## 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.

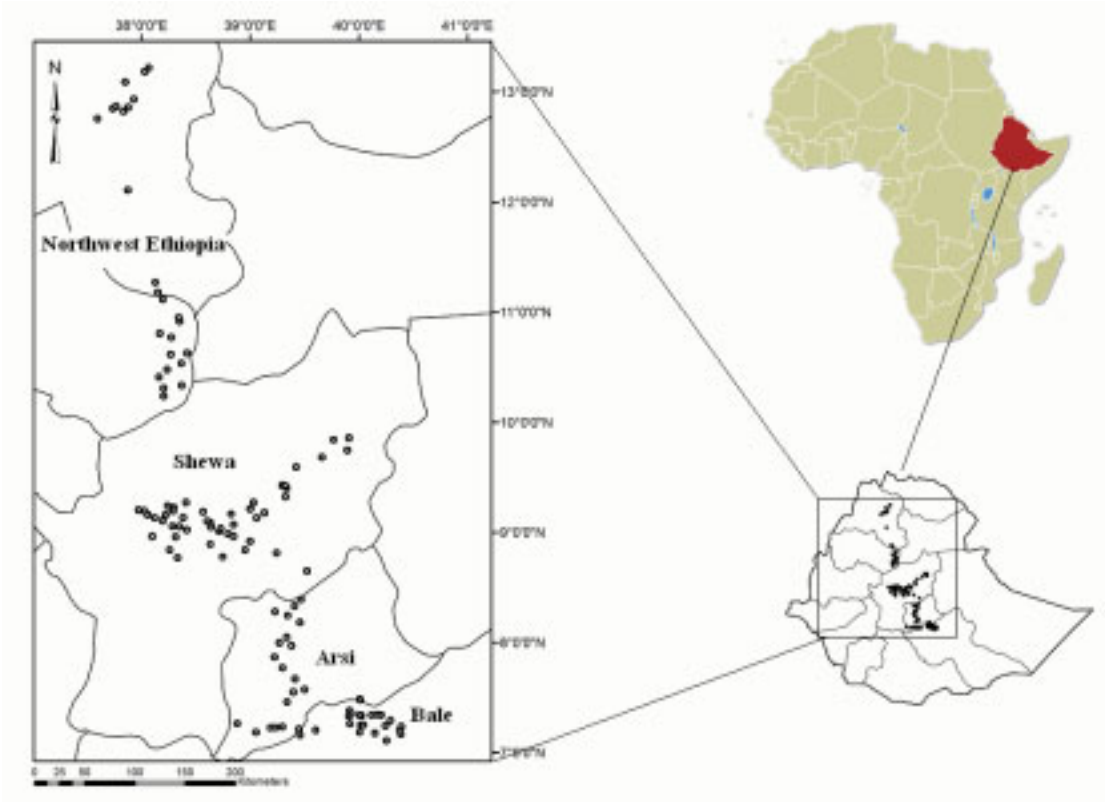


**Fig.** 1 Wheat stem rust collection sites in Arsi, Bale, Shewa and Northwest Ethiopia

**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.