the two experiments were considered for the data analysis. When there was infection type 0 (immune reaction), the test was repeated to exclude the possibility of disease escape.

Race designation was done by grouping the differential lines into five sub-sets in the following order: (i) *Sr5, Sr21, Sr9e, Sr7b*, (ii) *Sr11, Sr6, Sr8a, Sr9g*, (iii) *Sr36, Sr9b, Sr30, Sr17*, (iv) *Sr9a, Sr9d, Sr10, SrTmp*, (v) *Sr7a, Sr8b, Sr13, SrMcN* (Table 1).

Each isolate was assigned a five letter race code based on its reaction on the differential lines (Roelfs & Martens, 1988; Fetch & Dunsmore, 2004). For example, a low infection type on the four lines in a set is assigned with the letter 'B', while a high infection type on the four lines is assigned with a letter 'T'. Hence, if an isolate produces a low infection type (resistant reaction) on the 20 differential lines, the race will be designated with a five letter race code 'BBBBB'. Similarly, an isolate producing a high infection type (susceptible reaction) on 20 of the lines will have a race code 'TTTTT'. If an isolate produces a low infection type on *Sr36, SrTmp* and *Sr13*, but a high infection type on the remaining 17 differential lines, the race will be designated as 'TTKSR'.

Results

Stem rust was observed in all surveyed regions at variable levels. Disease incidence and severity were high in the traditional wheat monoculture belts of Arsi and Bale regions. In these two regions, 66 and 63 fields were inspected, and stem rust was present on 39 (59%) and 33 (52%) fields, respectively.

47% out of 66 fields inspected were infected with stem rust in parts of Shewa that are adjacent to Arsi, but the level was low in West and North Shewa (29% incidence). In Northwest Ethiopia stem rust was present only on 26 (34%) out of 75 fields inspected. Of 165 rust samples collected, 13 did not yield viable isolates at the time of inoculation in the laboratory. Hence, 152 isolates were used for the final race analysis.

Table 1 Code for the 20 differential hosts for *Puccinia graminis* f. sp. *tritici* in ordered sets of five.

		Infection types produced on near iso-genic Sr lines			
	Host set 1	*Sr5*	*Sr21*	*Sr9e*	*Sr7b*
	Host set 2	*Sr11*	*Sr6*	*Sr8a*	*Sr9g*
	Host set 3	*Sr36*	*Sr9b*	*Sr30*	*Sr17*
	Host set 4	*Sr9a*	*Sr9d*	*Sr10*	*SrTmp*
Pgt code[a]	Host set 5	*Sr7a*	*Sr8b*	*Sr13*	*SrMcN*
B		L[b]	L	L	L
C		L	L	L	H
D		L	L	H	L
F		L	L	H	H
G		L	H	L	L
H		L	H	L	H
J		L	H	H	L
K		L	H	H	H
L		H	L	L	L
M		H	L	L	H
N		H	L	H	L
P		H	L	H	H
Q		H	H	L	L
R		H	H	L	H
S		H	H	H	L
T		H	H	H	H

[a] Adopted from Roelfs & Martens, 1988; Fetch & Dunsmore, 2004

[b] L = low/resistant infection type (0 to 2+)

H = high/susceptible infection type (3- to 4+)

Virulence structure of stem rust pathogens

From 152 isolates studied, 22 races were identified. The 54 *Pgt* isolates collected from the Shewa region were assigned to 13 races. Similarly, the 39, 33 and 26 isolates collected from Arsi, Bale and Northwest Ethiopia belonged to eight, seven and eight races, respectively (Table 2). The highly virulent race called Ug99 (TTKSR) was the most abundant and widely

distributed race across the country with a frequency of 26.6%. The identity of Ug99 was confirmed based on its virulence on wheat differential lines carrying the resistance genes *Sr31* and *Sr38*. The other abundant races countrywide included TTHSR, RRTTR, PTHSR and KCCST, with frequencies of 17.7%, 11.1%, 7.9% and 4.6%, respectively. These five races accounted for almost 68% of the stem rust population of Ethiopia. The remaining 17 races composed the rest of the population (32%). Of these, the least abundant races were DPBTR, QLDGH and QQQCM, which were detected only at single locations, each. Similarly, races MLBDC, QMQTR and TCHTT were each detected only twice (Table 2).

There was a variation between the virulence spectra of races within the regions (Table 2). Of the 54 isolates studied in Shewa, race TTKSR (Ug99) and the closely related race TTHSR were pre-dominant, each with frequencies of 18.5% followed by race RRTTR with 14.8%. In Shewa, the six races DPBTR, MLBDC, QLDGH, QMQTR, TCHTT and TTTTR were the least abundant, each with frequencies of less than 5%. In Arsi, race TTKSR (Ug99) was again the most dominant (30.8%), followed by TTHSR (20.5%), PTHSR (12.8%) and RRTTR (10.3%). KCCST and TTHTR made up the least dominant races in this region with frequencies of 5.1%, each.

The race frequency in Bale showed a similar trend, as both TTKSR and TTHSR had the highest frequencies (45.4 and 18.2%, respectively). They were followed by PTHSR with 12.1%. In this region, the least dominant race was KCCST detected only once. The race pattern in Northwest Ethiopia was different from that of the other three regions. In this part of the country, KRHST was the dominant race accounting for 19.2% of the total population. The next abundant races were KCCST and TMHSR, each with 15.4%. QQQCM was the least frequent race, being detected only once.

Table 2 Races of *P. graminis* f. sp. *tritici* collected from Ethiopia in 2006 and 2007, and their virulence spectrum.

Race	Virulence spectrum (ineffective Sr resistance genes)	No.	%
Shewa			
DPBTR	9e, 11, 8a, 9g, 9a, 9d, 10, Tmp, 7a, 8b, McN	1	1.8
KHHTT	21, 9e, 7b, 6, 9g, 9b, 17, 9a, 9d, 10, Tmp, 7a, 8b, 13, McN	3	5.6
MLBDC	5, 7b, 11, 10, McN	2	3.7
PTHSR	5, 9e, 7b, 11, 6, 8a, 9g, 9b, 17, 9a, 9d, 10, 7a, 8b, McN	3	5.6
QLDGH	5, 21, 11, 30, 9d, 8b, McN	1	1.8
QMQTR	5, 21, 11, 9g, 36, 9b, 9a, 9d, 10, Tmp, 7a, 8b, McN	2	3.7
RKHSR	5, 9e, 7b, 6, 8a, 9g, 9b, 17, 9a, 9d, 10, 7a, 8b, McN	5	9.3
RRTTR	5, 21, 7b, 11, 6, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 7a, 8b, McN	8	14.8
TCHTT	5, 21, 9e, 7b, 9g, 9b, 17, 9a, 9d, 10, Tmp, 7a, 8b, 13, McN	2	3.7
THHST	5, 21, 9e, 7b, 6, 9g, 9b, 17, 9a, 9d, 10, 7a, 8b, 13, McN	5	9.3
TTHSR	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 17, 9a, 9d, 10, 7a, 8b, McN	10	18.5
TTKSR (Ug99)	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, 7a, 8b, McN	10	18.5
TTTTR	5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 7a, 8b, McN	2	3.7
Total		54	100
Arsi			
KCCST	21, 9e, 7b, 9g, 17, 9a, 9d, 10, 7a, 8b, 13, McN	2	5.1
PTHSR	5, 9e, 7b, 11, 6, 8a, 9g, 9b, 17, 9a, 9d, 10, 7a, 8b, McN	5	12.8
RMTTM	5, 21, 7b, 11, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 7a, McN	3	7.7
RRTTR	5, 21, 7b, 11, 6, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 7a, 8b, McN	4	10.3
TTHSH	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 17, 9a, 9d, 10, 8b, McN	3	7.7
TTHSR	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 17, 9a, 9d, 10, 7a, 8b, McN	8	20.5
TTHTR	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 17, 9a, 9d, 10, Tmp, 7a, 8b, McN	2	5.1
TTKSR (Ug99)	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, 7a, 8b, McN	12	30.8
Total		39	100
Bale			
KCCST	21, 9e, 7b, 9g, 17, 9a, 9d, 10, 7a, 8b, 13, McN	1	3.0
MRHLR	5, 7b, 11, 6, 9g, 9b, 17, 9a, 7a, 8b, McN	2	6.1
PTHSR	5, 9e, 7b, 11, 6, 8a, 9g, 9b, 17, 9a, 9d, 10, 7a, 8b, McN	4	12.1

RRTTR	5, 21, 7b, 11, 6, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 7a, 8b, McN	2	6.1
TTHSR	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 17, 9a, 9d, 10, 7a, 8b, McN	6	18.2
TTHTR	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 17, 9a, 9d, 10, Tmp, 7a, 8b, McN	3	9.1
TTKSR (Ug99)	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, 7a, 8b, McN	15	45.4
Total		33	100
Northwest Ethiopia			
HRTSH	21, 7b, 11, 6, 9g, 36, 9b, 30, 17, 9a, 9d, 10, 8b, McN	3	11.5
KCCST	21, 9e, 7b, 9g, 17, 9a, 9d, 10, 7a, 8b, 13, McN	4	15.4
KRHST	21, 9e, 7b, 11, 6, 9g, 9b, 17, 9a, 9d, 10, 7a, 8b, 13, McN	5	19.2
QQQCM	5, 21, 11, 6, 36, 9b, Tmp, 7a, McN	1	3.9
RRTTR	5, 21, 7b, 11, 6, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 7a, 8b, McN	3	11.5
TMHSR	5, 21, 9e, 7b, 11, 9g, 9b, 17, 9a, 9d, 10, 7a, 8b, McN	4	15.4
TTHSR	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 17, 9a, 9d, 10, 7a, 8b, McN	3	11.5
TTKSR (Ug99)	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, 7a, 8b, McN	3	11.5
Total		26	100

The race frequency in Bale showed a similar trend, as both TTKSR and TTHSR had the highest frequencies (45.4 and 18.2%, respectively). They were followed by PTHSR with 12.1%. In this region, the least dominant race was KCCST detected only once. The race pattern in Northwest Ethiopia was different from that of the other three regions. In this part of the country, KRHST was the dominant race accounting for 19.2% of the total population. The next abundant races were KCCST and TMHSR, each with 15.4%. QQQCM was the least frequent race, being detected only once.

Although most of the races were confined to specific locations, some had a wider spatial distribution. Six races (TTKSR (Ug99), TTHSR, RRTTR, PTHSR, KCCST and TTHTR) were present in two or more regions. Races such as TTKSR, TTHSR and RRTTR were present in all regions surveyed. PTHSR was present in Shewa, Arsi and Bale but not in Northwest Ethiopia. Similarly, KCCST was present in all regions except in Shewa. Race TTHTR was present only in Arsi and Bale regions. The remaining 16 races had only regional importance: nine, four, two and one were confined to Shewa, Northwest Ethiopia, Arsi and Bale regions, respectively (Table 2).

Virulence to Sr resistance genes

The virulence spectrum of the 22 races with supra regional (6 races) and regional (16 races) significance is given in Table 2. Most of the races were virulent to one or more of the resistance genes (Table 3). For instance, a race like TTTTR was virulent to all the resistance genes except *Sr13*. Similarly, races like TTKSR (Ug99), RRTTR, PTHSR and TTHSR, which were widespread, were virulent to most of the genes. On the other hand, races MLBDC and QLDGH were the least virulent ones, producing susceptible reactions only on five and seven iso-genic lines, respectively (Table 2).

It was also evident that a majority of the resistance genes was ineffective to most of the races identified in this study. For instance, the differential host carrying the resistance gene McNair 701 (*SrMcN*) was susceptible to all of the races. Similarly, nine differential hosts carrying the resistance genes: *Sr7a, Sr7b, Sr8b, Sr9a, Sr9b, Sr9d, Sr9g*, *Sr10*, and *Sr17* were susceptible to more than 90% of the races (Table 3). *Sr8b* was ineffective to 57.4, 76.9 and 84.8% of the races found in Shewa, Arsi and Bale, respectively. Unlike the three regions, this gene was ineffective only to 23.1% of the races found in Northwest Ethiopia, and hence can be considered as a source of resistance to this specific region. On the other hand, four resistance genes, *Sr13, Sr30, Sr36* and *SrTmp*, were found to be effective to most of the races detected in this study. Of the four genes, differential hosts carrying *Sr13* and *Sr36* were resistant to 85.5 and 81.6% of the races found across the country, respectively. Differential hosts carrying *Sr30* and *SrTmp* were resistant to 56.6 and 76.3% of the races, respectively.

Regionally, *Sr13* was effective to 97 and 95% of the races found in Bale and Arsi, but only to 71.5 and 65.4% of the races in Shewa and Northwest Ethiopia, respectively. Similarly, *Sr36* was effective to 94% of the races found in Bale, but its effectiveness dropped to 82.1, 77.8 and 73.1% of the races in Arsi, Shewa and Northwest Ethiopia, respectively. Only three of the 20 differential lines carrying resistance genes, *Sr13, Sr36* and *SrTmp*, were effective against race Ug99. Of the additional differential lines, *Sr24* was effective, whereas, *Sr31* and *Sr38* were ineffective against Ug99.

Discussion

The high level of rust incidence and severity recorded during the study period in central (Shewa) and southeast (Arsi and Bale) regions of Ethiopia might be due to the cultivation of wheat cultivars 'Kubsa' and 'Galama', which are susceptible to stem rust. These two susceptible cultivars covered 56% of the wheat in these regions (CIMMYT, 2005). The same report indicated that wheat cultivation throughout the year in these regions has contributed to the survival and build-up of stem rust populations.

Table 3 Frequency of virulence of *P. graminis* f. sp. *tritici* isolates collected from Shewa, Arsi, Bale and Northwest regions of Ethiopia to single gene wheat differentials

Differential host	Resistance gene	Shewa		Arsi		Bale		NWE[a]		Total	
		No.	%	No.	%	No.	%	No.	%	No.	%
McNair 701	*SrMcN*	54	100	39	100	33	100	26	100	152	100
ISr9a-Ra	*Sr9a*	51	94.4	39	100	33	100	25	96.2	148	97.4
CnSSr9g	*Sr9g*	51	94.4	39	100	33	100	25	96.2	148	97.4
W2691Sr10	*Sr10*	53	98.1	39	100	31	93.9	25	96.2	148	97.4
ISr7b-Ra	*Sr7b*	50	92.6	39	100	33	100	25	96.2	147	96.7
ISr9d-Ra	*Sr9d*	52	96.3	39	100	31	93.9	25	96.2	147	96.7
Barleta Benvenuto	*Sr8b*	52	96.3	36	92.3	33	100	25	96.2	146	96.1
LC/Kenya Hunter	*Sr17*	48	88.9	39	100	33	100	25	96.2	145	95.4
Line G sel	*Sr7a*	51	94.4	36	92.3	33	100	23	88.5	143	94.1
W2691Sr9b	*Sr9b*	50	92.6	37	94.9	32	97	22	84.6	141	92.8
ISr5-Ra	*Sr5*	50	92.6	37	94.9	32	97	14	53.8	133	87.5
ISr6-Ra	*Sr6*	46	85.2	34	87.2	32	97	18	69.2	130	85.5
ISr11-Ra	*Sr11*	39	72.2	37	94.9	32	97	22	84.6	130	85.5
CS_T_mono_deriv	*Sr21*	43	79.6	34	87.2	27	81.8	26	100	130	85.5
Verstein	*Sr9e*	41	75.9	32	82.5	29	87.9	19	73.1	121	79.6
ISr8-Ra	*Sr8a*	31	57.4	30	76.9	28	84.8	6	23.1	95	62.5
Bt Sr30 Wst	*Sr30*	21	38.9	19	48.7	17	51.5	9	34.6	66	43.4
Triumph	*SrTmp*	18	33.3	9	23.1	5	15.2	4	15.4	36	23.7
W2691SrTt-2	*Sr36*	12	22.2	7	17.9	2	6.1	7	26.9	28	18.4
W2691Sr13	*Sr13*	10	18.5	2	5.1	1	3	9	34.6	22	14.5

[a] NWE = Northwest Ethiopia

Wheat stem rust was on the decline for several decades due to the widespread use of the 1BL.1RS translocation carrying *Sr31* (Singh *et al.*, 2006) that was effective to all known *Pgt* races. The discovery of race Ug99 with virulence to *Sr31* in Uganda in 1999 (Pretorius *et al.*, 2000) represented a real threat to wheat production in the world, including Ethiopia where stem rust epidemics had not occurred since the resistant cultivar 'Enkoy' (Ayele, 2000) lost its resistance in 1993. Since the first report in Uganda, race Ug99 has now been detected in other parts of East Africa (Singh *et al.*, 2006), and beyond in Yemen (GRI, 2007) and Iran (SeedQuest, 2008). In Ethiopia Ug99 was first detected in 2003 at six dispersed sites (Singh *et al.*, 2006). The present study has also detected the race at five of the six locations. It is more dominant in the southeast and central parts of the country than in Northwest Ethiopia. The low frequency in the northwest was not surprising as this region is geographically isolated from the major wheat belts located in the southeast, where Ug99 was assumed to be introduced from Kenya. Therefore, Ug99 is a real threat to wheat growers of Ethiopia, and requires a very close attention to curb the threat.

The identification of 22 races from 152 samples is a clear indication of high virulence diversity within the *Pgt* population in Ethiopia. A comparison of the races identified in the present study with earlier reports (Admassu & Fekadu, 2005) revealed some differences. The earlier study used a three digit race designation, and reported the races TTT, TTR, PTT and PTR to be the widespread, whereas the current survey indicated TTK/SR and TTH/SR to be the dominant races. Such variation over time is not uncommon as the races prevalent in a specific season depend on the type of wheat cultivars grown in the season (Singh, 1991), and to some extent on the predominant environmental conditions, especially on temperature (Roelfs *et al.*, 1992). Virulence diversities within *Pgt* populations were also reported from countries such as South Africa, Mexico, USA and Canada (Le Roux *et al.*, 1987; Singh, 1991; Jin, 2005; Fetch, 2003; 2005). It is also important to note that the race spectrum in Ethiopia is clearly different from that reported in other parts of the world. Surveys in the USA (Jin, 2005) and Canada (Fetch & Dunsmore, 2004) detected fewer races, five and fifteen, respectively than in Ethiopia (22 races). According to the reports, the dominant races in North America were QCCJ/N and QFCS/R as opposed to races TTKSR (Ug99) and TTHSR in Ethiopia. TTTT is the only race detected in common between the USA and Ethiopia. Since we do not know which resistance genes local landraces and commercial wheat cultivars grown in Ethiopia contain, it is not possible to determine their effect on determining race composition of the *Pgt* population.

Recently, three NILs of the fifth set, *Sr7a*, *Srb8b* and *Sr13* were replaced by *Sr24*, *Sr31* and *Sr38*, respectively (Jin et al., 2008) thereby slightly changing the alphabetical designation of races. Under such circumstances Ug99 will have an alphabetical designation of TTKSK instead of TTKSR.

The two adjacent regions Arsi and Bale had six similar races out of seven and out of eight races detected in each, respectively. Their geographic proximity, an absence of barriers and cultivation of similar bread wheat cultivars between the two regions play significant roles for the high race similarity. The difference in race composition in Shewa compared to Arsi and Bale can possibly be explained by varietal difference, i. e., instead of bread wheat, which is grown in Arsi and Bale, durum wheat dominates the farming system in Shewa (Sewalem, 2006). Like in Shewa, there were four distinct races in Northwest Ethiopia. This region is geographically isolated from the other regions. Hence, the possibility of race migration to and from this region is much restricted. It is also interesting to note that two of the races (KRHST and TMHSR) that were unique to Northwest Ethiopia were also the most dominant ones in this region. Therefore, it is imperative for breeding programmes to take this regional differentiation into account while developing a strategy for breeding wheat for stem rust resistance.

Most of the races in Ethiopia varied from one another by single-gene changes. Race TTHSR is similar to PTHSR with additional virulence to *Sr21*, and TTHTR is similar to TTHSR with additional virulence to *SrTmp*. Race TTKSR is similar to TTHSR, and TTHSR to TTHSH with additional virulence to *Sr30* and *Sr7a*, respectively. Such single step changes in virulence were reported to be the main process of evolutionary change in *Pgt* populations (Green, 1975). But, the actual evolution of *Pgt* races in Ethiopia and co-evolution of races with hosts can hardly be discussed because of lack of data from independent molecular markers of the background genotypes and information on R-genes in Ethiopian wheat cultivars, respectively. This information could have given an insight to the evolution of virulence, lineage of races and the role of host genes in virulence evolution (Abbasi *et al.,* 2005; Kieper *et al.*, 2006).

After analyzing 152 isolates from regions representing the major wheat growing areas of the country, four important stem rust resistance genes namely: *Sr13, Sr30, Sr36* and *SrTmp* were found to confer resistance to most of the races prevalent in Ethiopia. *Sr13* and *Sr36* are

known to be genes effective to most races worldwide (Roelfs *et al.*, 1992; Rowell, 1982) except occasional high infection types in some countries (Knott, 1990) including Ethiopia (Huerta-Espino, 1992). The use of these genes as sources of resistance in other countries (Park & Wellings, 1992; McIntosh *et al.*, 1995) corroborates the recommendation to use the aforementioned genes in breeding for resistance to stem rust for the Ethiopian wheat production. It is risky, however, to use gene *Sr30* as a source of resistance as it is ineffective against Ug99. Apart from that, the other three genes can potentially be used as sources of resistance to stem rust. Moreover, lines carrying *Sr22, Sr24, Sr26, Sr33* and *Sr39* were resistant to Ug99 and most of the other races (data not shown). Further analyses of these genes will prove their effective use in Ethiopian wheat production. It is important to note that a variant of Ug99, that was detected in Kenya, and which is virulent to *Sr24* was not detected in this study. Though it is known that Ug99 and its variant originated in East Africa, there is no conclusive information which country is the actual source. Hence, detection of a Ug99 variant race in Kenya, but its absence in Ethiopia may shed little light in this regard. However, further analysis is needed to arrive at a conclusive point. Comparison of effective resistance genes, between Ethiopia and North America (Canada and the USA) shows that *SrTmp* is effective across all countries. Similarly, *Sr30* and *Sr36* are effective in Ethiopia and Canada, and Ethiopia and the USA, respectively. On the other hand, some resistance genes that are effective in the USA (*Sr6* and *Sr10*) (Jin, 2005) and in Canada (*Sr6, Sr9e* and *Sr11*) (Fetch & Dunsmore, 2004) are ineffective in Ethiopia. Conversely, *Sr13*, which is effective in Ethiopia, was reported as ineffective in North America.

The majority of wheat in Ethiopia is grown by subsistence farmers, for whom the use of chemical fungicides against stem rust is uneconomical. Hence, farmers need to be continuously supplied with resistant varieties to avoid epidemics of stem rust, especially in the light of the wide distribution of race Ug99. It is also evident that *Pgt* populations existing in Ethiopia are highly variable. Therefore, it is imperative for the national agricultural research system to monitor pathogen populations over time to track further virulence evolution and to ensure that currently effective resistance genes are applied within a system of resistance gene management.

Acknowledgement

The first author was supported by a scholarship from the Katholischer Akademischer Auslaender-Dienst (KAAD), Germany to conduct this research. We would like to thank the Ethiopian Institute of Agricultural Research for providing leave of absence for the first author to carry out the research. We also thank Dr. Thomas Fetch of Agriculture and Agri-Food Canada and Dr. Yue Jin, USDA, St. Paul for providing near-isogenic wheat lines.

References

Abbasi M, Stephen BG, Scholler M, 2005. Taxonomy, phylogeny, and distribution of *Puccinia graminis*, the black stem rust: new insights based on rDNA sequence data. Mycoscience **46**, 241 – 247.

Admassu B, Fekadu E, 2005. Physiological races and virulence diversity of *Puccinia graminis* f. sp. *tritici* on wheat in Ethiopia. *Phytopathologia Mediterranea* **44**, 313 – 318.

Ayele B, 2002. *Breeding Bread Wheat with Multiple Disease Resistance and High Yield for the Ethiopian Highlands: Broadening the Genetic Basis of Yellow Rust and Tan spot Resistance*. Goettingen, Germany: Goettingen University, PhD thesis.

CSA, 1998. *Report on area and crop production forecast for major grain crops*. Addis Ababa, Ethiopia: Statistical bulletin.

CSA, 2006. *Report on area and crop production forecast for major grain crops*. Addis Ababa, Ethiopia: Statistical bulletin.

CIMMYT, 2005. *Sounding the alarm on global stem rust: an assessment of race Ug99 in Kenya and Ethiopia and the potential for impact in neighboring countries and beyond*. Mexico city, Mexico.

Fetch TG, 2005. Races of *Puccinia graminis* on wheat, barley, and oat in Canada, in 2002 and 2003. *Canadian Journal of Plant Pathology* **27**, 572 – 580.

Fetch TG, 2003. Physiological specialization of *Puccinia graminis* on wheat, barley, and oat in Canada in 2000. *Canadian Journal of Plant Pathology* **25**, 174 – 181.

Fetch TG, Dunsmore KM, 2004. Physiological specialization of *Puccinia graminis* on wheat, barley, and oat in Canada in 2001. *Canadian Journal of Plant Pathology* **26**, 148 – 155.

Green GJ, 1975. Virulence changes in *Puccinia graminis* f. sp. *tritici* in Canada. *Canadian Journal of Botany* **53**, 1377 – 1386.

Global rust initiative (GRI). 2007. Dangerous wheat disease jumps red sea. Online [http://www.eurekalert.org/pub_releases/2007-01/imaw-dwd011607.php].

Huerta-Espino J, 1992. *Analysis of wheat leaf and stem rust virulence on a worldwide basis*. St. Paul, USA: University of Minnesota, PhD thesis.

Jin Y, 2005. Races of *Puccinia graminis* identified in the United States in 2003. *Plant Disease* **89**, 1125 – 1127.

Jin Y, Szabo LJ, Pretorius ZA, Singh RP, Ward R and Fetch TJ. 2008. Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici.* Plant Dis. 92:923-926

Keiper FJ, Haque MS, Hayden MJ, Park RF, 2006. Genetic diversity in Australian populations of *Puccinia graminis* f. sp. *avenae*. *Phytopathology* **96**, 96 – 104.

Knott DR, 1990. Near-isogenic lines of wheat carrying genes for stem rust resistance. *Crop science* **30**, 901 – 905.

Le Roux J, Rijkenberg FHJ, 1987. Pathotypes of *Puccinia graminis* f. sp. *tritici* with increased virulence for Sr24. *Plant Disease* **71**, 1115 – 1119.

McIntosh RA, Wellings CR, Park RF, 1995. *Wheat rusts: an atlas of resistance genes*. Canberra, Australia: CSIRO.

Park RF, Wellings CR, 1992. Pathogenic specialization of wheat rusts in Australia and New Zealand in 1988 and 1989. *Australasian Plant Pathology* **21**, 61 – 69.

Park RF, 2008. Breeding cereals for rust resistance in Australia. *Plant Pathology* **57**, 591–602.

Pretorius ZA, Singh RP, Wagoire WW, Payne TS, 2000. Detection of virulence to wheat stem rust resistance gene Sr31 in *Puccinia graminis* f. sp. *tritici* in Uganda. *Phytopathology* **84**, 203.

Roelfs AP, Martens JW, 1988. An international system of nomenclature for *Puccinia graminis* f. sp. *tritici*. *Phytopathology* **78**, 526 – 533.

Roelfs AP, Singh RP, Saari EE, 1992. *Rust diseases of wheat: Concepts and methods of disease management*. Mexico City, Mexico: CIMMYT.

Rowell JB, 1982. Control of stem rust by low receptivity to infection conditioned by a single dominant gene. *Phytopathology* **72**, 297 – 299.

SeedQuest, 2008. Ug99 wheat killer detected in Iran - Dangerous fungus on the move from East Africa to the Middle East. *SeedQuest News* Online. [http://www.seedquest.com/News/releases/2008/march/21996.htm]

Sewalem AM, 2006. *Genetic and phenotypic analyses of the wheat – Puccinia triticina Eriks*. Pathosystem. Bonn, Germany: University of Bonn, PhD thesis

Singh RP, 1991. Pathogenicity variation of *Puccinia recondita* f. sp. *tritici and P. graminis* f. sp. *tritici* in wheat-growing areas of Mexico during 1988 and 1989. *Plant Disease* **75**, 790-794.

Singh RP, Hodson DP, Jin Y, Huerta-Espino J, Kinyua MG, Wanyera R, Njau P, Ward RW, 2006. Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. *Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* **1**, 1 – 13.

Stakman EC, Stewart DM, Loegering WQ, 1962. *Identification of physiologic races of Puccinia graminis var. tritici*. Washington, USA: United States Department of Agriculture, Agricultural research service E-617 (revised).

van Ginkel MG, Getnet G, Tessema T, 1989. Stripe, stem and leaf rust races in major wheat producing areas in Ethiopia. *IAR Newsletter of Agricultural Research* **3**, 6 – 8.

Chapter 3

Genetic characterization of *Puccinia graminis* f. sp. *tritici* populations from Ethiopia by SSRs

B. Admassu[1,3], W. Friedt[2] and F. Ordon[3]

[1]Plant Protection Research Center, Ethiopian Institute of Agricultural Research, P. O. Box 37, Ambo, Ethiopia; [2]Justus-Liebig-University Giessen, Insitute of Crop Science and Plant Breeding I, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany; [3]Julius Kuehn-Institute, Federal Research Institute for Cultivated Plants (JKI), Institute for Resistance Research and Stress Tolerance, Erwin-Baur-Str. 27, 06484 Quedlinburg, Germany

Abstract

Stem rust (*Puccinia graminis* f. sp. *tritici*) is a devastating pathogen of bread wheat in major growing regions, particularly arid and semi-arid areas of Africa including Ethiopia. The genetic structure of *P. graminis* f. sp. *tritici* isolates sampled in three different regions of Ethiopia in 2006 and 2007 was investigated using 20 SSRs. The assays showed a high level of genetic diversity within *P. graminis* f. sp. *tritici* populations in Ethiopia. Tests for population subdivision revealed the absence of genetic differentiation among the populations on the basis of geographic separation as reflected by a low coefficient of genetic differentiation (≤0.046), and a single cluster consisting of all isolates. Gene flow among populations was estimated to be high (10 per generation). This study shows that the pathogen population of Ethiopia is characterized by a high genetic diversity within each population, and homogeneity across regions. The information obtained from this study may serve as a basis to develop better strategies for the deployment of resistance genes, e.g. using marker-assisted combination of resistance alleles to achieve better control of wheat stem rust in Ethiopia.

Key words: wheat stem rust, genetic diversity, microsatellites, population differentiation, resistance breeding

Part of this article is submitted for publication to the Journal of Phytopathology

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most widely grown cereal crops worldwide. In many warm environments where wheat is cultivated, stem rust disease caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn (*Pgt*) is the main limiting factor for wheat production (Roelfs et al. 1992). The disease is widespread throughout the world and causes significant yield reduction in tropical Africa in years of epidemics. Stem rust is also a major disease of wheat in Ethiopia, and it is widespread in the wheat growing regions, particularly in central, southeast and northwest Ethiopia (Admassu and Fekadu 2005). There are effective fungicides available to manage the stem rust fungus on wheat, but their use in developing countries like Ethiopia, where the farming systems are characterized by low input is not economically justifiable. In addition, due to the appearance and spread of Ug99, which is virulent to wheat that carries the 1BL.1RS translocation that contains *Sr31* (Singh et al. 2006) that was effective against all known *Pgt* races, stem rust represents a real threat to wheat production in Ethiopia. Recently, it was reported by Admassu et al. (2009) that this race is dominant throughout the country at a frequency of 26.6%. Hence, the occurrence and prevalence of Ug99 requires sources of resistance against this race that can be used in breeding programs. However, effective resistance breeding requires extensive information on the genetics of host-pathogen interaction.

Much of the knowledge accumulated regarding the population structure of *Pgt* was obtained by phenotyping (Stakman et al. 1962; Singh 1991; Fetch and Dunsmore 2004; Jin 2005; Admassu et al. 2009). Today, advancements in DNA fingerprinting techniques have allowed for a better understanding of the genetic structure of plant pathogens (Brake et al. 2001; Keiper et al. 2006; Bouajila et al. 2007; Szabo 2007). DNA-based techniques have increasingly become the tool of choice for understanding the genetic diversity of *Puccinia spp*, e.g., simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP) and single nucleotide polymorphism (SNPs) assays were used to study the genetic variability of *P. graminis* and *P. triticina* collections from different parts of the world (Keiper et al. 2006; Mebrate et al. 2006; Ordonez and Kolmer 2007; Szabo 2007; Visser et al. 2009). However, little is known about the genetic structure of the pathogen in African countries, where highly virulent races like Ug99 have originated. *Pgt* populations in Ethiopia are known

to exhibit a high level of variation in virulence (Admassu and Fekadu 2005; Admassu et al. 2009), but knowledge on the genetic diversity of the pathogen population is lacking.

The assumptions in this study were two fold: First, we expected the genetic diversity of *Pgt* populations in Ethiopia to be high like the variability in virulence (van Ginkel et al. 1989; Admassu and Fekadu 2005; Admassu et al. 2009). Second, there is variation in the prevalence of wheat species in the three regions; for example, central Ethiopia is dominated by *T. durum* with wide genetic base. On the other hand, southeast Ethiopia and northwest Ethiopia regions are dominated by *T. aestivum*. Such crop genetic variation is known to dictate the genetic background of plant pathogens through selection (McDonald 2004). In addition, northwest Ethiopia is geographically isolated from the other two regions, and we expected isolates from this region to be as unique as in the case of the virulence analysis study, which detected unique races in this region (Admassu et al. 2009). Hence, understanding the genetic structure of the pathogen populations is crucial for the development of effective control strategies for wheat stem rust.

Materials and Methods

Pgt isolates

Puccinia graminis f. sp. *tritici* samples were collected from farmers' wheat fields in Ethiopia in 2006 and 2007. Purification of the bulked samples and development of single pustule isolates is described in Admassu et al (2009). The list, origin and sources of 48 isolates utilized in the study are indicated in Table 1. For the sake of a better insight into the molecular diversity of *Pgt* populations, the 48 isolates were differentiated into three population groups based on their geographic origin. These three populations were: southeast Ethiopia (SEE), central Ethiopia (CEE) and northwest Ethiopia (NWE), each represented by 16, 21 and 11 isolates, respectively. Respective regions were described in Admassu et al. (2009).

Table 1 Origin, host cultivar and year of collection of *Puccinia graminis* f. sp. *tritici* isolates used for the SSR analyses

Region	Isolate	Origin	Host cultivar	Year of collection
Northwest Ethiopia	NWE01	Enarj Enawga	Dashen, BW	2006
	NWE02	Quami Cherq	Landrace, DW	2006
	NWE03	Bichena	Quamy, DW	2006
	NWE04	Awa	Unknown, BW	2007
	NWE05	Addis Zemen	Unknown, BW	2007
	NWE06	Angereb	ET-13A2, BW	2006
	NWE07	Walaj	Unknown, BW	2006
	NWE08	Adet	ET-13A2, BW	2006
	NWE09	Dejen	K6295-4A, BW	2006
	NWE10	Mahbere Berhan	Kubsa, BW	2006
	NWE11	Weyra	Bichena, DW	2006
Southeast Ethiopia	SEE01	Weltei	Unknown, BW	2006
	SEE02	Herero	Simba, BW	2006
	SEE03	Sinana	Oda, DW	2006
	SEE04	Adaba	Galema, BW	2006
	SEE05	Galema	Unknown, BW	2006
	SEE06	Robe	Sofumer, BW	2006
	SEE07	Ali Doyo	Ilani, DW	2006
	SEE08	Shorima	Unknown, BW	2006
	SEE09	Dhera	Unknown, BW	2006
	SEE10	Huruta	Kubsa, BW	2006
	SEE11	Gonde	Katar, BW	2006
	SEE12	Iteya	Kubsa, BW	2006
	SEE13	Temela	Digelu, BW	2006
	SEE14	Kulumsa	Kubsa, BW	2007
	SEE15	Kulumsa RC	Pavon 76, BW	2007
	SEE16	Asela	Galama, BW	2007
Central Ethiopia	CEE01	Akaki	Landrace, DW	2006
	CEE02	Dukem	Pavon 76, BW	2006

	CEE03	Dembi	Robe, DW	2006
	CEE04	Debre Zeit_1	Landrace, DW	2006
	CEE05	Debre Zeit_2	Gerardo, DW	2007
	CEE06	Weyo	Unknown, BW	2006
	CEE07	Melkasa ARC	Unknown, BW	2006
	CEE08	PPRC	Wetera, BW	2007
	CEE09	Ambo	Katar , BW	2006
	CEE10	Awash Buluto	Unknown, BW	2006
	CEE11	Ginchi	Kubsa, BW	2006
	CEE12	Addis Alem	ET-13A2, BW	2006
	CEE13	Alem Gena	Unknown, BW	2006
	CEE14	Geda Amba	Gerardo, DW	2006
	CEE15	Bonaya	Kubsa, BW	2006
	CEE16	Awash Melka	Kubsa, BW	2006
	CEE17	Adadi Mazoria	Israel, BW	2006
	CEE18	Tullubolo	Unknown, DW	2006
	CEE19	Legedadi	Landracel, DW	2006
	CEE20	Dire Mazoria	Unknown, BW	2006
	CEE21	Sendafa	Ude, DW	2006

DNA extraction

The total genomic DNA was extracted from 20 mg urediospores using the Nucleoplex^{TM} Plant DNA kit (Tepnel Life Sciences, Manchester, UK) according to the manufacturer's instruction with little modification, i.e., urediospores were first ground by shaking each sample with beads for 90s at a frequency of 27/s twice in a Mixer Mill MM 301 shaker (Retsch, Hannover, Germany). The concentration and quality of DNA were estimated using the NanoDrop ND-1000 spectrophotometer (PeQLab, Erlangen, Germany) and gel electrophoresis.

Microsatellite analysis

Twenty microsatellite markers (Table 2) developed for *Pgt* (Szabo 2007) were assayed in all individual isolates. Each PCR reaction contained 1 µl of 10x buffer, 1 µl of 25 mM $MgCl_2$, 0.2 µl of 10 mM dNTPs, 0.15 µl of forward primer (1.0 pmol/µl), 0.25 µl of reverse primer (10.0 pmol/µl), 0.08 µl of 5U HOT FIREPol®DNA polymerase (Solis BioDyne, Tartu, Estonia), and 1.5 µl template DNA (50 ng) in a final volume of 10 µl. For SSR amplification, M13 tailed forward primers were used, so that 0.1 µl of 'M13' primer (10.0 pmol/µl) (5' – CAC GAC GTT GTA AAA CGA C – 3') labelled with 5' fluorescent dyes of different colours (FAM, HEX or NED) were added to the reaction mix (Macdonald et al. 2006).

Amplification of DNA was conducted on a GeneAmp® PCR System 9700 (Applied Biosystems). PCR conditions for markers (*Pgt*SSR1, *Pgt*SSR3, *Pgt*SSR4, *Pgt*SSR11, *Pgt*SSR13, *Pgt*SSR14, *Pgt*SSR21, *Pgt*SSR47, *Pgt*SSR68, *Pgt*SSR119, *Pgt*SSR133, *Pgt*SSR134 and *Pgt*SSR147) were as follows: 94°C for 5 min; followed by touchdown PCR with 10 cycles of 30s at 94°C, 30s at 66°C, 30s at 72°C; and then 30 cycles with 30s at 94°C, 30s at 56°C, 30s at 72°C, and a final extension at 72°C for 15min. For the remaining microsatellites, the same touchdown PCR programme was used except the annealing temperature and number of cycles. Accordingly, for *Pgt*SSR6, *Pgt*SSR151, *Pgt*SSR162 and *Pgt*SSR180; 66 °C for 12 cycles and 60°C for 40 cycles were used.

For *Pgt*SSR12 and *Pgt*SSR 149; 70°C for 16 cycles and 62°C for 40 cycles, and finally for *Pgt*SSR180 64°C for 16 cycles and 56°C for 40 cycles were used. Amplification products were diluted 1:10 to 1:100 in HPLC gradient grade water (Carl Roth, Karlsruhe, Germany) depending on the intensity of the amplification product. PCR reactions of different colours (FAM, HEX and NED) were pooled at a ratio of 1:1:1 in a new plate and mixed with a mixture of Hi-Di™ formamide (Applied Biosystems) and GeneScan™ - 500 ROX™ size standard (Applied Biosystems) (0.03 µl ROX: 14 µl HiDi™ formamide). The ratio of the phosphoramidite fluorescent labels may vary depending on the intensity of the ampification product. The pooled PCR products were denatured at 94°C for 5 min and later subjected to capillary electrophoreses in an ABI PRISM® 3100 genetic analyzer (Applied Biosystems). Data were collected using 3130xl data collection software, v3.0 (Applied Biosystems). The size of the detected alleles was determined using the GeneMapper® software, v4.0 (Applied Biosystems).

Table 2 Number and size range of alleles of 48 *Puccinia graminis* f. sp. *tritici* isolates collected from Ethiopia assayed on 20 SSRs, number and frequency of null alleles, and the polymorphic information content of the SSR markers

Locus[a]	Size range (bp)	N_a[c]	No. of null alleles	PIC
*Pgt*SSR1	290 – 295	2	0	0.415
*Pgt*SSR3	351 – 353	2	7 (14.6)[d]	0.176
*Pgt*SSR4	375 – 378	4	0	0.694
*Pgt*SSR6	178 – 192	3	5	0.656
*Pgt*SSR11	169 – 190	4	0	0.636
*Pgt*SSR12	175 – 187	6	2 (4.2)	0.808
*Pgt*SSR13[b]	176 – 268	6	0	0.624
*Pgt*SSR14	216 – 238	4	0	0.734
*Pgt*SSR20[b]	172 – 190	5	0	0.681
*Pgt*SSR21	185 – 189	3	0	0.634
*Pgt*SSR47	206 – 211	2	0	0.507
*Pgt*SSR68	281 – 285	2	0	0.475
*Pgt*SSR119	328 – 345	5	0	0.770
*Pgt*SSR133[b]	295 – 376	6	0	0.602
*Pgt*SSR134	376 – 381	2	4 (8.4)	0.416
*Pgt*SSR147	226 – 238	4	0	0.712
*Pgt*SSR149	258 – 267	6	4 (8.4)	0.806
*Pgt*SSR151	274 – 280	2	0	0.434
*Pgt*SSR162	224 – 226	2	0	0.438
*Pgt*SSR180	228 – 260	4	0	0.733

[a] Source: Les Szabo, 2007

[b] Each locus detected unique alleles on separate single isolate

[c] N_a = Number of observed alleles

[d] The value in brackets show the frequency of null alleles

PIC = Polymorphic information content

Data analyses

Checking through the SSR data revealed absence of clones among isolates. Hence, data from all the 48 isolates were included in the statistical analyses. The absence or presence of allele(s) at a particular locus was recorded as 0 or 1. Distance matrices were calculated using the Neighbour Joining method, and later used for clustering of isolates by the unweighted pair group method with arithmetic averages (UPGMA) using the software NTSYSpc, v2.01e (Rohlf 1998). A consensus tree was generated by the majority-rule and strict consensus tree program using the software PHYLIP, v3.6 (Felstenstein 1995). Majority rule (extended) was selected as a consensus method. The goodness of fit of the cluster was estimated from the bootstrap values calculated in 1,000 permutations using the same software.

The usefulness of the SSR markers was estimated based on their polymorphic information content (PIC), which was calculated using the formula: PIC = $1 - \sum P_i^2$, where P_i is the frequency of the i^{th} allele. Population genetic analyses were done using the computer software POPGENE (Yeh et al. 1999). Accordingly, the genetic distance between populations (D) was estimated from allele frequencies using Nei's (1978) unbiased genetic distance for small samples. The genetic identity is 1 – D. Gene diversity at a given locus (h) was estimated using the formula: h = $1 - \sum X_i^2$, where X_i is the frequency of the i^{th} allele of a given locus. Population differentiation was calculated using the equation: $G_{st} = (H_t - H_s)/H_t$, where H_t refers to the total gene diversity, and H_s refers to the gene diversity within sub-populations. Gene flow between populations (N_m) was estimated using the formula: $N_m = 0.5\ (1 - G_{st})/G_{st}$. Other population genetic parameters that were estimated using POPGENE software included: number of observed and effective alleles, and percentage polymorphic loci. Multilocus, v1.3 (Agapow and Burt 2001) computer software was used to estimate the genotypic diversity of the populations.

Results

A total of 74 alleles were detected on 20 SSR loci on the set of 48 *Pgt* isolates. The number of alleles per locus ranged from 2 – 6, with an average of 3.7. Amplification product sizes varied between 169 to 381 bp. The polymorphic information content (PIC) of the 20 SSR primer pairs ranged from 0.176 to 0.808, with an average of 0.598. *Pgt*SSR12 and *Pgt*SSR3 had the highest (0.808) and lowest (0.176) PIC, respectively. Three of the 20 microsatellites amplified

null alleles (Table 2). The maximum genetic identity between two isolates (CEE02 and CEE19) was 94.1%. On the other hand, the lowest genetic identity, 22.2%, was observed between isolates SEE10 and CEE12.

Genetic diversity

The genetic structure of the three populations is summarized in Table 3. There was no marked difference in the 'within' population genotypic diversity between northwest Ethiopian (0.619) and southeast Ethiopian (0.600) populations. There was also no significant difference in their 'within' population gene diversity (NWE = 0.485 and SEE = 0.466). The central Ethiopian population had the highest gene (0.555) and genotypic (0.718) diversity compared to the other two regions. The analyses revealed a high number of different rust genotypes in each region. Absence of clones means each pathogen isolate belonged to a different genotype group. Hence, NWE, SEE and CEE had 11, 16 and 21 different genotypes, respectively (Table 2).

Population differentiation

The study revealed absence of population differentiation among populations as evidenced by the low coefficient of genetic differentiation (G_{ST}), which was estimated at 0.046 (Table 3). G_{ST} values of less than one are an indicator of a high degree of genetic similarity among populations and absence of population differentiation. Absence of population differentiation was also supported by high level of gene flow among populations. The gene flow among populations of the three regions per season was estimated at 10.4 when analysed using SSRs. The other genetic parameters described in this study were the number of observed and effective alleles. The latter represent the number of genetically distinct individuals that contribute gametes to the next generation. Out of 3.65 average observed alleles across populations on average, 2.63 (72.1%) alleles were effective. Looking at individual regions, central and southeast Ethiopian populations had the highest number of observed alleles, but the highest percentage of effective alleles was recorded in the northwest Ethiopian *Pgt* population (Table 3).

Furthermore, pair-wise population comparisons for genetic distance and identity indicated a high degree of identity among populations. The highest identity was observed between northwest Ethiopian and central Ethiopian populations with an estimated identity of 0.920. On the other hand, a relatively low degree of genetic identity was observed between northwest and southeast Ethiopian populations at 0.685 (Table 4).

Clustering of isolates by UPGMA placed the isolates in two major clusters at about 60% genetic similarity (data not shown). But a bootstrap analysis using the software PHYLIP, v3.6 assigned all isolates into a single cluster (Fig. 1). It is important to note here that the single isolate (SEE09) that was not included in the cluster is not a unique genotype. It is rather an outgroup, which by default is the first species encountered on the first tree, and it always appears when the un-rooted consensus tree programme is run (Felstenstein 1995). So this is not to be taken as interesting information.

Discussion

The microsatellite based analysis revealed a high level of genetic diversity within each population. Most of the SSR markers revealed a high polymorphic information content making them valuable for the characterization of *Pgt* isolates. The exception was *Pgt*SSR3, which had a very low PIC. The present study detected lower number of alleles than that detected on USA isolates (Szabo 2007) but higher than those identified in South African isolates (Visser et al. 2009).

Two important assumptions were made at the beginning of this study. The first was that the *Pgt* population in Ethiopia is genetically diverse, and secondly it was assumed that there is genetic differentiation based on the geographic origin of isolates. The present study proved the first hypothesis to be valid as the SSR analyses showed *Pgt* isolates in Ethiopia to be genetically diverse. The high genetic diversity was reflected by the high genotypic diversity, the large number of genotypes and the high gene diversity within populations. These results were similar to studies conducted in the USA (Szabo 2007) and Australia (Keiper et al. 2006) that found high genetic diversity among *Pgt* and *P. graminis* f. sp. *avenae* populations, respectively. The high genetic diversity in Ethiopian *Pgt* populations might be attributed to the co-evolution of the pathogen with durum wheat, which is indigenous to Ethiopia with a broad genetic base (Tessema 1991), and has been under cultivation for centuries (Nastasi 1964). van Ginkel et al. (1989) have also given a similar explanation, i.e., co-evolution of *Pgt* along with wheat being the reason for high virulence diversity in Ethiopian *Pgt* populations.

Table 3 Genetic structure of *Puccinia graminis* f. sp. *tritici* populations collected from three regions of Ethiopia (southeast Ethiopia, northwest Ethiopia and central Ethiopia) analysed by 20 SSR markers

Region	Sample size	N_a^* ±SD	N_e±SD	PL (%)	GnD±SD	NG	GtD±SD	G_{st}	N_m
Overall	48	3.65 ± 1.49[a]	2.63 ± 1.11[a]	100.00	0.560 ± 0.16[a]	48	0.745 ± 0.22[a]	0.046	10.4
SEE	16	3.20 ± 1.36[b]	2.08 ± 0.90[b]	100.00	0.466 ± 0.18[a]	16	0.600 ± 0.19[a]		
NWE	11	2.95 ± 1.19[b]	2.28 ± 0.96[b]	95.00	0.485 ± 0.21[a]	11	0.619 ± 0.16[a]		
CEE	21	3.45 ± 1.36[ab]	2.61 ± 1.16[ab]	100.00	0.555 ± 0.17[a]	21	0.718 ± 0.19[a]		

[a] Values followed by the same letter in a column are not significantly different

$^*N_a$ = Average number of observed alleles N_e = Average number of effective alleles PL = Polymorphic loci

GnD = Gene diversity NG = Number of genotypes GtD = Genotypic diversity

G_{st} = Genetic differentiation between populations N_m = Estimate of gene flow SD = Standard deviation

Hence, it is possible that *Pgt* in Ethiopia has evolved along with wheat, creating isolates with diverse genetic backgrounds. According to Burdon and Thrall (2000), host-pathogen co-evolution for fungal pathogens like rusts is reported to be strong due to cultivar replacement, and is one of the several evolutionary factors that affect the genetic structure of the pathogen. This might be the reason for the higher genetic diversity within the central Ethiopian *Pgt* population than the other two populations as central Ethiopia is dominated by *T. durum* cultivation, whereas, in southeast Ethiopia and northwest Ethiopia *T. aestivum* prevails (Tessema 1991; Mebrate et al. 2006). *T. aestivum* is an introduced species to Ethiopia with a narrow genetic base (Gebre-Mariam 1991; Tessema 1991). Eventually, host selection due to the narrow genetic make-up reduces the genetic diversity in the pathogen population. In a similar manner, high genetic diversity within the *P. tritici* population from central Ethiopia has been reported compared to south and southeast Ethiopian regions (Mebrate et al. 2006). Sexual reproduction is the other major source of genetic diversity in plant pathogens (McDonald 2004). The sexual life cycle of *Pgt,* in which nuclei of + and - mating types are reunited, encompasses five stages (uredospore, teliospore, basidiospore, pycniospore and aeciospore) and is completed on the alternate host (*Berberis vulgaris* L.). Berbery serves as a breeding ground for new races. In addition, accumulated mutation as evidenced in *Rhynchosporium secalis* in Tunisia (Bouajila et al. 2007), somatic hybridization (Anderson and Pryor 1992), and introduction of genetically distinct exotic isolates (Keiper et al. 2006; Visser et al. 2009) like Ug99 could be responsible for the observed genetic variation in the Ethiopian *Pgt* populations. The evidence for the introduction of exotic isolates with a unique genetic background and/or point mutation was in this study proven by the occurrence of single unique alleles in three isolates, one from northwest Ethiopia and two from southeast Ethiopia.

Table 4 Genetic identity among *Puccinia graminis* f. sp. *tritici* populations collected from three regions of Ethiopia (southeast Ethiopia, northwest Ethiopia and central Ethiopia) analysed by 20 SSR markers

Region	Combination of isolates	Genetic identity
NWE X SEE	176	0.685
NWE X CEE	231	0.920
SEE X CEE	336	0.870

Nei's (1978) unbiased measure of genetic genetic identity

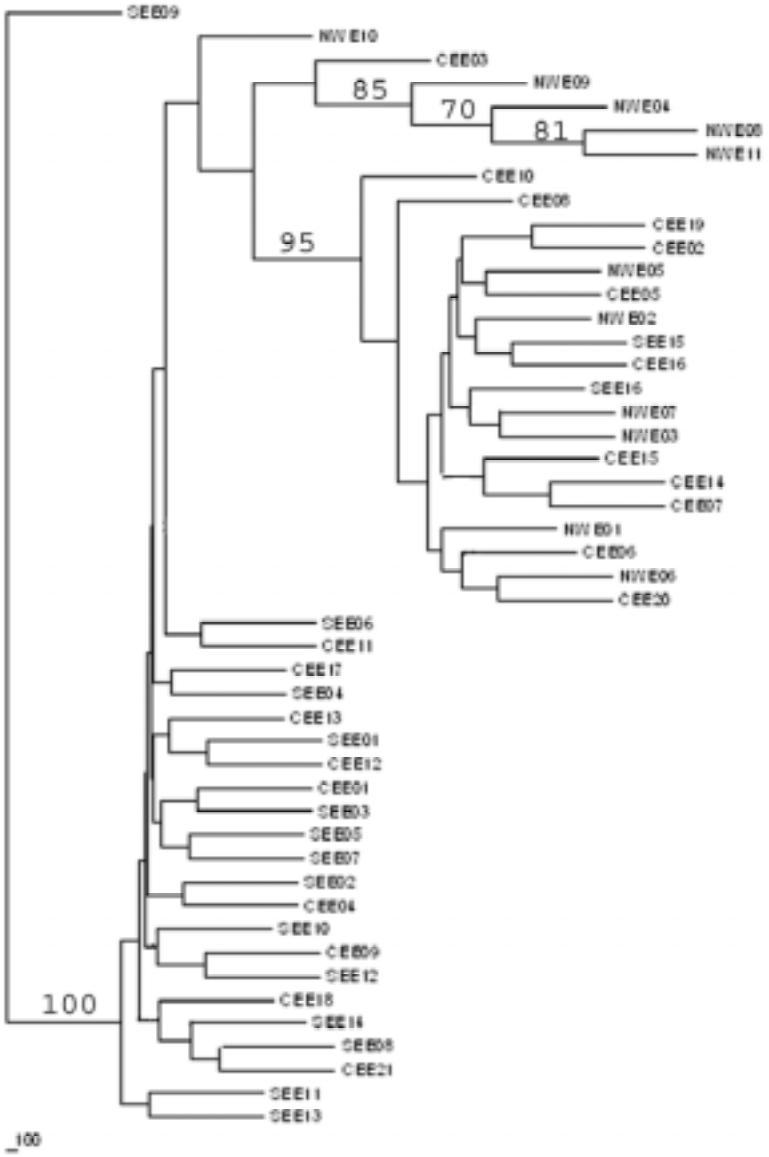

Fig. 1 Consensus tree of 48 *Puccinia graminis* f. sp. *tritici* isolates from Ethiopia based on data generated from 20 SSR markers. Bootstrap values supporting the cluster at higher than 70% after 1,000 iterations are shown above the internodes.

Over all, comparison to previous studies indicated that the 48 Ethiopian isolates are as diverse as the 25 isolates anlayzed by Szabo (2007), but have a higher genetic diversity than 29 isolates from South Africa (Visser et al. 2009). The high genetic diversity in the Ethiopian isolates compared to the South African ones might be due to cultivation of susceptible wheat land races in Ethiopia (Gebre-Mariam 1991) in contrast to cultivation of genetically uniform wheat cultivars in a small geographic area in South Africa (Visser et al. 2009).

The low level of genetic differentiation based on geographic separation observed in this study is in contrast to the hypothesis that *Pgt* populations in Ethiopia are differentiated based on geographic origin of isolates. Rather, the isolates from the three regions are closely related to one another as reflected by the high genetic identity among populations. The cluster generated using the SSR data also supported the absence of genetic differentiation in the Ethiopian *Pgt* populations. This suggests that the Ethiopian *Pgt* populations did not evolve independently, and are part of a larger pathogen genetic pool with a common ancestor, which might extend to include the larger east African region. Hence, future studies should focus on isolates from the sub-continent to determine their genetic lineage. Common ancestral origin is not unique to Ethiopian *Pgt* populations. Similar results were reported for pathogens of cereal rusts in South America, North America and Europe (McCallum 1999; Ordonez and Kolmer 2007). On the other hand, clonal lineages were reported for cereal rusts in South Africa and Australia (Brake et al. 2001; Keiper et al. 2006; Visser et al. 2009).

Absence of genetic differentiation in the Ethiopian *Pgt* populations might be attributed to gene flow among regions, which is reflected by the high migration rate (N_m) estimates. McDermott and McDonald (1993) indicated that if N_m is less than one, local population tend to differentiate; if N_m is greater than 1, there will be little differentiation among populations and migration is more important than genetic drift. Similarly, Wright (1951) stated that the movement of as few as one individual per generation is sufficient to prevent significant divergence between populations. The high estimate of gene flow among Ethiopian *Pgt* populations suggests that there have been only few restrictions to gene flow among regions. This is plausible as urediospores of *Pgt* are airborne and can travel long distance (Luig 1983). This was seen with the migration of a virulent race of *P. striiformis* f. sp. *tritici* from east Africa to the Middle East (Badebo 2002), and very recently with the spread of Ug99 from Uganda, first to Kenya and Ethiopia, and then to Asia (Mackenzie 2007). These are evidences that pathogen populations of neighbouring countries like Kenya, the Sudan, Uganda and

others do affect the genetic structure of the Ethiopian *Pgt* population and vice versa. Hence, to attain a sustainable control of stem rust in Ethiopia and in the wider east African region, coordinated efforts among countries in the region are imperative. This can include establishment of regional stem rust nursery to track the movement of races, and establishment of fast and formal information exchange network among regions.

Caution is needed to draw definitive conclusions to the causes of the variation and/or homogeneity of populations detected in this study as it is limited to a rather small number of samples collected in two years. Still, this study is the first of its type in the region and may serve as a basis for detailed studies in the future.

In conclusion, our study showed that *Pgt* populations in Ethiopia are characterized by a high genetic diversity within populations, and homogeneity across regions. According to McDonald and McDermott (1993) knowledge of the genetic structure of a pathogen helps to determine the rate of virulence evolution, and to predict how long a control measure is likely to be effective. The results obtained are reminders that *Pgt* populations in Ethiopia can easily adapt to deployed stem rust resistance genes. This might be one of the reasons for the resistance break in the Ethiopian wheat cultivars within two to three years of deployment (CIMMYT 2005). Hence, the agricultural research and development system needs to establish an anticipatory breeding programme as well as deploy cultivars possessing "horizontal" resistance to attain durable stem rust control in the country. Such broad resistance may be achieved either by selecting for polygenic (quantitative) resistance or by combining different major genes ("pyramiding") in new cultivars carrying multiple resistance genes.

Acknowledgement

The first author was supported by a scholarship from the Katholischer Akademischer Auslaender-Dienst (KAAD), Germany, to conduct this research. We would like to thank the Ethiopian Institute of Agricultural Research for providing leave of absence for the first author to carry out the research. We also thank Dr. Dragan Perovic for his help in the SSR analysis.

References

Admassu B, Fekadu E. (2005) Physiological races and virulence diversity of *Puccinia graminis* f. sp. *tritici* on wheat in Ethiopia. Phytopathol Mediterranea **44**:313-318.

Admassu B, Lind V, Friedt W, Ordon F. (2009) Virulence analysis of *Puccinia graminis* f. sp. *tritici* populations in Ethiopia with special consideration of Ug99. Plant Pathol **58**:362-369.

Agapow PM, Burt A. (2001) Indices of multilocus linkage disequilibrium. Molecular Ecol Notes **1**:101-102.

Anderson PA, Pryor AJ. (1992) DNA restriction fragment length polymorphisms in the wheat rust fungus, *Puccinia graminis* f. sp. *tritici*, Theor Appl Genet **83**:715-719.

Badebo, A. (2002) Breeding bread wheat with multiple disease resistance and high yield for the Ethiopian highlands: Broadening the genetic basis of yellow rust and tan spot resistance. Georg-August University of Goettingen, Germany, PhD Thesis.

Brake VM, Irwin JAG, Park RF. (2001) Genetic variability in Australian isolates of *Puccinia coronata* f. sp. *avenae* assessed with molecular and pathogenicity markers. Australasian Plant Pathol **30**:259-266.

Bouajila A, Abang MM, Haouasa S, Udupa S, Rezgui S, Baum M, Yahyaoui A. (2007) Genetic diversity of *Rhynchosporium secalis* in Tunisia as revealed by pathotype, AFLP, and microsatellite analyses. Mycopathologia **163**:281-294.

Burdon JJ, Thrall PH. (2000) Coevolution at multiple spatial scales: *Linum marginale Melampsora lini* – from the individual to the species. Evol Ecol **14**:261-281.

CIMMYT (2005). Sounding the alarm on global stem rust: an assessment of race Ug99 in Kenya and Ethiopia and the potential for impact in neighboring countries and beyond. 29 May 2005. Nairobi, Kenya, pp 1-26.

Felstenstein, J. (1995). PHYLIP: Phylogeny Inference Package, v3.6. Washington State University, Seattle, USA.

Fetch TG, Dunsmore KM. (2004) Physiological specialization of *Puccinia graminis* on wheat, barley, and oat in Canada in 2001. Can J Plant Pathol **26**:148-155.

Gebre-Mariam H. (1991) Wheat production and research in Ethiopia. In: Gebre-Mariam H, Tanner DG, Hulluka M. (eds.) Proc Wheat Research in Ethiopia: A Historic Perspective, 1991. Addis Ababa, Ethiopia, pp 1-16.

Jin Y. (2005) Races of *Puccinia graminis* identified in the United States in 2003. Plant Dis **89**:1125-1127.

Keiper FJ, Haque MS, Hayden MJ, Park RF. (2006) Genetic diversity in Australian populations of *Puccinia graminis* f. sp. *avenae*. Phytopathol **96**:96-104.

Luig NH. (1983) Epidemiology in Australia and New Zealand. In: Cereal rusts. Vol. II: Diseases, distribution, epidemiology, and control. Roelfs AP and Bushnell WR. (eds) Orlando, USA, Academic Press, pp 301-328.

Macdonald AJ, Sankovic N, Sarre SD, Fitzsimmons NN, Wakefield MJ, Graves JAM, Zenger KR. (2006) Y chromosome microsatellite markers identified from the tammar wallaby (Macropus eugenii) and their amplification in three other macropod species. Mol Ecol Notes **6**:1202-1204.

Mackenzie D. (2007) Billions at risk from wheat super-blight. New Sci **193**:35.

McCallum BD, Roelfs AP, Szabo LJ, Groth JV. (1999) Comparison of *Puccinia graminis* f.sp. *tritici* from South America and Europe. Plant Pathol **48**: 574-581.

McDermott JM, McDonald BA. (1993) Gene flow in plant pathosystems. Annu Rev Phytopathol **31**:353-373.

McDonald BA. (2004) Population Genetics of Plant Pathogens. The Plant Health Instructor **DOI**:10.1094/PHI-A-2004-0524-01.

McDonald BA, McDermott JM. (1993) Population genetics of plant pathogenic fungi. Biosc **43**:311-319.

Mebrate SA, Dehne HW, Pillen K, Oerke EC. (2006) Molecular diversity in *Puccinia triticina* isolates from Ethiopia and Germany. J Phytopathol **154**:701-710.

Nastasi V. (1964) Wheat production in Ethiopia. Information bulletin on the Near east wheat and barley improvement and production project **1**:13-23.

Nei M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**:583-590.

Ordonez ME, Kolmer JA. (2007) Simple sequence diversity of a worldwide collection of *Puccinia triticina* from durum wheat. Phytopathol **97**:574-583.

Roelfs AP, Singh RP, Saari EE. (1992) Rust diseases of wheat: Concepts and methods of disease management. CIMMYT, Mexico City, Mexico.

Rohlf FJ. (1998) NTSYSpc Version 2.01e: User Guide, New York, USA, Applied Biostatistics Inc.

Singh RP. (1991) Pathogenicity variation of *Puccinia recondita* f. sp. *tritici and P. graminis* f. sp. *tritici* in wheat-growing areas of Mexico during 1988 and 1989. Plant Dis **75**:790-794.

Singh RP, Hodson DP, Jin Y, Huerta-Espino J, Kinyua MG, Wanyera R, Njau P, Ward RW. (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources **1**:1-13.

Stakman EC, Stewart DM, Loegering WQ. (1962) Identification of physiologic races of *Puccinia graminis* var. *tritici*. United States Department of Agriculture, ARS, Washington, USA, pp 1-53.

Szabo LJ. (2007) Development of simple sequence repeat markers for the plant pathogenic rust fungus, *Puccinia graminis*. Mol Ecol Notes **7**:92-94.

Tessema T. (1991) Improvements of indigenous durum wheat landraces in Ethiopia. In: Engels JMM, Hawkes JG, Worede M. (eds) Proc 1st International Symposium on the Conservation and Utilization of Ethiopian Germplasm, 13-16 October 1986. Addis Ababa, Ethiopia, Cambridge University Press, pp 288-295.

van Ginkel MG, Getnet G, Tessema T. (1989) Stripe, stem and leaf rust races in major wheat producing areas in Ethiopia. IAR Newsletter of Agricultural Research **3**:6-8.

Visser B, Herselman L, Pretorius ZA. (2009) Genetic comparison of Ug99 with selected South African races of *Puccinia graminis* f.sp. *tritici*. Mol Plant Pathol 10:213-222.

Wright S. (1951) The genetical structure of populations. Ann Eug **15**: 323-354.

Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX. (1999) POPGENE: The user-friendly shareware for population genetic analysis. University of Alberta, Edmonton, Alberta, Canada.

Chapter 4

Genetic mapping of gene *Sr13* being effective against UG99 (*Puccinia graminis* f. sp. *tritici*) in wheat (*Triticum aestivum*)

B. Admassu[1, 2], D. Perovic[2], W. Friedt[3], F. Ordon[2]

[1] Plant Protection Research Center, Ethiopian Institute of Agricultural Research, P. O. Box 37, Ambo, Ethiopia; [2] Julius Kuehn-Institute, Federal Research Institute for Cultivated Plants (JKI), Institute for Resistance Research and Stress Tolerance, Erwin-Baur-Str. 27, 06484 Quedlinburg, Germany; [3] Justus-Liebig-University Giessen, Institute of Crop Science and Plant Breeding I, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany

Abstract

Puccinia graminis f. sp. *tritici*, the causative agent of stem rust in wheat, is known for its high virulence variability and ability to evolve new virulence to resistance genes. Thus, pyramiding of several resistance genes in a single line is the best strategy for a sustainable control of wheat stem rust. *Sr13* is one of the few resistance genes that are effective against wide ranging *Puccinia graminis* f. sp. *tritici* races, including the pestilent race Ug99. Its effectiveness to Ug99 makes it a valuable source for resistance to stem rust. Molecular markers play a pivotal role in the genetic characterization of the new sources of resistance as well as in stacking two or more resistance genes in a single line. Therefore, the aim of this study was to develop molecular markers for *Sr13* facilitating efficient pyramiding of Sr genes. Based on 158 F_2 individuals derived from a cross of Khapstein/9*LMPG x Morocco and SSR analyses the *Sr13* locus was mapped on chromosome 6A of wheat, and a genetic map comprising 37 cM was constructed with the closest marker Barc37 being located 3.0 cM distally of *Sr13*. These newly developed markers will increase the efficiency of incorporating *Sr13* into cultivars that are widely adopted but susceptible to hazardous Ug99 and/or assist for the development of new elite lines that are resistant to Ug99.

Keywords: Ug99, *Sr13*, stem rust, wheat, mapping

Part of this article will be published in a scientific journal

Introduction

Currently, world bread wheat production is threatened by wheat stem rust caused by the fungus *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn (*Pgt*) due to the spread of a new dangerous race designated as Ug99 (Singh et al., 2008). Stem rust was under control in most parts of the world through eradication of common barberry plants in north America (Roelfs et al. 1992) and deployment of resistance genes like *Sr31*, *Sr24, Sr2* and *Sr26* in many wheat producing countries (Bariana et al. 2004; Park 2008). The appearance of Ug99 and its variants that are virulent against the above mentioned genes (Pretorius et al. 2000; Jin et al. 2008) has brought the disease to the forefront as a potential threat to world wheat production. Ug99 was first discovered in Uganda in 1999 (Pretorius et al. 2000) and later spread to other parts of Africa and the Middle East (Singh et al. 2006; Nazari et al. 2009). It is projected to spread farther to the major wheat growing regions of Asia (Singh et al. 2008). Surveys and varietal tests in 18 African and Southeast Asian countries that are lying in the projected route showed that varieties exhibiting resistance to Ug99 account for only 5% of the total estimated area of 75 million ha wheat fields (Singh et al. 2008). In addition, out of a total of 5700 common and 2733 durum wheat landrace accessions collected from around the world only 251 (4.4%) and 290 (10.9%) respectively turned out to be resistant to Ug99 (Bonman et al. 2007). If this race is allowed to spread unchecked, it may have disastrous consequence in world wheat production. All these suggest the urgent need for incorporating effective resistance genes in wheat cultivars cultivated in countries where Ug99 already exists as well as in countries potentially threatened by it.

Experimental tests have shown that there are only few described resistance genes that are effective against Ug99, i.e. *Sr13*, *Sr14*, *Sr24, Sr36* and *SrTmp* (Singh et al. 2008). However, genes like *Sr24* and *Sr36* have rendered ineffective against variants of Ug99 that were detected very recently in Kenya (Jin et al. 2008; Jin et al. 2009). *Sr13* on the other hand is effective against both variants of Ug99 as well as against a wide range of races (McIntosh et al. 1995; Singh et al. 2006; Admassu et al. 2009). *Sr13*, which was assigned to the long arm of chromosome 6A by cytogenetic methods (McIntosh 1972) was derived from *T. turgidum* var. dicoccum cv. Khapli C.I.4013, and was later transferred to the common wheat line, Khapstein (McIntosh et al. 1995).

It is also important to note that some virulent races other than Ug99 are reported to *Sr13* in some countries (Huerta-Espino 1992; McInotsh et al. 1995). Hence, it can best be utilized in combination with other genes through gene pyramiding. The use of race-specific resistance genes in combinations (gene pyramiding) is described as the best strategy in wheat breeding to avoid fast breakdown to stem rust (Singh et al. 2008; Tsilo et al. 2008). For this, molecular markers provide a powerful tool to identify plants that carry combinations of resistance genes. In view of the effectiveness of *Sr13* against Ug99 and other wide ranging races, the identification of molecular markers closely linked to this gene will facilitate an enhanced incorporation of this gene into adapted wheat cultivars by marker assisted selection procedures. Therefore, the objective of this study was to identify microsatellite markers which are especially suited for marker assisted selection procedures as they are easy to handle and inherited in a co-dominant manner closely linked to *Sr13*.

Materials and Methods

Plant materials

An F_2 mapping population was derived by crossing the susceptible cultivar 'Morocco' with the near-isogenic wheat line Khapstein/9*LMPG (Khapstein) that carries the stem rust resistance gene, *Sr13*. A total of 158 segregating F_2 individuals were developed and used for marker development. Seeds from each F_2 plant were harvested separately to develop F_3 families.

Phenotyping

Spores of race Ug99 (also called TTKSK) were mixed with sterile water, and inoculated onto seven day old seedlings of F_2 plants, and the parents. Race Ug99 is virulent to cultivar Morocco, but avirulent to Khapstein. Besides this, 20 – 25 seedlings of each of the $F_{2:3}$ families were phenotyped with the same pathogen race to distinguish between homozygous and heterozygous resistant F_2 plants, and at the same time to confirm the disease scores on F_2 seedlings.

Immediately after inoculation, seedlings were incubated in the dark for 18 hours at 18^{o}C and high relative humidity in a humid chamber. After 18 hours of darkness, the seedlings were exposed to fluorescent light for three hours. Then they were transferred to a growth chamber

and grown constantly at 22 ± 1°C, a light intensity of 10,000 lx and a photoperiod of 16 hours. Infection types were scored 14 days after inoculation based on a standard 0-4 scale (Stakman et al. 1962; Roelfs 1988).

DNA extraction and microsatellite analysis

DNA from F_2 progenies and parents was extracted following the method developed by Stein et al. (2001) for wheat. The concentration and quality of DNA were estimated using the NanoDrop ND-1000 spectrophotometer (PeQLab, Erlangen, Germany) and gel electrophoresis.

37 microsatellite markers from the 6A chromosome of wheat (barc3, barc37, barc106, barc107, barc113, barc118, barc146, barc171, barc204, barc206, barc1055, cfa2114, cfd80, cfd82, cfd190, cfe132, cfe168, cfe179, cfe273, gwm132, gwm169, gwm334, gwm570, gwm617, wmc145, wmc150, wmc182, wmc201, wmc256, wmc398, wmc417, wmc753, wmc786, wmc580, wms427, wms459, wms494) were screened for polymorphism between parents. All polymorphic markers were assayed in all individual F_2 progenies and parents. Each PCR reaction contained 1 µl of 10x buffer, 1 µl of 25 mM $MgCl_2$, 0.2 µl of each 10 mM dNTPs, forward primer (1.0 pmol/µl), and reverse primer (10.0 pmol/µl), 0.18 µl of 5U HOT FIREPol®DNA polymerase (Solis BioDyne, Tartu, Estonia), and 1 µl template DNA (25 ng) in a final volume of 10 µl. For SSR amplification, M13 tailed forward primers were used, so that 0.18 µl of 'M13' primer (10.0 pmol/µl) (5' – CAC GAC GTT GTA AAA CGA C – 3') labelled with 5' fluorescent dyes was added to the reaction mix (Macdonald et al. 2006).
Amplification of DNA was conducted on a GeneAmp® PCR System 9700 (Applied Biosystems). PCR conditions were as follows: 94°C for 5 min; followed by touchdown PCR with 12 cycles of 30s at 94°C, 30s at 62°C, 30s at 72°C; and then 35 cycles with 30s at 94°C, 30s at 56°C, 30s at 72°C, and a final extension at 72°C for 10min. Amplification products were diluted 1:6 to 1:50 in HPLC gradient grade water (Carl Roth, Karlsruhe, Germany) depending on the intensity of the PCR product. 1 µl of the diluted PCR product was mixed with a mixture of Hi-Di™ formamide (Applied Biosystems) and GeneScan™ - 500 ROX™ size standard (Applied Biosystems) (0.03 µl ROX: 14 µl HiDi™ formamide). The mixture was then denatured at 94°C for 5 min and later subjected to capillary electrophoreses in an ABI PRISM® 3100 genetic analyzer (Applied Biosystems). Data were collected using 3130xl data collection software, v3.0 (Applied Biosystems). The size of the detected alleles was determined using the GeneMapper® software, v4.0 (Applied Biosystems).

Linkage analysis

The F_2 individuals were classified as homozygous resistant (identical allele size to the resistant parent), homozygous susceptible (identical allele size to the susceptible parent) and heterozygous resistant (having both parental allele sizes). Later the goodness-of-fit to the Mendelian segregation patterns of $F_{2:3}$ (1:2:1) and $F_{3:4}$ (3:1) were tested using Chi-squared (X^2) distribution analyses. Based on these data the genetic map was constructed using the software JoinMap, v.4 (Van Ooijen 2006) applying the Kosambi function (Kosambi 1944).

Results

Phenotypic analysis

The results of the inoculation test showed that cultivar 'Morocco' was highly susceptible (4+ infection type) to race Ug99 whereas the parental line 'Khapstein' was resistant showing an infection type of 2. The 158 F_2 individuals segregated at a ratio of 121R:37S which fits to a Mendelian 3r:1s ratio (Table 1). Phenotyping of the F_3 families showed that the F_2 population had a genetic make-up of homozygous resistance (44 plants), heterozygous resistant (77 plants) and homozygous susceptible (37 plants). Chi-square test indicated that this ratio fits to a 1:2:1 segregation ratio (Table 1). These results clearly indicate that resistance to Ug99 in this population is due to a single dominant resistance gene in the resistant parental line. Assay results of the *Sr13* locus and the five marker loci fitted the 1:2:1 ratio with P-values > 0.20 (Data not shown).

Linkage analysis

The parents, Morocco and Khapstein, as well as susceptible and resistant bulks were screened with 37 microsatellites which were previously mapped on 6A. Out of 37 markers, ten (27%) were polymorphic (nine co-dominant and one dominant).

The allele sizes detected in the parents when assayed with these markers is indicated on Table 2. All of the polymorphic SSRs were assayed across the mapping population of 158 F_2 progenies. Three markers (barc118, cfe168 and gwm334) deviated significantly from the expected 1:2:1 or 3:1 ratio hence they were eliminated from the linkage analysis. Five of the remaining polymorphic markers revealed linkage to *Sr13* (Fig. 1). All of the mapped markers were co-dominant. The stem rust resistance gene *Sr13* was mapped 3.0 cM distal to the

closest marker, i.e. barc37, and 6 cM proximal to wmc256. Besides this, linkage to barc107 at a distance of 7.0 cM and to gwm570 and barc146 at distances of 18.0 and 19.0 cM, respectively was detected (Fig. 1).

Discussion

Sr13 is an important source of resistance to stem rust in the face of Ug99. In addition to its proven effectiveness against Ug99, virulence for *Sr13* appears to be extremely rare except in a few countries (Luig 1983; Huerta-Espino 1992; Admassu et al. 2009). Because of this it has already been exploited in some Australian wheat cultivars as a source of resistance; but cultivars carrying this gene in general have low thousand grain weight (McIntosh et al. 1995). This may be a reason for the under utilization of *Sr13* despite its potential for stem rust control. Therefore, long lasting backcrossing procedures are needed to combine *Sr13* with superior agronomic performance. This can be considerably enhanced by using marker assisted selection procedures (e.g. Ordon et al. 2003; Dong et al. 2007).

In the present study such markers were developed and *Sr13* was assigned to chromosome 6AL of wheat. This map location is consistent with previous mapping studies based on cytogenetic techniques positioning *Sr13* on chromosome 6AL of wheat (McIntosh 1972; Klindworth et al. 2007). Chromosome 6A carries additional stem rust resistance genes *Sr8* and *Sr26* (McIntosh 1972, McIntosh 1995; Mago et al. 2005). Another gene designated as *Srdp2* (Rondo et al. 1966), which was initially thought to be allelic to *Sr13* (McIntosh 1995), but later confirmed as a different gene (Huerta-Espino 1992) was also located on this chromosome.

Table 1 Segregation ratio and chi square test analysis of F_2 and F_3 progenies inoculated with race Ug99

Generation	Resistant	Segregating	Susceptible	Total	Expected ratio	X^2	P-value
F_2	121	-	37	158	3:1	0.211	0.646
F_3	44	77	37	158	1:2:1	0.722	0.697

Table 2 Size of alleles detected from the resistant (Khapstein/9*LMPG) and susceptible (Morocco) parents when assayed with the ten polymorphic markers

	Allele size	
Marker	Resistant parent	Susceptible parent
Barc37	175	181
Barc107	201	193
Barc118	152	168
Barc146	182	180
Cfe168*	144_153	153
Cfe179	340	328
Gwm334	133	139
Gwm570	171	163
Wmc256	134	144
Wmc580	323	338

* Dominant marker

The order of markers obtained in our study is in agreement with those previously published (Somers et al. 2004; Zhang et al. 2008). The order of three markers out of four corresponds to the consensus map of Somers et al. (2004). According to the latter authors barc37 is located proximal to barc146, however, the present study located barc37 distal to barc146 as shown in Fig. 1. Such discrepancy in marker order on a genetic map is not uncommon as witnessed by Tsilo et al. (2008) and Song et al. (2005).

The deviation of co-linearity in marker order between the two maps could be explained by the fact that the map of Somers et al (2004) was a consensus map constructed by joining four independent genetic maps of wheat. On the other hand, the order of four markers, barc146, barc107, wmc256 and gwm570, is in complete agreement with the consensus map of Zhang et al. (2008). Barc37 was not present in this map, and hence, its position could not be compared to our result or to that of Somers et al. (2004). Overall, our marker order is in agreement with previously published maps. Aaccording to Sourdile et al. (2004), the bin locations of the two

flanking markers proximal to *Sr13*, barc107 and barc37, were tentatively placed in the combined bin of C-6AL5-0.55 and 6AL4-0.5-0.90 on chromosome 6A of Sourdille's physical map. Only the marker mapped distal to *Sr13*, wmc256, was placed in the 6AL4-0.55-0.90 bin. Therefore, the bin location of *Sr13* is not conclusive.

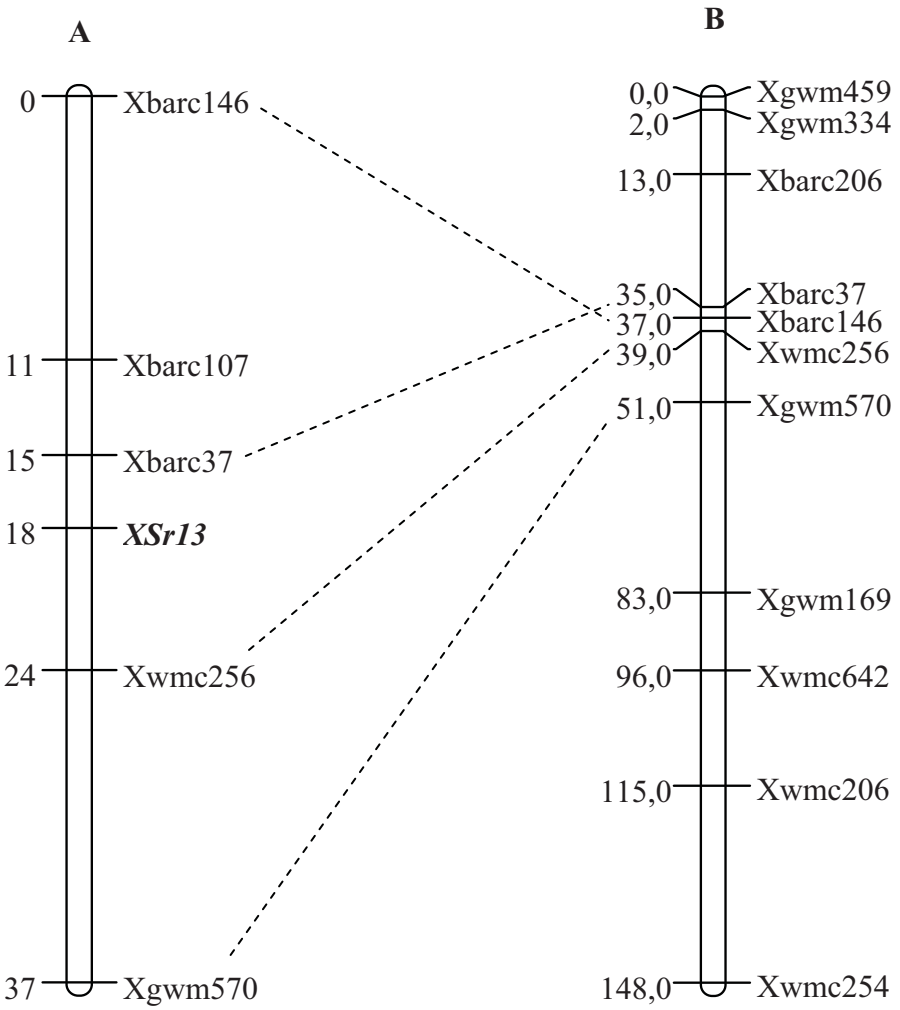

Fig. 1 A genetic linkage map of *Sr13* compared to the consensus map of chromosome 6A.
A. A genetic linkage map of *Sr13* constructed by using simple sequence repeat (SSR) markers from the long arm of chromosome 6A. The map was constructed from 158 F_2 individuals derived from a cross between Khapstein/9*LMPG and Morocco.
B. A consensus map of chromosome 6A modified after Somers et al. (2004)

Linkage of *barc37,* barc107 and wmc256 is sufficient for the detection of *Sr13* in segregating populations especially as these markers flank the gene. A combined use of these markers may lead to a reduction in the selection of false positives, but may also lead to a larger linkage drag (Zeven et al. 1983; Hospital 2001). The five markers reported here may be good candidates for marker assisted selection. Previously published literature for other stem rust resistance genes suggested the same (Mago et al. 2005; Tsilo et al. 2007; Bansal et al. 2008). However, the diagnostic value of these markers has to be carefully validated across cultivars with diverse genetic backgrounds known to carry the specific resistance gene, *Sr13*.

Even though there is virulence to *Sr13* in some countries, its effectiveness against Ug99 makes it a highly valuable source of resistance in the present day breeding programmes. But, using rust bioassays while stacking two or more resistance genes is a time consuming practice, and sometimes it is difficult to determine the presence of all resistance genes in the new cultivar unambiguously, especially for stem rust resistance genes that are broadly effective (Mago et al. 2005; Tsilo et al. 2007). PCR-based markers help to identify and stack these genes. Therefore, these markers will be useful for marker-assisted integration of *Sr13* into newly developing elite wheat breeding lines or increase the efficiency of incorporating *Sr13* to commercial cultivars that are widely adopted but susceptible to Ug99, thereby help to achieve durable stem rust control in wheat.

Acknowledgement

The first author was supported by a scholarship from the Katholischer Akademischer Auslaender-Dienst (KAAD), Germany, to conduct this research. We would like to thank the Ethiopian Institute of Agricultural Research for providing leave of absence for Belayneh Admassu to carry out the research.

References

Admassu B, Lind V, Friedt W, Ordon F (2009) Virulence analysis of *Puccinia graminis* f. sp. *tritici* populations in Ethiopia with special consideration of Ug99. Plant Pathol 58:362-369

Bariana HS, Willey N, Venkata BP, Lehmenseik A, Standen G, Lu M (2004) Breeding methodology to achieve durability for rust resistance in wheat, Cereals 2004. In: Black et al. (eds) 54th Australian cereal chemistry conference and 11th wheat breeders assembly 2004, ACT, Canberra, pp 8–12

Bansal UK, Bossolini E, Miah H, Keller B, Park RF, Bariana HS (2008) Genetic mapping of seedling and adult plant stem rust resistance in two European winter wheat cultivars. Euphytica DOI 10.1007/s10681-008-9736-z

Bonman JM, Bockelman HE, Jin Y, Hijmans RJ, Gironella AIN (2007) Geographic distribution of stem rust resistance in wheat landraces. Crop Sci 47:1955–1963

Dong Y, Zhao X, Wang J, Yuan G, Zhang X (2007) Improvement for agronomic traits of partial waxy wheat by combination of backcrossing with a PCR-based DNA marker. Journal of Genetics and Genomics 34: 836 – 841

Hospital F (2001) Size of donor chromosome segments around introgressed loci and reduction of linkage drag in marker-assisted backcross programs. Genetics 158:1363–1379

Huerta-Espino J (1992) Analysis of wheat leaf and stem rust virulence on a worldwide basis. Dissertation, University of Minnesota

Jin Y, Szabo LJ, Pretorius ZA, Singh RP, Ward R, Fetch TJr. (2008) Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici.* Plant Dis 92:923-926

Jin Y, Szabo LJ, Rouse MN, Fetch TJr., Pretorius ZA, Wanyera R, Njau, P (2009) Detection of virulence to resistance gene *Sr36* within the TTKS race lineage of *Puccinia graminis* f. sp. *tritici*. Plant Dis 93:367-370.

Klindworth DL, Miller J, Jin Y, Xu SS (2007) Chromosomal locations of genes for stem rust resistance in monogenic lines derived from tetraploid wheat accession ST464. Crop Sci 47:1441-1450

Kosambi DD (1944) The estimation of map distances from recombination values. Annu Eugen 12:172–175

Luig NH (1983) Epidemiology in Australia and New Zealand. In: Roelfs AP and Bushnell WR (eds) Cereal rusts. Vol. II: Diseases, distribution, epidemiology, and control, Academic Press, Orlando, pp 301-328

Macdonald AJ, Sankovic N, Sarre SD, Fitzsimmons NN, Wakefield MJ, Graves JAM, Zenger KR (2006) Y chromosome microsatellite markers identified from the tammar wallaby (*Macropus eugenii*) and their amplification in three other macropod species. Mol Ecol Notes 6:1202-1204

Mago R, Bariana HS, Dundas IS, Spielmeyer W, Lawrence GJ, Pryor AJ, Ellis JG (2005) Development of PCR markers for the selection of wheat stem rust resistance genes Sr24 and Sr26 in diverse wheat germplasm. Theor Appl Genet 111: 496–504

McIntosh RA (1972) Cytogenetical studies in wheat VI. Chromosome location and linkage studies involving *Sr13* and *Sr8* for reaction to *Puccinia graminis* f. sp. *tritici*. Australian J Biol Sci 25: 765-773

McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts: an atlas of resistance genes. CSIRO, Canberra

Nazari K., Mafi M., Yahyaoui A., Singh RP, Park RF (2009) Detection of Wheat Stem Rust (*Puccinia graminis* f. sp *tritici*) Race TTKSK (Ug99) in Iran. Plant Dis 93: 317

Ordon F, Pellio B, Werner K, Schiemann A, Friedt W, Graner A. 2003. Molecular breeding for resistance to soil-borne viruses (BaMMV, BaYMV, BaYMV-2) of barley (*Hordeum vulgare* L.). J Plant Dis and Protection 110: 287 – 295.

Park RF (2008) Breeding cereals for rust resistance in Australia. Plant Pathol 57: 591 – 602

Pretorius ZA, Singh RP, Wagoire WW, Payne TS (2000) Detection of virulence to wheat stem rust resistance gene Sr31 in *Puccinia graminis* f. sp. *tritici* in Uganda. Phytopathology 84: 203

Roelfs, AP (1988) Genetic control of phenotypes in wheat stem rust. Annu Rev Phytopathol 26:351–367

Roelfs AP, Singh RP, Saari EE (1992) Rust diseases of wheat: Concepts and methods of disease management. CIMMYT, Mexico City

Singh RP, Hodson DP, Jin Y, Huerta-Espino J, Kinyua MG, Wanyera R, Njau P, Ward RW (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 1: 1 – 13

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Njau P, Wanyera R, Herrera-Foessel SA, Ward WR (2008) Will stem rust destroy the world's wheat crop? Advances in Agronomy 98: 271 – 309

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

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550–560

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Functional and Integrative Genomics 4:12-25.

Stakman EC, Stewart DM, Loegering WQ (1962) Identification of physiologic races of *Puccinia graminis* var. *tritici*. Washington, USA: United States Department of Agriculture, Agricultural research service E-617 (revised).

Stein N, Herren G, Keller B (2001) A new DNA extraction method for high-throughput marker analysis in a large-genome species such as *Triticum aestivum*. Plant Breeding 120: 354 – 356.

Tsilo TJ, Jin Y, James A, Anderson JA (2007) Microsatellite markers linked to stem rust resistance allele *Sr9a* in wheat. Crop Sci 47: 2013 - 2020

Van Ooijen JW (2006) JoinMap ® 4. Software for the calculation of genetic linkage maps in experimental populations. Kyazma B. V., Wageningen

Zeven AC, Knot DR, Johnson R (1983) Investigation of linkage drag in near isogenic lines of wheat by testing for seedling reaction to races of stem rust, leaf rust and yellow rust. Euphytica 32: 319 - 327

Zhang W, Chao S, Manthey F, Chicaiza O, Brevis JC, Echenique V, Dubcovsky J (2008) QTL analysis of pasta quality using a composite microsatellite and SNP map of durum wheat Theor Appl Genet 117:1361–1377

Chapter 5

Postulation of stem rust (*Puccinia graminis* f. sp. *tritici*) resistance genes in Ethiopian wheat cultivars and breeding lines

B. Admassu[1,2], W. Friedt[3] and F. Ordon[2]

[1] Ethiopian Institute of Agricultural Research, Plant Protection Research Center, P. O. Box 37, Ambo, Ethiopia; [2] Julius Kuehn-Institute, Federal Research Institute for Cultivated Plants (JKI), Institute for Resistance Research and Stress Tolerance, Erwin-Baur-Str. 27, 06484 Quedlinburg, Germany; [3] Justus-Liebig-University Giessen, Department of Plant Breeding, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany

Abstract

Stem rust caused by *Puccinia graminis* f. sp. *tritici* is one of the major biotic limiting factors for wheat production in Ethiopia. Host plant resistance is the best option to manage stem rust from its economic and environmental point of view. Wheat cultivars in Ethiopia are released for production without information on race specific genes against stem rust. Hence, genes responsible for resistance in commercial wheat cultivars are not known. The objective of this study was to postulate stem rust resistance genes present in Ethiopian commercial wheat cultivars and advanced breeding lines in order to get information on the possibilities of broadening the genetic base of resistance and on combining resistances by crossing elite cultivars. 30 durum wheat (19 commercial cultivars and 11 breeding lines) and 30 bread wheat (20 commercial cultivars and 10 breeding lines) were tested for gene postulation. Stem rust infection types produced on wheat cultivars and breeding lines by ten *P. graminis* f. sp. *tritici* races were compared with infection types produced on 40 near isogenic lines carrying single stem rust resistance genes. A total of 11 stem rust resistance genes (*Sr5, Sr7a, Sr7b, Sr8a, Sr9e, Sr11, Sr21, Sr27, Sr29, Sr30* and *Sr37*) were postulated to be present either singly or in combination in the durum and bread wheat cultivars and breeding lines. Except *Sr30*, the other postulated genes were susceptible to most of the prevalent *P. graminis* f. sp. *tritici* races in Ethiopia. Since *Sr30* is also ineffective against Ug99, a gene management strategy that incorporates a combination of genes (gene pyramiding) that provide sufficient protection should be devised to achieve a durable control of stem rust. In addition, the significance of *Sr27*, *Sr29* and *Sr37* has to be investigated for Ethiopian agriculture.

Part of this article will be published in a scientific journal

Introduction

Stem rust caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*) is one of the major and economically important diseases of wheat in Ethiopia (CIMMYT 2005). Host resistance is the best option of disease control. An effective deployment of resistance genes for the management of stem rust in wheat requires knowledge about the resistance status and the diversity of resistance genes in cultivars under consideration. Moreover, knowledge on the prevailing races is crucial as pathogens like *Pgt* are known to evolve their virulence frequently, thereby compromising the durability of resistance. This has been documented on a number of occasions (Pretorius et al. 2000; Jin et al. 2008; Jin et al. 2009). Therefore, achievement of durable resistance against wheat stem rust requires constant characterization of the pathogen, and identification and deployment of new resistance genes that are effective against the prevailing virulent races.

Gene postulation is the classical method of detecting resistance genes likely present in crop cultivars. It is based on the gene-for-gene specificity, where the infection types produced by pathogen isolates on cultivars under study are compared to infection types produced by the same isolates on near-isogenic lines carrying single known resistance gene (Pathan and Park 2007). Provided that well characterized pathogen isolates with diverse combinations of virulence and avirulence are used, this method enables the postulation of genes present in cultivars. In addition to postulating the gene(s) present in a cultivar, gene postulation allows the identification and characterization of new resistance genes, helps to study the resistance spectrum in cultivars, and other aspects of host pathogen interactions (Singh et al. 2001). Gene postulation has been commonly used to postulate resistance genes in wheat to stem, yellow and leaf rusts (Kolmer 2003; Pathan and Park 2007; Qamar et al 2008) and other crop-disease complexes (Jensen et al 1992; Dreiseitl and Steffenson 2000).

Hexaploid wheat (*T. aestivum* L) and tetraploid wheat mainly represented by durum wheat (*T. turgidum var. durum*) are the two major wheat species cultivated in Ethiopia. Bread wheat cultivars are developed from introduced genotypes from international sources, mainly from CIMMYT. Although wheat lines released by the CIMMYT programme are selected based on their stem rust resistance (Singh et al. 2008), it is essential to have data on the local pathotypes, and it is even a greater advantage to know the actual genes responsible for

resistance in each cultivar. On the other hand, most of the durum wheat cultivars were developed from local landraces as Ethiopia is the center of diversity of this species (Harlan 1969; Tesemma and Bechere 1998). The national breeding programme undertakes multi location tests in hot spot areas to determine the resistance level of newly developed wheat cultivars to stem rust and other diseases. Race specific tests are not conducted. Hence, there is no data that shows which stem rust resistance genes are responsible for the resistance of released cultivars. Therefore, the objective of the present study was to postulate seedling resistance genes present in Ethiopian wheat cultivars and advanced breeding lines.

Materials and Methods

Wheat germplasm

60 wheat genotypes, 30 bread and 30 durum wheat cultivars and breeding lines, were tested to determine their resistance spectrum to stem rust. Of these, 39 were commercially grown cultivars and 21 were advanced breeding lines (Table 1). 40 near iso-genic wheat lines carrying known stem rust resistance genes were used as tester lines (Table 2.). Universally susceptible cultivar ´Morocco´ with no known stem rust resistance gene was used as a susceptible check.

Pathogen isolates

Ten *Pgt* races (PTHSR, RMTTM, RRTTR, TTHSR, HRTSH, DPBTR, KRHST, KCCST, QQQCM, TTTTR) were used to test the 60 wheat genotypes and 40 near isogenic tester lines in a greenhouse. The races were derived from stem rust samples collected from commercial farms in Ethiopia. Purification of bulked samples, development of single pustule isolates, characterization and nomenclature of the isolates was described in Fetch and Dunsmore (2004). The ten races were selected based on their virulence spectra on the various stem rust resistance genes (Admassu et al. 2009).

Table 1 List of bread and durum wheat cultivars and breeding lines, and their pedigree tested for postulation of stem rust resistance genes

Genotype	Pedigree
Durum wheat cultivars	
Cocorit 71	RAE/4* TC 60// STW 63/3/ AA ”S” DZ 27617 -18-64-OM
Gerardo	VZ 466/61- 130XLD SX GII “ S” CM 9605
Ld 357	LD-357/ CL 8155 NO 58-40
Boohai	COO “S” / CANDEAL II CD 3062- BS OGR
Foka	CIT 71 CANDEAL II CD 3369
Kilinto	ILUMILO/INRAT 69// BHA /3/ HORA /4/ CIT 71/ JORO , DZ 918
Bichena	ILUUMILO / COCORIT 71 DZ 393-4
Tob 66	-
Quamy	ADS // PGO / CANDEAL II /7/ JO “S”/ CR “S”// GS “S”/SBA81 /3/ FG”S” /4/ FG”S” /CR “S” /5/ FG “S” DOM “S /6/ HUI “S” CD 75533-A
Assasa	CHO “S”/ TARUS//YAV “S” 3/FG”S” /4/ FGS/CR “S” /5/ DZ 2085
Robe	HORA/ CIT “S” // JO ‘S’ / GS ‘S’ /3/ SOME ‘S’ /4 / HORA RESPINEGRO// CM 9908 /3/ RAHUM DZ 1640
Ude	CHEN / ALTAR- 84// JO69 CD 95294-9M-030Y-040 PAP-2Y-OB
Yerer	CHEN/TEZ // GULL /3/ CII CII CD 94026-4Y- 04OM-O3OY –PAP-04
Ilani	IMILO/RAHUM//A4#72/3/GERARDO
Oda	DZ046881/IMLO//CIT 71/3/RCHI/LD 357//IMLO/4/YEMEN/CIT'S'//PLC'S'/3/TAGANROY
Obsa	ALTAR 84//ALTAR 84/SERI/3/6*ALTAR 84
Ejersa	LABUD/NIGRIS 3// GAN CD98206

Bekelcha	98 OSN GEDILFA/GUEROU
Leliso	COCORIT 71/3/GERARDO//61-130/G//"S"/4/BOOHAI/HORA//GERARDO/3/BOOHAI
Durum wheat breeding lines	
CDSS97-B00845S	-
CDSS97-B00983S	-
CDSS97-B00983S...3Y..6Y	-
CD196B00S5S	-
CDSS96-B00540S	-
CDSS96-B00540S...3B...2Y	-
CDSS96B00540S...3B...2Y...AY	-
13-1DZOS-ODZR—ODZO-5DZR	-
13-1DZOS-ODZR—ODZO-1DZR	-
49-2DZOS-ODZR—ODZO-1DZR	-
49-2DZOS-ODZR—ODZO-2DZR	-
Bread wheat cultivars	
Enkoy	(HEBRARD SEL/WIS245XSUP51)X(FR-FNM)^{2}.A
Pavon 76	VCM//CNO//7C/3/KAL/BB
Simba (HAR 2536)	PRL/VEE6//MYNA/VUL
Katar (HAR 1899)	Cook/Vee''S''/Dove''S''/Seri/3/Bjy''S''
Galama (HAR 604)	4777(2)//FNK/GB/3/PVN''S''
Kubsa (HAR 1685)	ND G9144//KAL/BB/3/YACO''S''/4VEE#5''S''

Sirbo (HAR 2192)	VS73.600/MRL/3/BOW///YR/TRF
Wetera (HAR 1920)	MON’’S’’-BUC’’S’’
Bobitcho (HAR 2419)	PEG/PF70354/KAL/BB/ALD/3/MRNG
Digelu (HAR 3116)	-
Meraro (FH 11-6-24)	-
KBG-01	300/SM+501M)/HAR 1709
Abola (HAR 1522)	BOW’’S’’/BUC’’S’’
ET-13A2	ENKOY/UQ105
Tussie (HAR 1407)	COOK/VEE’’S’’//DOVE’’S’’/SERI
K6295-4A	ROMANYxGB-GAMENYA
Hawi (HAR 2501)	CHIL/PRL
Madda Walabu	TL/3/FN/TH/NAR59*2/4/BOL”S”
Sofumer	LIRA “S”/TAN “S”
Dure	BOW “S”/YD ‘S’//Z ‘S’
Bread wheat breeding lines	
IBWSN1225	Croc//AE.Squarrosa(224)//OPA1A/3/KAVZ*2/...
HRWSN675	PGO/SER//BAO/3/DUCULA
IBWSN1375	VEE#8//JUP/BJY/3/F3.71/TRM/4/2*WEAVER/5/...
ESWYT275	CROC.1/AE.Squarossa(224)//OPA1A/3/KAVZ*2/...
IBWSN75	ACC.8528
HRWYT165	ALD/CEP75630//CEP75234/PT7219/3/BVC

HRWYT465	Croc.1/AE.Sqarossa(213)//PGO/3/SODA1/...
ESWYT295	VEE#8//JUP/BTY/3/F3.71/TRM/4/2*Weaver/5/...
HRWSN565	ESDA/LIRA//MILAN/3/VEE#5/SARA
HK-14-R278	HAR1871/Jagger

Table 2 Near-isogenic lines (NILs) and corresponding resistance genes used for gene postulation of Ethiopian wheat cultivars and advanced breeding lines

NIL	Sr-gene	NIL	Sr-gene
Isr5RA	*Sr5*	LCsr 19MG	*Sr19*
W2691 sr6	*Sr6*	LCSR 20MG	*Sr20*
Mendos/w2691/w3498	*Sr7a*	T. Monococcum Deriv	*Sr21*
Isr 7bRA	*Sr7b*	SW sr22T.B.	*Sr22*
Isr 8aRA	*Sr8a*	BT sr24	*Sr24*
Barleta Benvenuto	*Sr8b*	NA	*Sr 26*
Isr9aRA	*Sr9a*	WRT.238.5	*Sr27*
W2691 sr9b	*Sr9b*	Pusa/EDCH	*Sr29*
W2691 sr9d	*Sr9d*	BTsr30 WST	*Sr30*
Verstein	*Sr9e*	Line-E/KUZ	*Sr31*
CNS Sr9g	*Sr9g*	ER.5155	*Sr32*
W2691Sr 10	*Sr10*	Tetra Canth TCH/Ag.Squarros	*Sr33*
ISr 11RA	*Sr11*	Compare	*Sr34*
BTSr 12TC	*Sr12*	W3763	*Sr35*
W2691sr13	*Sr13*	W2691Sr36TT1	*Sr36*
Line. A Selection	*Sr14*	W2691Sr36TT2	*Sr37*
W2691 srNK	*Sr15*		*RL 6076*
Isr 16RA	*Sr16*		*Sr 39*
LC/Kenya Hunter	*Sr17*		*Sr Tmp*
LC Sr18RL	*Sr18*		*Sr McN*

Postulation of resistance genes

Five seeds of each wheat cultivar, advanced breeding line and near isogenic line were sown in pots, and grown in a greenhouse. Inoculation and disease assessment was done according to Admassu et al. (2009). Postulation of seedling resistance genes in the wheat genotypes was done using the classical gene-for-gene method. The presence of one or more known resistance gene was postulated by comparing the IT pattern of the isolate-test cultivar with that of the IT pattern of an isolate-differential line combination (Pathan and Park 2007). A high IT on the test cultivar indicated that it did not have any of the resistance genes for which the test isolate is avirulent. Hence, cultivars or breeding lines exhibiting the same reaction pattern as a specific differential line were postulated to carry that respective Sr-gene.

Results

Based on the multipathotype tests, 11 seedling stem rust resistance genes (*Sr5, Sr7a, Sr7b, Sr8a, Sr9e, Sr11, Sr21, Sr27, Sr29, Sr30* and *Sr37*) and some unknown genes were postulated to be present in some of the genotypes either alone or in combinations.

Group 1: Cultivars with single seedling resistance gene

Two stem rust resistance genes (*Sr8a* and *Sr27*) were postulated singly in five cultivars. The stem rust resistance gene *Sr8a* was postulated to be present in three bread wheat cultivars, 'Abola', 'Tussie' and 'Madda Walabu'. These cultivars gave low IT to six of the ten races (Table 3). This pattern was identical to the differential cultivar 'Barleta Benvenuto' that carries the stem rust resistant gene *Sr8a*, indicating the presence of *Sr8a* in these cultivars. Cultivars 'Enkoy' and 'Gerardo', bread and durum wheat cultivars respectively, displayed low ITs to nine of the ten races. Only race QQQCM produced high IT against these two cultivars. This pattern was similar to that displayed by the differential host 'WRT.238.5' that carries the resistance gene *Sr27* (Table 3). Hence, cultivars 'Gerardo' and 'Enkoy' were postulated to carry the stem rust resistance gene *Sr27*.

Group 2: Cultivars and breeding lines with two seedling resistance genes

Five cultivars (Simba, Katar, Wetera, Bobitcho and Dure) and two breeding lines (HRWYT165 and ESWYT295) produced low ITs with races RMTTM, HRTSH, DPBTR, KRHST, KCCST and QQQCM. A differential line carrying *Sr5* had produced low ITs to four of the above six races (HRTSH, DPBTR, KRHST and KCCST) while another differential line 'Verstein' that carries the resistance gene *Sr9e* had produced low ITs with the remaining two races (RMTTM and QQQCM) (Table 4). Hence, the combination of the IT patterns of the two differential lines matched to that of the IT pattern of the genotypes in this group. Therefore, these seven genotypes were postulated to carry the stem rust resistance genes *Sr5* and *Sr9e* in combination. All of the genotypes in this group are bread wheat.

Table 3 Infection types (IT) produced on genotypes postulated to carry single resistance gene, and on the tester NIL (control) with ten *Pgt* races

Genotype	Race										Postulated gene
	PTHSR	RMTTM	TTHSR	HRTSH	DPBTR	KRHST	KCCST	QQQCM	RRTTR	TTTTR	
Abola	3[c]	;	3	2	3	2	1	2-	2	3	*Sr8a*
Tussie	3	;1	3	1+2	3	1	;	1	1	3	*Sr8a*
Madda Walabu	3	1	3	1	3	1	1	2	1	3+	*Sr8a*
ISr 8aRA[a]	**3**	**1**	**3**	**2**	**3**	**2**	**2**	**2**	**2**	**3**	
Enkoy	1	;	1+	2-	2	1	1	3-	2	2	*Sr27*
Gerardo	1-	;	1	1	0;	1	2	3	1	1	*Sr27*
WRT.238.5[b]	**2**	**;**	**1**	**2**	**2**	**2**	**1**	**3**	**2**	**2**	

[a] NIL used as differential for *Sr8a*

[b] NIL used as differential for *Sr27*

[c] ITs ; to 2+ = resistant reaction

3- to 4+ = susceptible reaction

Table 4 Infection types produced on genotypes postulated to carry two resistance genes, and on the tester NIL (control) with ten *Pgt* races

Genotype	Race										Postulated gene
	PTHSR	RMTTM	TTHSR	HRTSH	DPBTR	KRHST	KCCST	QQQCM	RRTTR	TTTTR	
Simba	3[g]	;	4	1	2	2+	2-	1	3	3	Sr5 + Sr9e
Katar	3-	1	3	1	1	2	2	2	3	4	Sr5 + Sr9e
Wetera	3	1	3	1	0;	2	;	1	3	3	Sr5 + Sr9e
Bobitcho	3	1	3	12-	1	1+	1	1	3	3	Sr5 + Sr9e
Dure	3	;	3	1	1	;	1	1	3	3	Sr5 + Sr9e
HRWYT165	3	1	4	1	1	1	;	1	3	3	Sr5 + Sr9e
ESWYT295	3+	;	4	1	;	1	2	1	4	4	Sr5 + Sr9e
ISr 5RA[a]	**3-**	**3**	**3**	**2**	**1**	**2**	**2**	**3**	**3**	**3**	
Verstein[b]	**3+**	**1**	**4**	**2**	**3**	**3**	**3+**	**2**	**4+**	**4+**	
Pavon 76	3	2+	3+	1	3	3+	2	2	3	3	Sr9e + Sr11
Galama	3	;1	3	2	3	3	1	2	3+	3	Sr9e + Sr11
Verstein[b]	**3+**	**1**	**4**	**2**	**3**	**3**	**3+**	**2**	**4+**	**4+**	
ISr 11RA[c]	**4**	**4**	**4**	**3**	**4**	**3+**	**2+**	**3**	**4**	**4**	
HRWYT465	3+	3	3	2+	1	3	3	1	34-	3	Sr7a + Sr7b
Mendos/w2691/w3498[d]	**3**	**3**	**3+**	**2+**	**3**	**3**	**3**	**3**	**3+**	**3+**	
ISr 7bRA[e]	**3**	**3**	**3**	**3**	**2**	**3+**	**3**	**1**	**3**	**3**	

Assassa	1	1	12-	1	2	1	2	1	1	3	Sr9e+Sr30
Verstein[b]	**3+**	**1**	**4**	**2-2**	**3**	**3**	**3+**	**2**	**4+**	**4+**	
BTSr30 WST[f]	**1**	**3+**	**1**	**4**	**2-**	**1**	**2**	**;**	**2**	**4**	

[a] NIL used as differential for *Sr5*

[b] NIL used as differential for *Sr9e*

[c] NIL used as differential for *Sr11*

[d] NIL used as differential for *Sr7a*

[e] NIL used as differential for *Sr7b*

[f] NIL used as differential for *Sr30*

[g] ITs ; to 2+ = resistant reaction

3- to 4+ = susceptible reaction

The other group of genotypes with two stem rust resistance genes consisted of two cultivars 'Pavon 76' and 'Galama' and an advanced breeding line 'HRWSN675', both of which are bread wheat. They gave low ITs with *Sr9e* and *Sr11* avirulent races (Table 4), indicating the presence of these two stem rust resistance genes in the three geotypes. Similarly, an advanced bread wheat breeding line 'HRWYT465' and a durum wheat cultivar 'Assassa' produced low ITs with *Sr7a* and *Sr7b*, and *Sr9e* and *Sr30* avirulent races, respectively (Table 4). Therefore 'HRWYT465' was posulated to carry *Sr7a* and *Sr7b* while 'Assassa' carries *Sr9e* and *Sr30*.

Group 3: *Cultivars with more than two seedling resistance genes*

The durum wheat cultivar 'Boohai' was postulated to carry three resistance genes in combination. *Sr8a*, *Sr21* and *Sr37* were postulated because 'Boohai' had low ITs to all races that are avirulent to these three genes (Table 5). The IT pattern of the bread wheat cultivar 'Digelu' matched the combination of IT patterns of differential cultivars that carry the resistance genes *Sr5*, *Sr21*, *Sr29* and *Sr37* (Table 5), indicating the presence of these genes in the cultivar 'Digelu'. Another durum wheat cultivar 'Foka' gave low ITs to all races that are avirulent to genes *Sr5*, *Sr9e*, *Sr21* and *Sr37* (Table 5), hence, it was postulated to carry a combination of these four resistance genes.

Table 5 Infection types produced on genotypes postulated to carry more than two resistance genes, and on the tester NIL (control) with ten *Pgt* races

Genotype	Race										Postulated gene
	PTHSR	RMTTM	TTHSR	HRTSH	DPBTR	KRHST	KCCST	QQQCM	RRTTR	TTTTR	
Boohai	1[g]	2	3+	2	2	2	1	2	1	2	Sr8a+Sr21+ Sr37
Digelu	2	3	2	2	1	;1	1	2	3-	1	Sr5+Sr21+ Sr29+Sr37
Foka	2	2+	3	22+	1	2	1	1	3	2	Sr5+Sr9e+ Sr21+Sr37
ISr8aRA[a]	**3**	**1**	**3**	**2**	**3**	**2**	**2**	**2**	**2**	**3**	
ISr5RA[b]	**3**	**1**	**3**	**2**	**3**	**2**	**2**	**2**	**2**	**3**	
T. Monococcum Deriv[c]	**2**	**3**	**3**	**4**	**2**	**3+**	**3**	**3-**	**3**	**3**	
W2691Sr36TT2[d]	**3**	**3**	**3**	**3**	**3**	**3**	**2**	**2**	**3**	**2**	
Pusa/EDCH[e]	**4-**	**3**	**;**	**3**	**2+**	**3**	**3+**	**3**	**3-**	**3**	
Verstein[f]	**3+**	**1**	**4**	**2**	**3**	**3**	**3+**	**2**	**4+**	**4+**	

[a] NILs used as differential for *Sr8a*

[b] NILs used as differential for *Sr5*

[c] NILs used as differential for *Sr21*

[d] NILs used as differential for *Sr37*

[e] NILs used as differential for *Sr29*

[f] NILs used as differential for *Sr9e*

[g] ITs ; to 2+ = resistant reaction

3- to 4+ = susceptible reaction

Table 6 Infection types produced on genotypes postulated to carry unknown resistance genes with ten *Pgt* races

Genotype	Race										Postulated gene
	PTHSR	RMTTM	TTHSR	HRTSH	DPBTR	KRHST	KCCST	QQQCM	RRTTR	TTTTR	
Ejersa	1[a]	;	;	;	1	1	3	1	1	;	Unknown
Sirbo	3	2	3	1	;	2	1	3	3+	2	Unknown
Meraro	3	2	3	2	2	1	1	3-	3+	1	Unknown
ET-13A2	2+	12-	3	1	2	2	;	3	3	2	Unknown
KBG-01	3-	;	2	1	1	2	3	2	3	2	Unknown
Hawi	2	1	3	1	2	1	2	3	3+	;	Unknown
Sofumer	2+	1	3+	1	3	1	0;	1	3	;	Unknown
IBWSN1225	3	3	3	3-	3+	2+	1	1	3	3-	Unknown
IBWSN1375	2	1	3	;	3	2	1	;	3	;	Unknown
ESWYT275	3-	3	3	3	3	1	1	2	3	4	Unknown
HRWSN565	3	;	2	1	1	1	4	;1	3	2	Unknown

[a] ITs ; to 2+ = resistant reaction

3- to 4+ = susceptible reaction

Group 4: Cultivars and breeding lines with unidentified resistance genes

This group comprised 11 genotypes: one durum wheat cultivar, six bread wheat cultivars and four bread wheat breeding lines (Table 6). They were categorized as genotypes with unidentified resistance genes because they had low IT to at least one of the races, but the IT patterns produced on these genotypes did not conform to any of the IT patterns exhibited on the tester lines.

Group 5: Genotypes without resistance genes

All races produced high ITs on three bread wheat breeding lines, which was similar to the universally susceptible cultivar Morocco (Table 7). Hence, the three lines in this group 'GIBWSN75', 'HRWYT465' and 'HK-14-R278' were postulated to have no known resistance genes when tested with the ten races used in this study.

Group 6: Genotypes resistant against all races

24 durum and 2 bread wheat genotypes were placed in this group. All the genotypes in this group displayed low ITs against all of the races (Table 8). It was difficult to postulate the resistance gene(s) responsible for this as there were five differential lines that carry the stem rust resistance genes *Sr22*, *Sr24, Sr26, Sr33* and *Sr39* that had similar IT patterns to that of the genotypes. Either a single gene or a combination of these five genes may be responsible for the resistance displayed by these cultivars.

Table 7 Infection types produced on genotypes postulated to have no known resistance genes, and on the tester cultivar 'Morocco' (control) with ten *Pgt* races

Genotype	Race										Postulated gene
	PTHSR	RMTTM	TTHSR	HRTSH	DPBTR	KRHST	KCCST	QQQCM	RRTTR	TTTTR	
IBWSN75	4-[b]	3+	3+	3	3	3	4	3	4	3	None
HRWYT465	3+	3	3	3+	34-	3	3	4-	3	3	None
HK-14-R278	3	3	3-	3+	3	3	3-	3-	4-	4+	None
Morocco [a]	**4-**	**3**	**3**	**3+**	**3**	**3**	**3**	**3**	**3**	**4**	

[a] A universally susceptible wheat cultivar with no known stem rust resistance gene

[b] ITs ; to 2+ = resistant reaction

3- to 4+ = susceptible reaction

Discussion

The present study identified 11 seedling stem rust resistance genes in Ethiopian wheat cultivars and advanced breeding lines to be present either alone or in combination. The frequency of occurrence of *Sr9e* was the highest among the postulated genes (occurring in 18.3% of the genotypes) followed by *Sr5* and *Sr8a* each occurring in 15 and 6.7% of the genotypes, respectively. Other genes that occurred at low frequencies include *Sr21* and *Sr37* (5%), *Sr11* and *Sr27* (3.3%), and *Sr7a*, *Sr7b*, *Sr29* and *Sr30* (1.7%). Only three genotypes, (5%) were postulated to have no known stem rust resistance genes.

A big proportion of the genotypes (26 durum wheat cultivars and breeding lines, and one bread wheat cultivar) were effective against all *Pgt* races, which made it difficult to postulate the types of genes present in these genotypes. The low ITs on these genotypes could be either due to one or more of the Sr-genes that had similar IT patterns (*Sr22*, *Sr24*, *Sr26*, *Sr33* and *Sr39*) on the genotypes. Moreover, it could also be due to a combination of other two or more resistance genes that produced low ITs with all races. For example, a combination of ITs of differential cultivars carrying *Sr14*, *Sr15* and *Sr35* produced low ITs with all of the races. Hence, additional races each with virulence to one of the five resistance genes but avirulent to the other four are required to identify the likely source of resistance in these genotypes. On the other hand, a significant proportion of the genotypes (18.3%) were postulated to carry unknown resistance genes. This requires further analysis using additional races with a wider virulence spectra than the present races to determine the type(s) of gene(s) that are responsible for the low ITs displayed by the genotypes against some of the races.

Table 8 Infection types produced on genotypes resistant against all races, and on the tester NILs (control) with ten *Pgt* races

Genotype	Race									
	PTHSR	RMTTM	TTHSR	HRTSH	DPBTR	KRHST	KCCST	QQQCM	RRTTR	TTTTR
Cocorit 71	;[f]	;	1	1	0;	1	1	1	1	;
LD375	;	;	;	;	;	1	1	1	1	2-
Kilinto	1	1	1	1	1	2	1	2	1	;
Bichena	1	;	1	1+	2	1	1	2	2-	1
Tob 66	1	1	1	1	2	1	2	1	1	1
Quamy	1	1	;	1	1	1	;	1	1	1
Robe	;	;	1	1	;	2	1	1	2-	1
Ude	1	1	1	1+2	1	2	1	1	1	1
Yerer	1	;	1	1	;	1	2	;	1	1
Ilani	1	;	;1	1	1	2	2	2	1	;
Oda	1	1	;	1	;	1	1	1	1	;
Obsa	1	;	1	;	1	1	2-	1	;	;
Bekelcha	1	1	1	;	1	;	2-	1	1	;
Leliso	1	1	1	1+2	1-	1	1	1	1	1
CDSS97-B00845S	1	0;	1	1	1	1	2+	1	2-	;

CDSS97-B00983S	1	;	1	1	1	;	2	1	1	;
CDSS97-B00983S...3Y..6Y	1	1	1	;	1	1	2	1	1	;
CD196B00S5S	1+	;	1	;1	1	1	2	;	1	0;
CDSS96-B00540S	1	;	;	1	0;	1	1	;	1	;
CDSS96-B00540S...3B...2Y	1	;	1	1	;	;	0	;	;	1
CDSS96B00540S...3B...2Y...AY	2-	;	1	;	;	;	1	;	1+	1
13-1DZOS-ODZR—ODZO-5DZR	2	;1	1	;	;	2	1	12-	2	1
13-1DZOS-ODZR—ODZO-1DZR	1	;	2-	1	;	;	2	1	2-	1
49-2DZOS-ODZR—ODZO-1DZR	1	1	1	1	1	1	2	;	;1	;
49-2DZOS-ODZR—ODZO-2DZR	1	;	1	1	;	2	2	1	2-	1
K6295-4A	2	1	1+	2-	2	2	;	2	2	1
SW Sr22T.B[a]	**1**	**;**	**2**	**2**	**2**	**1**	**2**	**2**	**2**	**2**
BT Sr24[b]	**;**	**2**	**2**	**;**	**1**	**1**	**2**	**;**	**1**	**1**
***Sr26*[c]**	**1**	**1**	**1**	**2**	**2**	**2**	**2**	**2**	**2**	**2+**
Tetra Canth TCH/Ag.Squarros[d]	**2-**	**;**	**1**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
***Sr39*[e]**	**;1**	**2**	**2**	**1**	**2**	**2**	**2**	**2**	**1**	**0**

[a] NIL used as differential for *Sr22*

[b] NIL used as differential for *Sr24*

[c] *Sr26* gene

[d] NIL used as differential for *Sr33*

[e] *Sr39* gene

[f] ITs ; to 2+ = resistant reaction

3- to 4+ = susceptible reaction

A significant variation in resistance spectra was observed between durum and bread wheat genotypes. In general, durum wheat genotypes showed a broader resistance spectrum than bread wheat. This might be associated with the fact that most of the durum wheat genotypes were developed from local landraces, which have co-evolved with indigenous pathogen populations. This finding was in agreement with previous reports that established the importance of Ethiopian cultivated tetraploid wheat accessions as good sources of stem rust resistance (Knott 1996; Beteselassie et al. 2007; Bonman et al. 2007; Klindworth et al. 2007). On the other hand, bread wheat genotypes were introduced to the country via different ways including genotypes developed by international breeding programmes. Hence, their narrow resistance spectrum against indigenous pathogen isolates was not surprising.

Most of the resistance genes that were postulated in this study are known to confer seedling resistance against a wide range of races. However, *Sr27*, *Sr37* and *Sr39* have not been exploited in cultivated durums or common wheat (McIntosh et al. 1995). Hence, their significance should be further investigated.

A previous study by Beteselassie et al. (2007) had detected five of the stem rust resistance genes that were postulated in this study (*Sr7b*, *Sr24*, *Sr27*, *Sr29* and *Sr30*) in Ethiopian durum and emmer wheat accessions. The significance of some of the postulated genes for agriculture was established earlier (McIntosh et al. 1995). Unfortunately, an earlier study had shown that most of the postulated resistance genes (*Sr5*, *Sr7a*, *Sr7b*, *S8a*, *Sr9e*, *Sr11* and *Sr21*) were ineffective against most of the prevalent races in Ethiopia (Admassu et al. 2009). The exception here is *Sr30*, which was relatively effective against most of the races prevalent in Ethiopia. The problem with *Sr30* is its ineffectiveness against race Ug99 (Singh et al. 2008). Hence, a gene management strategy that incorporates a combination of genes (gene pyramiding) that provide sufficient protection against the races prevalent in the country has to be devised to achieve a durable control of stem rust. In addition, the significance of the other remaining postulated genes, *Sr27*, *Sr29* and *Sr37*, which had not been utilized for agriculture up to now, needs to be investigated from the perspective of Ethiopian pathogen population-wheat interaction. In addition to gene pyramiding, varietal diversification should be encouraged to get the advantage of horizontal resistance from vertical resistance.

References

Admassu B, Lind V, Friedt W and Ordon F. 2009. Virulence analysis of *Puccinia graminis* f. sp. *tritici* populations in Ethiopia with special consideration of Ug99. Plant Pathol 58: 362 - 369

Beteselassie N, Fininsa C and Badebo A. 2007. Sources of stem rust resistance in Ethiopian tetraploid wheat accessions. African Crop Science Journal 15: 51-57

Bonman JM, Bockelman HE, Jin Y, Hijmans RJ and Gironella AIN. 2007. Geographic distribution of stem rust resistance in wheat landraces. Crop Science 47: 1955 – 1963

Dubin HJ, Johnson R and Stubbs RW. 1989. Postulated genes for resistance to strupe rust in selected CIMMYT and related wheats. Plants Disease 73: 472-473

CIMMYT. 2005. Sounding the alarm on global stem rust: an assessment of race Ug99 in Kenya and Ethiopia and the potential for impact in neighboring countries and beyond. Mexico city, Mexico.

Dreiseitl A and Steffenson BJ. 2000. Postulation of leaf rust resistance genes in Czech and Slovak barley cultivars and breeding lines. Plant Breeding 119: 211 – 214

Fetch TG and Dunsmore KM. 2004. Physiological specialization of *Puccinia graminis* on wheat, barley, and oat in Canada in 2001. Canadian Journal of Plant Pathology 26: 148 – 155

Harlan JR. 1969. Ethiopia: A center of diversity. Econ. Bot. 23: 309 – 314

Jensen HP, Christensen E and Jorgensen H. 1992. Powdery mildew resistance genes in 127 Northwest European spring barley varieties. Plant breeding 108: 210 - 228

Jin Y, Szabo LJ, Pretorius ZA, Singh RP, Ward R and Fetch TJr. 2008. Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici.* Plant Dis 92:923-926

Jin Y, Szabo LJ, Rouse MN, Fetch TJr., Pretorius ZA, Wanyera R and Njau P. 2009. Detection of virulence to resistance gene *Sr36* within the TTKS race lineage of *Puccinia graminis* f. sp. *tritici*. Plant Dis 93:367-370

Klindworth DL, Miller J, Jin Y and Xu SS. 2007. Chromosomal locations of genes for stem rust resistance in monogenic lines derived from tetraploid wheat accession ST464. Crop Science. 47:1441-1450

Knott, DR. 1996. The transfer of stem rust resistance from the Ethiopian durum wheat St. 464 to common wheat. Canadian Journal of Plant Science 76:317-319

Kolmer JA. 2007. Postulation of leaf rust resistance genes in selected soft red winter wheat. Crop Science 43: 1266 – 1274

McCartney CA, Sommers DJ, Mccallum BD, Thomas J, Humphreys DG, Menzies JG and Brown PD. 2005. Microsatellite tagging of the leaf rust resistance gene Lr16 on wheat chromosome 2BSc. Molecular Breeding 15: 329-337

McIntosh RA, Wellings CR and Park RF. 1995. Wheat rusts: an atlas of resistance genes. CSIRO, Canberra, Australia

Mebrate SA, Dehne HW, Pillen K and Oerke EC. 2008. Postulation of seedling leaf rust resistance genes in selected Ethiopian and German bread wheat cultivars. Crop Science 48: 507 – 516

Pathan AK and Park RF. 2007. Evaluation of seedling and adult resistance to stem rust in European wheat cultivars. Euphytica 155: 87-105

Pretorius ZA, Singh RP, Wagoire WW and Payne TS. 2000. Detection of virulence to wheat stem rust resistance gene Sr31 in *Puccinia graminis* f. sp. *tritici* in Uganda. Phytopathology 84: 203

Pamar M, Ahmad SD, Shah AH, Wellings CR and Batool F. 2008. Postulation of strip rust resistance genes in some Australian bread wheat cultivars and their response to temperature. Pak. J. Bot. 40: 2573 – 2585

Singh D, Park RF and McIntosh RA. 2001. Postulation of leaf (brown) rust resistance genes in 70 wheat cultivars grown in the United Kingdom. Euphytica 120: 205-218

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Njau P, Wanyera R, Herrera-Foessel SA and Ward WR. 2008. Will stem rust destroy the world's wheat crop? Advances in Agronomy 98: 271 – 309

Stakman EC, Stewart DM and Loegering WQ. 1962. *Identification of physiologic races of Puccinia graminis var. tritici*. Washington, USA: United States Department of Agriculture, Agricultural research service E-617 (revised).

Tesemma T and Bechere E. 1998. Developing elite durum wheat selections (composites) for Ethiopian peasant farm use: Raising productivity while keeping diversity alive. Euphytica 102: 323 - 328

Wanyera R, Kinyua MG, Jin Y and Singh RP. 2006. The spread of stem rust caused by *Puccinia graminis* f. sp. *tritici*, with virulence on Sr31 in wheat in Eastern Africa. Plant Dis 90: 113

General Summary

Wheat in Ethiopia is grown on ca 1.4 million ha with a national average productivity of 1.7 tons/ha. The low productivity is attributed to a number of biotic and abiotic factors. Stem rust caused by the fungus *Puccinia graminis* f. sp. *tritici* (*Pgt*) is one of the major biotic factors that limit wheat production. Stem rust is problematic in the mid to low altitude wheat producing areas. It can cause yield reduction of up to 70% on susceptible cultivars. *Pgt* is known for its high virulence and genetic diversity wherever it is found. Mutation, sexual reproduction and selection are the major causes of diversity. These phenomena in turn lead to the evolution of new virulent races that overcome the already deployed stem rust resistance genes. Such events had occurred in the past resulting in the appearance of new virulent races like 15B that caused devastating stem rust epidemics in 1950s in North America, and very recently the appearance of Ug99 in East Africa. Hence, sustainable control of stem rust in wheat requires a continuous monitoring and characterization of the pathogen, auditing of available stem rust resistance genes in commercial wheat cultivars, and application of marker assisted selection to increase the efficiency of breeding for stem rust resistance.

This study was conducted (i) to determine the virulence and genetic structure of *Pgt* populations in Ethiopia, (ii) to develop a genetic linkage map of the stem rust resistance gene *Sr13* effective against Ug99 and (iii) to postulate genes that are responsible for stem rust resistance in Ethiopian bread and durum wheat cultivars and breeding lines.

A total of 152 single pustule isolates collected from Arsi, Bale, Shewa and northwest Ethiopia were analysed using 20 near isogenic wheat lines (NILs). Race analysis was done based on the standard procedure. The analysis detected 22 *Pgt* races from all regions. Eight races each from Arsi and northwest Ethiopia, and seven and 13 races from Bale and Shewa respectively were identified. Some of the races were confined only to one region. For example, races DPBTR, RMTTM, MRHLR and QQQCM were detected only in Shewa, Arsi, Bale and northwest Ethiopia, respectively. On the other hand, races like TTKSR and RRTTR were spread across all regions. The virulence spectrum and frequency of occurrence of races was directly correlated, i.e., races with low virulence spectrum like DPBTR were the ones that occurred at lower frequencies and vice versa. The new virulent race Ug99 (TTKSR) was

predominant with frequency of 26.5% followed by races TTHSR and RRTTR with frequencies of 17.7 and 11.1%, respectively.

Most of the stem rust resistance genes in this study were ineffective against the majority of races prevalent in Ethiopia. Fore example *SrMcN* was ineffective against all of the isolates. The other resistance genes were ineffective against 62.3 – 97.4% of the stem rust isolates studied. Only four stem rust resistance genes were found to confer resistance to the majority of the isolates. These were *Sr13*, *Sr36*, *SrTmP*, *and Sr30*. Three of these four resistance genes (*Sr13*, *Sr36* and *SrTmP*) can be used as potential sources of resistance to stem rust in breeding programmes. Ineffectiveness of *Sr30* against Ug99 makes it risky to use it as a source of resistance for Ethiopian agriculture. Therefore, efforts should be exerted to incorporate the three resistance genes to the already adopted wheat cultivars or into new wheat breeding lines.

Genetic characterization of *Pgt* isolates was done using 48 isolates that were selected based on their virulence spectrum and geographic origin. Data generated by assaying DNA of the isolates on 20 microsatellite markers were used to validate two assumptions that proposed high genetic diversity within and among *Pgt* populations, and differentiation of *Pgt* populations based on their geographic origin. The results showed high genetic diversity within populations, but low genetic distance among populations. The genic and genotypic diversity within populations ranged from 0.466 – 0.555 and 0.600 – 0.718, respectively. These results support the assumption of high genetic diversity within populations. On the other hand, genetic distance between populations ranged from 0.080 – 0.315. This was an indicator for genetic similarity among populations. Absence of genetic differentiation was supported by a low coefficient of population differentiation (0.046) and a high estimate of gene flow (10.4). High genetic diversity within populations and homogeneity across regions is a phenomenon that could create a situation where deployed stem rust resistance genes adapt to the pathogen easily and lead to resistance break-down. Hence, it is necessary to deploy cultivars containing two or more genes pyramided together or to incorporate horizontal resistance.

In the absence of diagnostic molecular markers, the classical gene postulation technique was used to postulate resistance genes in Ethiopian wheat cultivars and breeding lines. After testing 60 wheat genotypes (39 cultivars and 21 breeding lines), 11 stem rust resistance genes (*Sr5, Sr7a, Sr7b, Sr8a, Sr9e, Sr11, Sr21, Sr27, Sr29, Sr30* and *Sr37*) were postulated to occur

either singly or in combination in some of the wheat cultivars and breeding lines. *Sr9e* was postulated in 18.3% of the cultivars. The next frequently detected resistance genes were *Sr5* and *Sr8a* at frequencies of 15 and 6.7%, respectively. They were followed by the other stem rust resistance genes at low frequencies ranging from 1.7 – 5%. A total of 26 durum wheat cultivars and breeding lines (86.7%) and one bread wheat cultivar (3.3%) were effective against all of the *Pgt* races, indicating the wider resistance spectrum of durum wheat than bread wheat cultivars. Eleven wheat genotypes had unidentified resistance genes, whereas only three breeding lines were postulated to possess no known resistance genes.

The stem rust resistance genes postulated in this study can not be deployed singly, because all of them are ineffective either against the majority of *Pgt* races prevalent in Ethiopia or against the most virulent and wide-spread races like Ug99. Hence, it is essential to develop a strategy that incorporates a combination of genes like gene pyramiding and/or cultivar mixture that provide sufficient protection against the races prevalent in the country to achieve durable control of stem rust.

Sr13 was one of a few stem rust resistance genes that confer resistance against Ug99 and other *Pgt* races prevalent in Ethiopia. Development of a genetic map and identification of microsatellite markers linked to the gene increases the efficiency of incorporation of this gene to wheat cultivars grown in Ethiopia. To achieve this, a cross between the resistant cultivar 'Khapstein/9*LMPG' and the susceptible cultivar 'Morocco' was done and 158 F_2 plants were developed. Results of the phenotypic analysis showed that segregation of F_2 plants and F_3 families fitted to a 3:1 and 1:2:1 ratio, respectively, indicating the presence of a single dominant gene. Out of a total of 37 microsatellite markers screened, 10 were polymorphic between parents and bulks. Of these 10 polymorphic markers, five (barc37, wmc256, barc107, gwm570 and barc146) were mapped on the long arm of chromosome 6A. Barc37 and wmc256 were the closest markers that flanked *Sr13* distally and proximally at distances of 3.0 and 6.0 cM, respectively. The other three markers barc107, gwm570 and barc146 were mapped at 7.0, 18.0 and 19.0 cM, respectively. The two closely linked flanking markers could potentially be used for MAS.

Zusammenfassung

Weizen wird in Äthiopien auf ca. 1,4 Mio ha mit einem Durchschnittsertrag von 1,7 dt/ha angebaut. Die geringe Ertragsleistung ist auf eine Vielzahl biotischer und abiotischer Stressfaktoren zurückzuführen, wobei insbesondere Schwarzrost, verursacht durch *Puccinia graminis* f. sp. *tritici* (*Pgt)* ertragslimitierend wirkt. Schwarzrost ist besonders in den niedrigen und mittleren Höhenlagen problematisch, wobei Ertragsverluste bis 70% beim Anbau anfälliger Sorten beobachtet werden. Schwarzrost zeichnet sich weltweit durch eine hohe Virulenz und genetische Diversität aus, wodurch neue virulente Rassen entstehen, welche bekannte Schwarzrost-Resistenzgene überwinden, wie z.B. im Fall des Auftretens der Rasse 15B, welche in den 1950er Jahren in Nordamerika zu verheerenden Schwarzrostepedemien geführt hat, oder jüngst im Falle des Auftretens von Ug99 in Ostafrika. Dies macht im Hinblick auf eine effektive Kontrolle des Schwarzrostes eine kontinuierliche Überwachung und Charakterisierung des Pathogens erforderlich, eine Erfassung der in Sorten vorhandenen Resistenzgene und deren Wirksamkeit und die Entwicklung molekularer Marker für Schwarzrostresistenzgene, um diese beschleunigt in Sorten einlagern bzw. kombinieren zu können und damit den Schwarzrost zu kontrollieren.

Die vorliegende Arbeit hatte daher zum Ziel (i) die Virulenz und genetische Struktur äthiopischer Schwarzrostpopulationen zu erfassen, (ii) molekulare Marker für das Resistenzgen *Sr13* zu entwickeln, welches gegen Ug99 wirksam ist, und (iii) Gene in äthiopischen Brot- und Durumweizensorten und Zuchtlinien zu bestimmen.

Insgesamt wurden 152 Einsporisolate, die in den Regionen Arsi, Bale, Shewa und Norwest-Äthiopien gesammelt wurden, auf 20 nahe isogenen Linien (NILs) unter Verwendung der Standardmethode zur Rassendifferenzierung analysiert. Über alle Regionen wurden 20 Schwarzrostrassen (jeweils 8 in Arsi und Nordwest-Äthiopien, 7 in Bale und 13 in Shewa) identifiziert. Einige dieser Rassen waren dabei regionsspezifisch, so wurden die Rassen DPBTR, RMTTM, MRHLR und QQQCM jeweils nur in Shewa, Arsi, Bale oder Nordwest-Äthiopien detektiert, während TTKSR und RRTTR in allen Regionen nachgewiesen werden konnten. Das Virulenzspektrum und die Häufigkeit des Auftretens waren direkt korreliert, d.h. Rassen mit einem geringen Virulenzspektrum wie DPBTR traten auch nur in geringer Frequenz auf, während hochvirulente Rassen auch häufig auftraten. Die neue virulente Rasse

Ug99 (TTKSR) war über alle Anbauregionen dominierend mit einer Häufugkeit von 26,5%, gefolgt von TTHSR (17,7%) und RRTTR (11,1%).

Die meisten in dieser Arbeit untersuchten Schwarzrostresistenzgene waren gegenüber den in Äthiopien dominierenden Rassen nicht mehr wirksam, so waren z.B. Träger des Resistenzgens *SrMcN* gegenüber allen Isolaten anfällig. Die anderen Resistenzgene – mit Ausnahme der Resistenzgene *Sr13, Sr36, SrTmP* und *Sr30* - waren gegenüber 62,3 – 97,4 % der Rassen anfällig. Drei (*Sr13, Sr36* und *SrTmP*) der genannten vier Resistenzgene sind dafür geeignet die Basis der Schwarzrostresistenz in äthiopischem Weizen zu erweitern, da sie auch gegenüber Ug99 wirksam sind.

Eine genetische Charakterisierung der Schwarzrostisolate erfolgte anhand von 48 Isolaten, die entsprechend ihrer geographischen Herkunft und ihres Virulenzspektrums ausgewählt wurden. Basierend auf der Analyse von 20 SSRs wurden die Annahmen geprüft, dass in Äthiopien eine hohe genetische Diversität innerhalb und zwischen den Schwarzrostpopulationen besteht und eine Differenzierung entsprechend dem geographischen Ursprung. Die Ergebnisse zeigten eine hohe genetische Diversität innerhalb der Populationen und eine geringe genetische Distanz zwischen denselben. Die genetische Diversität innerhalb der Populationen lag im Bereich von 0,466 bis 0,555 und 0,600 bis 0,781. Diese Ergebnisse stützen die Annahme einer hohen genetischen Diversität innerhalb der Populationen. Demgegenüber wurde die genetische Distanz zwischen den Populationen im Bereich von 0,080 – 0,315 bestimmt, was auf eine geringe genetische Distanz zwischen den Populationen hindeutet. Dieses Fehlen genetischer Differenzierung zwischen Populationen wird auch durch den geringen Koeffizienten für die Populationsdifferenzierung (0,046) und einen hohen Wert für den Genfluss (10,4) bestätigt. Eine hohe genetische Differenzierung innerhalb und eine geringe Differenzierung zwischen Populationen ermöglicht einen beschleunigten „Resistenzdurchbruch“ (Resistenzverlust), so dass es nötig ist zwei oder mehr Resistenzgene zu kombinieren bzw. horizontale Resistenzen zu nutzen.

Da nicht für alle Schwarzrostresistenzgene molekulare Marker vorhanden sind, wurden klassische Verfahren zur Genidentifikation in äthiopischen Weizensorten und Zuchtlinien verwendet. Basierend auf der Analyse von 60 Genotypen (39 Sorten und 21 Zuchtlinien) wurden 11 Resistenzgene postuliert (*Sr5, Sr7a, Sr7b, Sr8a, Sr9e, Sr11, Sr21, Sr27, Sr29, Sr30* and *Sr37*), die entweder einzeln oder in Kombination in den analysierten Genotypen

vorkommen. *Sr9e* trat in 18,3% der Genotypen auf, gefolgt von *Sr5* (15,0%) und *Sr8a* (6,7%). Die weiteren Resistenzgene traten in 1,7-5,0% der Genotypen auf. Insgesamt 26 Durumweizensorten und Zuchtlinien (86,7%) und eine Brotweizensorte (3,3%) waren gegenüber allen analysierten Rassen resistent. Dies deutet darauf hin, dass ein größeres Resistenzspektrum in *Triticum durum* als in *T. aestivum* vorliegt. In 11 Weizensorten konnten die Resistenzgene nicht eingehender identifiziert werden, während dies nur bei 3 Zuchtlinien der Fall war. Die identifizierten Resistenzgene können nicht einzeln genutzt werden, da sie entweder gegenüber den in Äthiopien dominierenden Rassen oder gegenüber Ug99 unwirksam sind. Es ist daher nötig Strategien zu entwickeln, welche eine Kombination von Resistenzgenen ermöglichen, z.B. Pyramidisierung von Resistenzgenen oder Sortenmischungen, um auf diese Weise eine dauerhafte Widerstandsfähigkeit zu erzeugen.

Sr13 gehört zu den wenigen Resistenzgenen, die gegenüber den in Äthiopien dominierenden Rassen einschließlich Ug99 wirksam sind. Die Entwicklung molekularer Marker erlaubt eine beschleunigte Nutzung dieses Resistenzgens in der äthiopischen Weizenzüchtung. Zur Markerentwicklung wurde der resistente Genotyp ´Khapstein/9*LMPG´mit der anfälligen Sorte ´Morocco´ gekreuzt und 158 F_2-Pflanzen und deren Nachkommen in F_3 analysiert. Die beobachteten Spaltungsverhältnisse zeigten eine Anpassung an ein Spaltungsverhältnis von 3r:1s bzw. 1r:2rs:1s, so dass von einem dominanten Resistenzgen ausgegangen werden kann. Von insgesammt 37 auf dem Chromosom 6A lokalisierten SSR-Markern waren 10 polymorph zwischen den Eltern und den DNA-Bulks. Von diesen konnten fünf auf dem langen Arm von Chromosom 6A lokalisiert werden (barc37, wmc256, barc107, gwm570 und barc146). Dabei flankieren barc37 und wmc56 den Resistenzlocus *Sr13* in einem Abstand von 3cM bzw. 6cM. Die SSRs barc107, gwm570 und barc146 wurden in einem Abstand von 7,0 cM, 18,0 cM und 19,0 cM von *Sr13* kartiert. Die beiden genannten flankierenden Marker erlauben eine effektive markergestützte Selektion auf *Sr13*.

Appendix

Coordinate and elevation of stem rust sample collection sites in Arsi, Bale, Shewa and northwest Ethiopia regions

Region	Location	Coordinate	Elevation
Arsi	Asella	N07 56.075, E039 07.925	2550
	Belallo	N07 51.446, E039 07.930	2515
	Brkitu	N07 46.372, E039 09.247	2550
	Sagure	N07 44.958, E039 09.410	2265
	Temela	N07 40.382, E039 10.452	2505
	Lemu	N07 36.263, E039 13.674	2525
	Dodota	-	2680
	Ardaita	-	2352
	Edo Negga	N07 16.703, E039 16.236	2517
	Asasa	N07 05.554, E039 11.391	2371
	Waba	E07 00.824, E039 01.602	2506
	Wagae	N07 01.613, E038 58.302	2578
	Kofele	N07 04.712, E038 46.421	2465
	Deyea	N08 11.760, E039 14.815	1800
	Handode	N08 10.604, E039 14.603	2140
	Iteya	N08 07.251, E039 13.835	2196
	Shaki	N08 05.592, E039 13.441	2200
	Gonde	N08 02.895, E039 12.090	2260
	Kulumsa_1	N08 02.010, E039 09.812	2200
	Kulumsa_2	N08 01.927, E039 09.718	2200
	Huruta	N08 08.794, E039 21.960	1988
	Bika	N08 13.929, E039 20.00	-
	Kulumsa RC	N08 01.120, E039 09.655	2200
	Wetera	N08 09.107, E039 38.919	
	Arsi Robe	N07 52.845, E039 37.556	2460
	Dhera	N08 19.268, E039 19.272	1686
	Sero	N08 07.715, E039 16.219	2165
	Lode	N08 07.529, E039 26.182	2472

	Ataba Gora	N07 56.152, E039 36.100	2465
	Bullallo	N07 59.258, E039 34.346	2506
	Tullu Jebi	N08 06.388, E039 27.405	2534
	Boru Lencha	N08 07.872, E039 18.140	2166
	Shorima	N08 05.305, E039 13.369	2209
	Dosha	N0 54.460, E039 07.427	2510
	Kechema	N07 49.569, E039 08.673	2439
	Ashebeka	N07 43.366, E039 09.431	2463
	Tiyo	-	2432
	Kubsa	N07 12.912, E039 14.397	2488
	Bokolla	N07 00.265, E039 07.069	2497
Bale	Dodolla	N06 58.896, E039 11.919	2450
	Herero	N06 59.807, E039 19.994	2383
	Adaba	N07 00.587, E039 24.185	2411
	Sinana	N07 06.568, E040 13.126	2400
	Sinana ARC	N07 07.178, E040 13.513	2465
	Seka	N07 04.154, E040 12.108	2450
	Gafera	N07 04.487, E040 09.732	2431
	Obra Shaya	N07 04.613, E040 08.826	2432
	Shallo	N07 04.995, EE040 07.234	2428
	Galema	N07 05.751, E040 03.778	2455
	Robe 1	N07 06.863, E040 01.290	2478
	Robe 2	N07 09.100, E039 59.515	2490
	Weltei	-	2450
	Ali Doyo	N07 13.467, E039 57.718	2386
	Ali	N07 14.914, E039 55.269	2481
	Ali Agarfa	N07 15.315, E039 54.379	2486
	Ilani	N07 15.825, E039 51.526	2510
	Seso	N07 17.177, E039 50.209	2422
	Agarfa College	N07 19.232, E039 53.052	2411
	Agarfa Town	E07 16.318, E039 49.359	2450
	Fade	N07 09.370, E039 57.179	2450
	Homma	E07 08.196, E039 55.254	2537

	Abakera	N07 07.519, E039 52.117	-
	Washa	-	-
	Bokola	N07 00.824, E039 05.530	2478
Shewa	Akaki	N08 51.432, E038 48.170	2090
	Dukem	N08 48.441, E038 53.418	1964
	Dembi	N08 46.281, E038 56.077	1907
	D. Zeit	N08 146.174, E038 59.921	1850
	Melkasa ARC	N08 24.906, E039 19.709	1550
	PPRC	N08 57.840, E037 51.496	2250
	Senkelle	N08 58. 939, E037 53.007	1985
	Ambo	N08 58.931, E037 52.614	2190
	Awaro	N08 58.002, E037 54.760	2225
	Asgori	N08 58.705, E038 01.190	2454
	Meti	N08 58.506, E037 58.857	2435
	Degawuchi	N08 59.885, E038 03.924	2351
	Awash Buluto	N09 01.662, E038 07.657	2224
	Ginchi R. C	N09 01.434, E038 10.805	2208
	Ginchi	N09 01.143, E038 08.804	2234
	Korae	N09 03.405, E038 04.600	2397
	Worka Korae	N09 04.247, E038 03.307	2396
	Olonkomi	N09 00.212, E038 16.102	2144
	Kimoye	N09 00.990, E038 20.300	2168
	Addis Alem	N09 03.011, E038 25.822	2382
	Holetta	N09 03.505, E038 30.265	2393
	Menagesha	N09 04.091, E038 35.203	2584
	Alem Gena	N08 54.976, E038 39.642	2277
	Geda Amba	N08 51.803, E038 39.710	2241
	Bonaya	N08 48.695, E038 38.589	2125
	Gejja	N08 45.943, E038 39.217	2281
	Awash Melka	N08 41.762, E038 36.144	2063
	Adadi Mazoria	N08 39.470, E038 35.608	2112
	Sebeta	N08 53.800, E038 35.094	2112
	Tefki	N08 50.741, E038 28.961	2070

	Teji	N08 48.813, E038 21.270	2070
	Tullubolo	N08 40.376, E038 13.449	2140
	Chollo	N08 44.401, E038 09.440	2174
	Boda Honcha	N08 53.280, E038 05.862	2497
	Worka	N08 58.933, E038 07.486	2242
	Legetafo	N09 04.245, E038 53.628	2472
	Legedadi	N09 05.484, E038 56.234	2473
	Dire Mazoria	N09 06.638, E038 59.172	2520
	Sendafa	N09 09.115, E039 01.399	2584
	Cholle	N09 09.934, E039 05.889	2583
	Aleltu	N09 12.467, E039 09.617	2555
	Mojjo	N08 36.595, E039 06.271	1802
	Gelan	N08 0.146, E038 50.063	2097
	Melka Oda	N07 13.863, E038 37.991	1965
	Weyo	N07 25.050, E038 40.428	1871
	Lencha	-	2400
	Wegolli	-	2550
	Rachoo	-	2050
	Jato Dirki	-	1820
	Tikur Inchini	-	2450
	Inewari	-	2690
	Albasa	-	2680
	Dilfeka	-	1780
Northwest Ethiopia	Awa	N11 59.463, E037 42.646	2270
	Angereb	N12 38.835, E037 42.646	2212
	Walaj	N12 40.333, E037 30.354	2430
	Ambeza	N12 43.491, E037 29.921	2560
	Dabat	N12 59.390, E037 46.304	2620
	Addis Zemen	-	2190
	Dejen	N10 09.590, E038 09.227	2430
	Dejen Juncture	N10 14.025, E038 09.227	2471
	Enarj Enawga	-	2450
	Inebrga	N10 16.977, E038 07.923	2410

	Yetmen	N10 20.024, E038 08.883	2420
	Dibissa	N10 22.418, E038 09.338	2437
	Mahibere Berhan	N10 24.055, E038 10.010	2439
	Bichena	N10 27.662, E038 11.869	2530
	Weyra	N10 31.938, E038 09.472	2560
	Telima	N10 36.073, E038 09.453	2564
	Debre Work	N10 39.582, E038 10.032	2525
	Dinjubit	N10 44.670, E038 08.803	2555
	Quamy Cherk	N10 47.616, E038 05.960	2562
	Gunde Woyin	N10 54.791, E038 05.434	2636
	Gezamin	N10 57. 835, E038 02.441	2520
	Titar Eyesus	N10 70. 954, E038 16. 392	2568
	Ney Mariam	N11 00.331, E037 94. 060	2512
	Mota	N11 04.743, E037 52.961	2430
	Adet	N11 13. 819, E037 43.778	-
	Magew	N11 83. 819, E038 06. 778	2407

Acknowledgement

I would first like to express my heartfelt gratitude to PD. Dr. Frank Ordon for allowing me to work in his laboratory, for his great interest in this project, constant technical guidance throughout the project period, and finally for his personal kindness that made my stay at JKI comfortable.

My special thanks goes to Prof. Dr. Dr. h.c. Wolfgang Friedt for his willingness to be my university advisor, for his thorough and critical review of this dissertation as well as his help in academic issues related to my study.

I would like to extend my thanks to Dr. Dragan Perovic for his technical guidance in molecular research works and for his friendly and kind behavior. I would also like to acknowledge Nina Meyer, Dr. Monique Juergens and Dr. Ilona Kraemer for their help in acquainting me with the Li-COR, ABI genetic analyzer and other laboratory equipments. Thanks are also to Ilona Renneberg for her assistance in the laboratory. I also extend my thanks to Dr. Edgar Schliephake for his help in statistical analyses.

The help rendered to me by colleagues from the National Plant Protection Research Center at Ambo: Emebet Fekadu, Netsanet Bacha, Elisabeth Terefe and Tizazu Tafesse during sample collection is greatly acknowledged. Thanks to Tegest and Zeritu for taking care of the experiments in the greenhouse at Ambo.

I would like to acknowledge Frau Elke Strauch and Herr Burkhard Strauch who made my stay in Quedliburg feel like home away from home. I am grateful for their kindness and companion during my stay in Quedlinburg.

I am indebted to my sisters Simegnish Admassu and Nibret Getahun, and my brother-in-laws Dr. Workneh Ayalew and Asfaw Shebeshe who gave me the highly needed encouragement, support and advice at all stages. I highly value the friendship and support I got throughout the study period from my friends Dr. Tesfamariam Mekete, Yonas Wobalem, Shimelis Getnet, Henok Abraha, Alemayehu Belay, Amsalu Fekadu and Dereje Bejiga.

I would like to thank the Catholic Academic Exchange Service (KAAD), Ethiopian Institute of Agricultural Research (EIAR) and Julius Kuehn Institute for Cultivated Plants (JKI) for financially supporting my study and research.

Last but not least my praise goes to my wife Mahlet Solomon for her patience, understanding and love, and for taking care of our daughter, Beza Belayneh, alone in my absence. I am blessed to have you in my life!

Above all thanks to the Almighty God with whom everything is possible!

Resume

Name	Belayneh Admassu Yimer
Date of birth	21.08.1973
Place of birth	Zigem, Gojjam, Ethiopia
Sex	Male
Nationality	Ethiopian
Marital status	Married and one daughter

Education

Sept. 1979 – June 1983	Kilaj Elementary School, Gojjam, Ethiopia (Grades 1-4)
Sept. 1983 – June 1984	Meseret Elementary School, Gondar, Ethiopia (Grades 5 & 6)
Sept. 1984 – Apr. 1990	Fassiledes Junior & Comprehensive High School, Gondar, Ethiopia
Sept. 1990 – July 1994	B. Sc degree (Plant Sciences) with distinction, Alemaya University of Agriculture, Alemaya, Ethiopia
Oct. 2000 – Sept. 2002	M. Sc degree (Phytopathology) with Summa cum laud, Leibniz University of Hanover, Hanover, Germany
Sept. 2006 – Feb. 2010	PhD (Agricultural Science), Justus-Liebig-University of Gießen, Gießen, Germany (Research conducted at Julius Kuehn-Institute for Cultivated Plants - JKI, Quedlinburg, Germany)

Professional Experience

Nov. 1994 – March 1995	Agronomist, Gondar Fuelwood Plantation and Integrated Rural Development Project, Ethiopia
April 1995 – Jan. 1997	Expert, Dangla Woreda Agricultural Office, Ethiopia
Feb. 1997 – Jan. 2000	Junior Researcher in Plant Pathology, National Plant Protection Research Center (PPRC), Ethiopian Institute of Agricultural Research (EIAR), Ambo, Ethiopia
Dec. 2002 – Feb. 2006	Research Plant Pathologist, PPRC, EIAR, Ambo, Ethiopia