Chapter 1

General introduction

1.1 Wheat

Wheat is one of the most important cereals world-wide and it is grown in many areas (Curtis 2002). The wide adaptation of wheat permits its cultivation from the equator to 60°N and 44°S and at elevations from sea level to 3000 m and greater. According to estimates of Central Statistics Authority (CSA) (2010), wheat is one of the major cereal crops grown world-wide in terms of total production amount used for food. The world‘s largest wheat producer is China followed by India, the United States, France, Russia, Canada, Germany, Turkey, Kazakstan, Ukraine and Pakistan (FAOSTAT 2010). Currently wheat is second to rice (*Oryza sativa* L.) and ahead of maize (*Zea mays* L.) as the main human food crop. Its consumption continuously increased during the past decades. The demand for wheat is expected to grow faster than any other major agricultural crop. According to Food and Agriculture Organization of the United Nations (FAO 2011) report, world wheat production in 2011 stands at 676 million tons, representing a growth of 3.4 percent from 2010. Curtis et al. (2002) similarly reported that in future global wheat production is expected to reach 850 million tons a year by 2030. In Ethiopia, wheat is the third most grown crop, after teff and sorghum, both in terms of acreage and production volumes (CSA 2010). In global terms, wheat is the leading source of cereal proteins in human food, having higher protein content than maize or rice. In total, 16% and 26% of total dietary calories in developing and developed countries, respectively, come from wheat (Ortiz et al. 2008). Wheat is the most traded food crop internationally and is used as an emergency food in aid for developing countries (Hailu 2011). It is nutritious, easy to store and transport and can be processed into various types of food. Its grain is used to make flour for leavened, flat and steamed breads, biscuits, cookies, breakfast cereal, pasta, noodles, bio-fuel, and for fermentation to make alcoholic beverages such as beer and liquors (Tsegaye and Berg 2007b).

Ethiopia is the second largest wheat producer in the sub-Saharan Africa, following South Africa (White et al. 2001) with an area of 1.5 million ha and annual production of about 2.9 metric tons (Joshi et al. 2011). Bread (*Triticum aestivum* L.) and durum (*Triticum durum* Desf.) wheat are the two dominant wheat species cultivated in Ethiopia. Other species of the genus *Triticum* like *T. aethiopicum* Jakubz. (syn. *T. abyssinicum* (Vav.) Vav), Hammer et al. 2011), *T. dicoccon* Schrank, *T. turgidum* L., *T. durum* convar. *durocompactum* Flaksb., *T.*

polonicum L. and *T. compactum* Host are also cultivated in small pocket areas by small scale farmers (Porceddu and Perinno 1973; Tessema and Jemal 1982). Most of the tetraploid wheat under cultivation consists of a mixture of types (Tesemma and Belay 1991). More than one million hectares of land is allocated for wheat production per year, making up 13% of the total crop production. During the recent past, tetraploid wheat used to occupy more than 60% of the total wheat area in Ethiopia (Tessema and Belay 1991). Recently, it has been reported that tetraploid and hexaploid species are occupying approximately 30% and 70%, respectively, of the total area of wheat under cultivation in Ethiopia (CSA 2010). About 85% of hexaploid wheat is grown in south and southeast highlands, whereas tetraploid wheat is produced predominantly on the central and north-western highlands of the country (Gebre-mariam 1991). Though wheat is widely produced in the highlands and mid-altitudes, there is a considerable irrigated wheat production potential in lowlands (White et al. 2001).

The cultivation of tetraploid wheat generates greater yield than other wheats in areas of low precipitation (300–500 mm). Good yields can be obtained by irrigation, but this is rarely found. The area annually planted with durum wheat worldwide is estimated to be 13.5 million ha. The European Union devotes around 3.5 million ha to its cultivation with a production of around 9.2 million metric tons. Average global yields have been increased from 1.4 tons/ha during the 1970s to more than 2 tons/ha in recent years leading to great increase in total production (Royo et al. 2009).

Wheat belongs to cereal grass Gramineae (Poaceae) family and the genus *Triticum.* Tetraploid wheat has 28 chromosomes and the genomes are designated AB. It evolved from the ancestral wild emmer *Triticum dicoccoides* (Koern. ex Aschers. et Graebn.) Schweinf., which originated from *Triticum urartu* Thum. ex Gandil. as donor of A genome (14 chromosomes) and a species related to *Aegilops speltoides* Tausch that donated the B genome (14 chromosomes) (Hopf and Zohary 1993). Thus it is an allotetraploid (AA BB). Tetraploid wheat is economically and nutritionally important for the production of pasta, macaroni, couscous, bulgur, frekeh and other local foods. Most of the tetraploid wheats grown today belong to *T. durum*, with amber-colored and larger seed than most other wheat. It is used for pasta production because of its hard grain texture, amber color, and other grain quality traits related to endosperm protein (Gashawbeza et al. 2006). Ethiopian farmers also use the straw of wheat for animal feed and in some rural areas it is used as a hatching material. Recently, this crop has been the subject of renewed interest because of its valuable production and adaptation to low-rainfall, semi-arid environments, and its unique products (Martos et al. 2005; Mardi et al. 2011). In South Europe, West Asia and North Africa, durum wheat is mainly grown under rainfed conditions, characterized by unpredictable rainfall and a large incidence of abiotic and biotic stresses. Drought and heat during the grain filling period, nutrient deficiencies, soil problems, diseases and pests are the main yield constraints (Royo et al. 2009).

Ethiopia is considered as one of the world’s secondary centers of diversity for tetraploid wheats (Vavilov 1997). Of the tetraploid wheat species, *T. durum* is the most extensively cultivated (Tessema 1991). It is a traditional crop for the Ethiopian farmers and is grown on the heavy black soils (Vertisols) of the highlands at altitudes ranging between 1800-2800 m, exclusively under rainfed agriculture (Tessema and Belay 1991). Its production however has been limited to locally made foods. The current increase in food processing industries and importance of pasta products by the urban people of the country has increased the importance of tetraploid wheat production.

Although Ethiopia is rich in genetic resources of tetraploid wheat and has suitable environments for wheat production, the country does not produce enough wheat to satisfy national demand. Nearly 1 million tons are imported annually, mainly on a concessional basis or as food aid (Joshi et al. 2011). Additionally, about 85% of the tetraploid wheats are landraces adapted to specific areas. The productivity of wheat in Ethiopia is low due to biotic (diseases, insect pests and weeds), abiotic (drought, low soil fertility and heat) stresses and low adoption and availability of new agricultural technologies due to technical, socioeconomic, and institutional problems. Among these factors, diseases play a significant role in yield reduction (Admassu 2010). During the past two decades severe stripe rust and stem rust epidemics have occurred and caused significant yield losses (40–60%) (Joshi et al. 2011). But on the other hand, the demand for tetraploid wheats is continuously increasing because of the new emerging food processing industries. Therefore, there are efforts to improve their productivity and quality.

1.2 Stem rust of wheat

There are numerous wheat diseases caused by various pathogens. Of these, rust diseases remain the most important diseases of wheat worldwide because of their wide distribution, their capacity to form new races that can attack previously resistant varieties, their ability to move long distances and their potential to develop rapidly under optimal environmental conditions that result in serious yield losses (Kolmer et al. 2009). In all regions in which wheat is grown rusts have caused periodic severe epidemics and major losses have occurred (Roelfs et al. 1992). New pathogenic races are usually developed by mutation and selection for virulence against rust resistance genes in wheat. In recent years, new races of wheat leaf rusts, wheat stripe rust and wheat stem rust have been introduced into wheat growing areas in different continents (Todorovska et al. 2009). These introductions have complicated the efforts of breeders to develop wheat cultivars with durable resistance and have significantly reduced the number of the effective rust resistance genes available at present.

Wheat rust pathogens belong to the genus *Puccinia*, family Pucciniaceae, order Uredinales and class Basidiomycetes. The genus *Puccinia* includes three important species of rust fungi that attack wheat. The rust *Puccinia graminis* Pers. f. sp. *tritici* causes the stem (black) rust that tends to occur in the warmer, moister regions; *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* causes the leaf (brown) rust disease that occurs in all wheat-growing areas with moist climates, and *Puccinia striiformis* Westend. f. sp. *tritici* causes stripe (yellow) rust that occurs at high rainfall in cooler regions (McIntosh 1998). All three rust fungi are obligate parasites, which mean they are completely dependent on living tissue for reproduction and they survive by the production of huge amounts of wind-dispersed spores known as urediniospores (Roelfs et al. 1992).

Stem rust was historically one of the most destructive and most feared diseases in various countries on all continents where wheat is grown. The fear of stem rust is understandable because an apparently healthy looking crop of a susceptible cultivar about three weeks prior to harvest could reduce to a black tangle of broken stems and shrivelled grains by harvest (Singh et al. 2008a). Stem rust was historically a major problem in all of Africa, the Middle East, all of Asia except Central Asia, Australia and New Zealand, Europe, and the Americas (Saari and Prescott 1985).

According to Roelfs (1978) stem rust had caused serious epidemics in North America in the 1950s causing yield losses of up to 50%. Losses incurred due to stem rust in Ethiopia were estimated up to 70% on susceptible wheat cultivars at times of disease epidemics (Bechere et al. 2000; Admassu 2010). Although the last major stem rust epidemic occurred in Ethiopia during 1993/94 (Shank 1994; Badebo 2002) when a popular wheat variety ‘Enkoy’ fell out of production, the rest of the world has practically remained unhurt from stem rust for over three decades (Singh et al. 2008a) until the appearance of a new virulent race named Ug99 that overcomes the previously effective stem rust resistance gene *Sr31*. Even if 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).

1.2.1 Life cycle and symptoms of *Pgt*

The understanding of a disease cycle or commonly referred to as life cycle, is necessary to effectively manage and control disease. The life cycle of *P. graminis* f. sp. *tritici* mostly consists of continual uredinial generations. The fungus has five types of spores at different developmental stages: pycniospores, aeciospores, urediniospores (uredospores), teliospores, and basidiospores (Fig. 1). It is heteroecious which means that two unrelated hosts, such as wheat and barberry, are required to complete its life cycle. Wheat is the primary host where the pathogen spends most time, and barberry is the secondary host. Urediniospores are the active spores which are produced by infected wheat plants and which the fungus uses for re-infection of other wheat plants. Teliospores are produced at the later developmental stage of the fungus as survival mechanism for unfavorable environmental conditions when susceptible hosts are unavailable. Aeciospores stalked in chains produced on barberry are transported by wind to start a new disease cycle in susceptible wheat varieties.

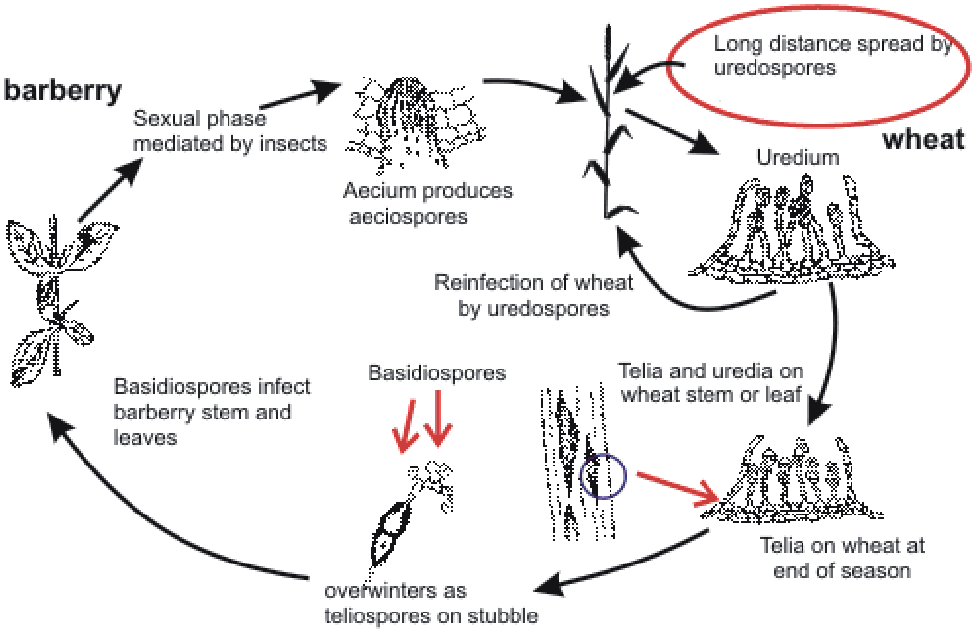

Fig. 1 Life cycle of the stem rust fungus, *Puccinia graminis* (Source: Cereal Disease Encyclopedia)

Stem or black rust of wheat caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. and Henn. (*Pgt*), as the name implies, infects the stem but is not always confined to the stem. It can infect leaves, sheaths, glumes, and awns. Elongate, reddish brown pustules with ragged margins (Fig. 2) symptoms are occurring in early stages of the disease. With the termination of the disease, black sooty teliospores are formed and the bursting pustules take on a black colour. The host epidermis is ruptured by the pustule, giving the stems and sheaths a rough-textured surface. Stem rust pustules are larger than leaf rust pustules and often erupt on both upper and lower leaf surfaces. It has a high output of urediniospores per day which can remain viable for long periods and are carried for long distances by winds (Singh et al. 2008a). Wind is a great spreading agent of stem rust and causes concern as to how easy and far stem rust can spread. The minimum, optimum, and maximum temperatures for urediniospore germination are 2, 15–24, and 30 ^{0}C; and for sporulation 5, 30, and 40 ^{0}C (Roelfs et al. 1992), thus providing a vast range of favorable environmental conditions. Urediniospores initiate germination within 1–3 hours of contact with free moisture over a range of temperatures. In field conditions, 6–8 hours of dew period or free moisture from rains is required for the completion of infection process (Singh et al. 2008a).

Fig. 2 Elongated brick-red stem rust pustules on infected stems and leaves

1.2.2 Ug99 and its distribution

In 1999, a new virulent race of stem rust was identified from wheat fields in Uganda popularly known as Ug99 (Pretorius et al. 2000) after the year and country of discovery. Using North American scientific nomenclature, this new race was designated as TTKS (Wanyera et al. 2006). It is the only known race of *Pgt* that has virulence for gene *Sr31* known to be located in the translocation 1BL.1RS from rye (*Secale cereale* L.) (Singh et al. 2006). Following its detection, investigations in neighbouring countries in East Africa revealed that the same race migrated to Kenya in 2001 and to Ethiopia in 2003 (Singh et al. 2006). By 2006 it was detected in Sudan and Yemen (http://www.fao.org), and in 2007 and 2009 its presence was confirmed in Iran and South Africa (Nazari et al. 2009; Pretorius et al. 2010) (Fig. 3) following the migration path suggested by Singh et al. (2006). Similar trajectories from Ug99 sites in Iran indicate that Iran can be gateway for Ug99 migration to Afghanistan, Pakistan, Central Asia, the Caucasus, or Russia (Singh et al. 2008a; 2008b).

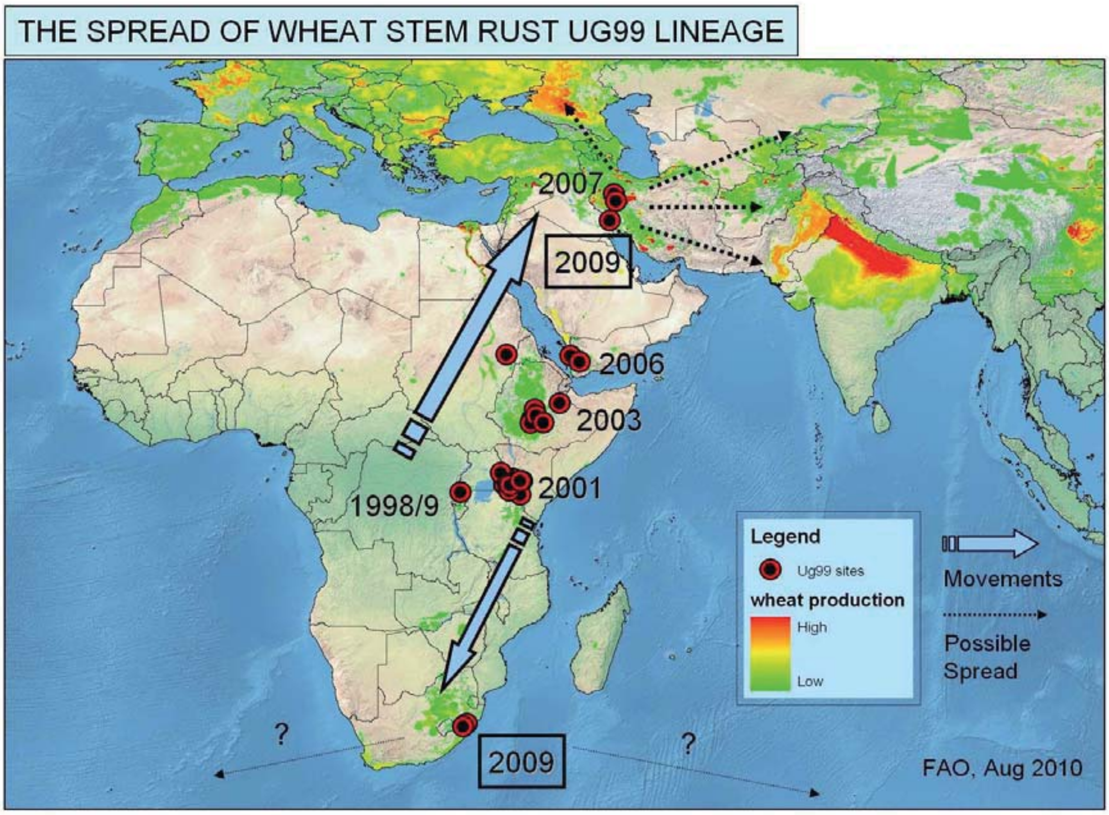

Fig. 3 The spread of *Puccinia graminis f*. sp. *tritici* race Ug99 and its derivatives (Source: http://www.fao.org/agriculture/crops/rust/stem/rust-report/stem-ug99racettksk/en/)

Unfortunately, race Ug99 not only carries virulence to gene *Sr31* but also this unique virulence is present together with virulence for most of the genes of wheat origin. This unique virulence profile of Ug99 (*Pgt* race TTKSK and derivatives) makes it a tremendous threat to wheat production worldwide. Six additional variants are now recognized in the Ug99 lineage. All exhibit an identical DNA fingerprint, but differ in virulence patterns. Additional important resistance genes e.g., *Sr24* and *Sr36* have now been defeated by variants of Ug99. Virulence by race Ug99 has been also detected for gene *Sr38* which was introduced in wheat from *Triticum ventricosum* and which is present in several European and Australian cultivars and a small portion of new CIMMYT germplasm (Singh et al. 2008a). Based on these TTKS lineages that were identified from isolates in Kenya the designation of TTKS was modified. The original race was re-designated as TTKSK, and the other two races as TTKST (with additional virulence on *Sr24*) (Jin et al. 2008) and TTTSK (with additional virulence on *Sr36*) (Jin et al. 2009). A major concern is that a significant proportion of global wheat germplasm is potentially at risk from race Ug99. Reynolds and Borlaug (2006) estimated that this area might amount to 50 million ha of wheat grown globally i.e., about 25% of the world's wheat area. The development of lines with adequate and durable resistance to Ug99 presents a unique challenge to wheat scientists worldwide. The majority of genes conferring resistance to Ug99 comes from wild relatives (Singh et al. 2008b). Additionally, many of the effective resistance genes are present on large translocations and are associated with linkage drag.

1.3 Stem rust resistance genes in wheat

Currently, about 50 stem rust resistance (*Sr*) genes have been identified and mapped to specific chromosomes (McIntosh et al. 2008). However, the new race, Ug99, overcomes many known *Sr* genes. Genes *Sr5*, *Sr6*, *Sr7a*, *Sr7b*, *Sr8a*, *Sr8b*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9g*, *Sr10*, *Sr11*, *Sr12*, *Sr15*, *Sr16*, *Sr17*, *Sr18*, *Sr19*, *Sr20*, *Sr23*, *Sr30*, *Sr31*, *Sr34*, *Sr38* and *SrWld-1* were all defeated by Ug99 (Pretoruis et al. 2000; Jin and Singh 2006; Jin et al. 2007; Singh et al. 2011). Therefore, from the identified 50 stem rust resistance genes, only a few are effecctive against Ug99. *Sr2*, *Sr13*, *Sr22*, *Sr25*, *Sr26*, *Sr35*, *Sr39* and *Sr40* are some of the genes that are effective against Ug99 (Singh et al. 2006, 2008a; Yu et al. 2010, 2011). Recently, two new genes, *Sr47* and *Sr52*, with a high level of resistance to race TTKSK were identified by Klindworth et al. (2012) and Qi et al. (2011), respectively.

The majority of these resistance genes confer race specific resistance, and only *Sr2* belongs to the slow rusting/partial/minor resistance gene category (Bansal et al. 2008). *Sr2* derived from *Triticum turgidum* and located on chromosome 3BS, has been widely used in the wheat breeding program in CIMMYT, Mexico for global improvement of wheat stem rust resistance. Moreover, the *Sr2* complex in combination with other resistance genes showed effective protection against Ug99 (Singh et al. 2006).

1.4 The application of molecular markers in wheat improvement

The availability of high-throughput molecular markers linked to resistance genes and their genetic location could make the selection process faster and more cost effective. In addition, different genes can be combined in a pyramiding strategy for resistance breeding. The most