4. DISCUSSION

4.1. Population structure of *Puccinia striiformis* f. sp. *tritici*

Ethiopian pathotypes carrying virulence to one or more of the following known stripe rust genes were identified in this study: *Yr*2+, *Yr*3a, *Yr*3b, *Yr*4a, *Yr*6, *Yr*6+2, *Yr*7, *Yr*8, *Yr*9, *Yr*9+2, *Yr*10, *Yr*17, *Yr*24, *Yr*26, *Yr*27, *Yr*32, *Yr*A, *Yr*Cv, *Yr*Sd, and *Yr*Su. Virulence to *Yr*17 had not been detected in Ethiopia until 2005, although virulence frequency close to 100% has been reported for this gene in some Northern European countries (Villaral et al. 2002). In the present study 14% mean virulence frequency to *Yr*17 was observed. Genes *Yr*8, *Yr*Cv and *Yr*Su had high virulence frequency, although they were characterised as effective source of resistance against stripe rust in Ethiopia in previous studies (Yahyaoui et al. 2000). This difference suggests drastic pathotype shift that occurred between 2000 and 2005. Other isolates carrying virulence to *Yr*2+, *Yr*3a, *Yr*3b, *Yr*4a, *Yr*6, *Yr*6+2, *Yr*7, *Yr*8, *Yr*9, *Yr*9+2, *Yr*10, *Yr*24, *Yr*26, *Yr*27, *Yr*A, and *Yr*Sd identified in this study were not unique to Ethiopia. For instance, virulence to *Yr*2, *Yr*6, *Yr*7, and *Yr*9 occur in most wheat producing areas of the world (Chen et al. 2002). In Germany virulence has been reported to *Yr* genes *Yr*2, *Yr*3, *Yr*4, *Yr*6, *Yr*7, *Yr*8, *Yr*9, *Yr*17, *Yr*Cv, *Yr*Su, and *Yr*Sd (Flath and Bartels 2002).

*Yr*1, *Yr*5, *Yr*15, and *Yr*Sp stripe rust resistance genes were found effective against all isolates collected across the major wheat growing regions of Ethiopia. The findings were in agreement with the work of Chen (2005), Afsharri (2008) and Chunmei et al (2008) who reported that virulences to *Yr*5 and *Yr*15 genes rarely occur in most wheat producing areas of the world as a rare phenomenon. *Yr*5 showed high resistance against all or most of the yellow rust isolates in China, North America, and Turkey (Macer 1966, Wang et al. 1996, Zeybek and Fahri 2004, Chen 2005).
DISCUSSION

On the contrary virulence close to 100% was observed to Yr1 in Europe (Flath and Bartels 2002, Woz´niak-Strzembicka 2003). The use of these resistance genes alone or in combinations may help to reduce yield losses due to stripe rust in Ethiopia. However, the gene pyramiding approach has a clear advantage to develop commercial cultivars with durable resistance (Jacobs and Parlevliet 1993). Genes Yr3a, Yr4a, Yr10, Yr17, and Yr26 confer high resistance to the tested Ethiopian isolates. Yr genes Yr6+2, Yr9+2, and Yr24 provide moderate resistance and still can be considered for gene pyramiding in the future breeding programme. However, their low protective efficiency to the existing stripe rust population underline the urgent need to search for novel sources of resistance.

Mutations, migrations, recombinations, and direct selections can change the pathogen virulences of the rust population. By classifying pathotypes and grouping them according to the number of virulence genes present in each isolate (Tables 7 and 9), high similarity between pathotypes is observed. Thus, the pathotype with 16 virulences (21751773) differs from the pathotype with 15 virulences (21670773) by the presence of virulence to gene Yr10. The pathotype with 15 virulences (61770473) differs from the pathotype with 14 virulences (21770473) by the presence of the Yr3a virulence. The number of virulences (14) in 21770473 and 23750473 are increased by the addition of virulences to Yr2+9 and Yr4a in to pathotype 21750473 (13), respectively. Similarly the most frequent pathotype 21650473 in the sub-group with 12 virulence genes differs from pathotype 01650473 with 11 virulences by having virulence to Yr2+. This pathatotype differ from pathotype 21654473 with 13 virulence by having virulence to Yr17. Likewise, pathotype 21750473 increased to 14 by the addition of virulence to YrCv in 21750573. The difference among pathotypes by a single Yr gene might explains a single step mutation pattern.

There were remarkable differences in the composition of pathotypes in different regions (Table 10). Out of the 39 pathotypes, only four were detected in two or three locations. The most
frequent pathotype in the south (01650473) was not found at any other region. Similarly the two most frequent pathotypes in the central region (00651751 and 21770440) were not detected in the other three regions. The most frequent pathotype in the north (01654463) was not identified at any other location. The most abundant pathotype (21770473) with 12.1% was found in the south and southeast regions. The regional differences in the composition of pathotypes indicate that the wheat improvement programme may capitalize on strategies that target these regional pathotype populations’ differences to developed commercial cultivars for specific regions. However, in view of small sample size used further studies with more locations is essential. In addition the composition of pathogen populations can change through time and this can also be an important consideration for breeding programs. In this regard future studies should focus on special and temporal dynamics of stripe population within and between years in the country.

In plant pathogen populations that are subject to continuous directional selection from increasing virulence, it is inevitable that the most complex pathotypes will eventually predominate if no other selective factors are present. In the present study pathotype with a high complexity value of 14 is more frequent. The lowest complexity value of 0 to 6 is not contained by any pathotypes (Table 9). These results are in agreement with the work of Chen (2005) who found in recent years the predominance of *P. striiformis* f. sp. *tritici* with a wide virulence spectrum compared to those with a narrow virulence spectrum in North America. However, this phenomenon does not support the general concept that isolates with fewer virulence genes are more aggressive and have better fitness than isolates with more virulence genes (Vanderplank 1963, Line and Qayoum 1992). This concept was later demonstrated with *P. striiformis* f. sp. *hordi* in North America (Chen 2004). The predominance of a wider or narrow virulence spectrum is probably more influenced by selection pressure from the genotypes of the host population at a specific location. Chen (2005) reported that, if the host population contains relatively few resistance genes, races with only the virulence genes to match these resistance genes should be favoured by
selection and therefore, should tend to be predominant. In the contrary, if the host population contains many resistance genes, races capable of overcoming more of these genes should become predominant.

Significant phenotypic and genetic differences were detected within and between populations of different regions (Table 11 and 12). However, within populations, the genetic index (Kosman diversity) was negatively correlated with the phenotypic diversity indices (Shannon’s, Simpson’s and Gleason’s) and hence did not yield the same rank order of diversity. The southeast region which had the highest Shannon and Gleason diversity indices was rated as lowest divers system with Kosman diversity. Similarly, the isolates from the north and central regions which were characterized as the most diverse with Kosman diversity index received the lowest Shannon’s and Gleason’s diversity values. A general lack of significant correlations between phenotypic and genetic diversities within populations is in part attributed to the differences in procedures followed to determine each diversity index. For instance, all phenotypic indices are calculated from occurrence and frequency of different pathotypes in each population, regardless of how many virulence factors the phenotypes share in common. The Kosman diversity index on the other hand take into account the number of virulence factors the isolates share in common in addition to the relative frequencies of different pathotypes in each population. Our results are in agreement with the work of Manisterski et. al. (2000) who found high correlation between Shannon’s and Simpson index values but not to the Kosman diversity index. In a similar study, Andrivon and de Vallavielle Pope (1993) found a general lack of significant correlation between differences in complexity and diversity as measured by the Shannon diversity in three populations of Erysiphe graminis, the causal agent of powdery mildew on barley in France. Andrivon and de Vallavielle Pope (1995) also found a general lack of correlation between virulence complexity and diversity for other rust and mildew diseases.
`Within` diversity comparison, pathotypes from the north and central regions had higher genetic diversity (Kosman diversity index) than pathotypes from the south and southeast regions. This may be attributed to the cultivation of both indigenous landrace cultivars and commercial durum wheat cultivars along with bread wheat cultivars in the central and northern regions of the country. Durum wheat genotypes in these regions are known to have high genetic diversity (Tessema and Belay 1991) and hence, isolates with high complimentary genetic diversity co-exist with a wide range of genotypes. Manisterski et. al. (2000) have noted strong influence of genes in cultivated wheat cultivars, landraces and wild and relative species on diversity of virulence in a pathogen population of *Puccinia recondita* f. sp. *tritici* in Israel.

Unlike the north and central regions, genetically low diverse pathotypes were observed from the south and southeast regions. This could be ascribed to the cultivation of wheat cultivars with a low degree of genetic diversity. In these regions, the large-scale of semi-commercial bread wheat monocropping system is the dominating mode of production and thus time has played a significant role in reducing crop diversity in farmers’ fields (Ensermu et al. 1998). A recent disease survey in the south and southeast regions showed that 56% of the small-scale farmers grow two major bread wheat cultivars, Kubsa and Galama (Temesgen, Pers. comm.). These regions account for more than 75% of the total area under bread wheat cultivation in the country (Ensermu et al. 1998). Most of the bread wheat cultivars in the country lack adequate genetic variation against stripe rust resistance because they have the same genetic background (Gebremariam 1991, Badebo 2002). Selection by growing cultivars with a similar genetic background induces shift of pathotypes and increases pathogen complexity (Müller et al. 1996).

A maximum Rogers index value of 1.00 between the central and the south regions indicates that no pathotype was common between these two regions. Unlike the durum wheat belt of the central region the south wheat belt is commonly occupied by bread wheat cultivars. The lowest
DISCUSSION

Rogers index value of 0.77 was observed for populations from the south and southeast which have two pathotypes in common (Table 10). The lowest genetic distance was also measured with Kosman distance index was obtained from these two locations. This was also demonstrated by the cluster analysis that indicated group similarity within and between pathotypes from these two regions. This may suggest that the stripe rust population present in these two regions have high adaptation to *T. aestivum* commercial cultivars which are grown under both small-scale and commercial state farmers.

4.2. Gene postulation

Resistance-specificity of the host, as expressed by distinct qualitative disease reactions on seedlings, i.e. infection types (IT), when challenged by a series of pathogen isolates, has often formed the basis for genetic analysis and gene postulation of both the host and the pathogen (Day 1974, Jensen et al. 1992, Johnson and Knott 1992). To postulate the genes from the cultivars, in the present study cultivars and differential genotypes were challenged with 20 stripe rust isolates at the seedling stage. The set of the isolates represents different origins and virulence spectra, and proven to be effective in discriminating different sources of resistance, thereby providing useful information for the current wheat breeding programmes. The 18 bread wheat cultivars tested were postulated to have stripe rust resistance genes *Yr2, Yr3a, Yr4a, Yr6, Yr7, Yr8, Yr9, Yr27, Yr32, YrA*, and *YrSu* in different combinations while the five durum wheat cultivars were assumed to have genes *Yr3a, Yr8, Yr32, Yr27*, and *YrA*. The most commonly encountered stripe rust resistance gene in the bread wheat cultivars was *Yr2*. This gene was present in eight of the bread wheat cultivars alone or in combinations with additional genes. Gene *Yr2* introduced through 'Kalyansona', is widely present in the CIMMYT derived germplasm (Rajaram et al. 1983). According to this study, *Yr2* is known not to provide enough protection against a wide range of pathogen genotypes indicating the need for searching more effective stripe rust resistance genes to be incorporated in Ethiopian bread wheat cultivars.
Although *Yr* genes *Yr1*, *Yr5*, *Yr15*, and *Yr17* found to be effective under Ethiopian conditions, there is no indication for the presence of these genes in Ethiopian wheat cultivars. By contrast, Hovmøller (2007) identified genes *Yr1*, *Yr15*, and *Yr17* in European wheat cultivars.

The validity of host-pathogen interaction data strongly depends on large prerequisites, which include correct identity and purity of the seed stocks, pure and well-characterized pathogen isolates, appropriate experimental conditions for temperature, humidity and light, to which the yellow rust/wheat interaction may be sensitive (Stubbs 1967), and correct interpretation of the disease reaction in terms of compatibility or incompatibility. Here, the purity of the seed samples was insured by carrying out homogeneity test using 6 SSR markers that were selected based on their amplifications on the different regions of the wheat chromosomes (Appendix 4). There was no perfect uniformity in 5 out of 8 varieties for at least one marker. In addition the identified genes *Yr6* and *Yr7* in the cultivar Pavon-76 that was confirmed also by earlier work of Dubin et al (1989) and Badebo et al. (1990) demonstrated the validity of the gene postulation. (Where is that shown ???)

The stripe rust resistance genes *Yr5*, *Yr15*, and *Yr26* were non-compatible to all the isolates tested which makes it difficult to postulate these genes in the cultivars Tusie, Sirbo, ET-13, and Wabe which had also resistance genes effective against all isolates tested. However the molecular analysis revealed that the mapped *Yr* genes on chromosomes 7BL in cultivars Wabe confirmed that the resistance in this cultivar is governed by different resistance gene other than *Yr5* (2BL), *Yr15* (1BL), and *Yr26* (1BS). The resistance genes were not identified for three of the bread (Wetera, Megal, and Suf-Omer) and ten of the durum wheat cultivars due to the lack of matching reaction patterns with any of the differential genotypes used for the gene postulation.
4.3. Adult plant resistance (APR)

In the case of *P. striiformis* on wheat, where other sources of host resistance are expressed at the adult plant stages (Johnson 1984), percentage of leaf area infected in single-isolate inoculated field trails have been used as a basis for describing the presence of adult plant resistance (Priestley et al. 1984). In the present study all the cultivars were challenged with a mixture of four virulent isolates. At least one isolate was virulent to the cultivars at the seedling stage except to the four cultivars that were non-compatible to all the isolates tested. The high disease severity (60-90%) which was observed on the susceptible check both in the greenhouse and field, indicates that the infection level was high enough to assess the resistance in the cultivars tested. Cultivars that had ≤10% leaf area infection were regarded as carrying adult plant resistance genes (Johnson 1993). In the present study, the bread wheat cultivars Mada walabu, Dodata, Abola, KBG-01, Bobicho, Hawii, Kater, Galama, Megal, and Suf-Omer had ≤10% disease severity, indicating the presence of APR genes in these cultivars. The bread wheat cultivars Dereseligh (*Yr*2), Kubsa (*Yr*2+), K6290-Bulk (*Yr*6+2), Simba (*Yr*6+2), Tura (*Yr*6, *Yr*9) and Dashen (*Y8*) exhibited 20% or more disease severity. This threshold level indicates, APR in these cultivars does not play a major role in keeping the seedling stage resistance at the adult stage.

On the other hand, the durum wheat cultivars were susceptible at seedling stage to most of the races tested but about 60% of the cultivars have less than 10% disease severity when artificially inoculated with the same set of isolates at adult stage. This may suggest unlike bread wheat cultivars that stripe rust resistance in durum wheat cultivars is mainly governed by adult plant resistance genes.
4.4. Mapping of Yr genes using microsatellite markers (SSR)

Following discovery of the Flor’s gene-for-gene relationship between the hosts and the pathogen genotypes, breeding and use of resistant varieties underwent enormous progress. Using this method new stripe rust race-specific resistance genes were identified and transferred to breeding populations. Recently, resistance genes have been identified and mapped from the host plants using molecular markers. Molecular markers linked with stripe rust resistance genes were reported for Yr5 (Chen et al. 2003), Yr9 (Shi et al. 2001), Yr10 (Wang et al. 2002), Yr15 (Peng et al. 2000), Yr17 (Seah et al. 2001), Yr18 (Suenaga et al. 2003), Yr24 (Zakari et al. 2003), Yr26 (Ma et al. 2001), Yr28 (Singh et al. 2000), Yr32 (Eriksen et al. 2004), Yr33 and Yr34 (McIntosh et al. 2004), YrH52 (Peng et al. 2000), and Yrns-B1 (Börner et al. 2000). Closely linked markers can be used for marker-assisted selection (Chen 2005). In the present investigation, stripe rust resistance genes were mapped on the long arms of wheat chromosomes 3B (Suf-Omer), 1BL (Wetera) and 7BL (Wabe) using SSR markers. The only seedling resistance gene for stripe rust resistance so far mapped on chromosome 3B is YrSte2. The chromosome arm of YrSte2 which is identified from Stephens has not been determined (Chen 2005, McIntosh et al. 2006).

Yr3a, Yr3b and Yr3c were identified on chromosome 1B although their specific positions were not yet determined. Macer (1975) and Peng et al. (2000) were able to map seedling resistance genes Yr9 and Yr15 on the long arm of chromosome 1B for the wheat genotype Riebesel and T. dicoccoides, respectively. Chen et al. (1995) and Singh et al. (2005) mapped adult plant resistance gene Yr21 and Yr29. The chromosomal location for Yr21 was not given while Yr29 was mapped on the long arm of 1B. In the current study, the races that were virulent on Yr3a, Yr3b, and Yr9 at the seedling stage, were avirulent on Wetera. On the contrary, two of the races which were virulent on Wetera were avirulent to Yr15. Hence the Yr genes identified in Wetera might be different from these genes. However, allellism test with the donor parents Capelle-Desprez (Yr3a), Hybrid 46 (Yr3b) and Yr9/6*Avocet S (Yr9) is a must to confirm this.
Chen (2005) reported the seedling resistance gene *Yr6* and an adult plant resistance gene *Yr39* on the short and long arm of chromosome 7B, respectively. In a similar study, Lupton and Macer (1962) as well as Chen et al. (1995) identified the seedling resistance gene *Yr2* on chromosome 7B from the variety Heines VII. According to the present seedling resistance test, all the isolates which were virulent on Heines VII were avirulent on Wabe. Therefore, the identified *Yr* gene from this cultivar in the long arm of chromosome 7B might be different from the mapped *Yr2* gene. However, it is suggested to conduct further allelism test, by crossing the source cultivars of the present study with the donor parent of the *Yr2* (Heines VII) to further differentiate the genes.

The identified resistance genes from cultivar Suf-Omer, Wetera, and Wabe confer high resistance to the tested isolates. However, further validation of the identity potential value of these genes is crucially important. The markers *Xgwm181* and *Xgwm340* closely linked to *Yr* gene in Suf-Omer may be useful in pyramiding this gene with other stripe rust resistance genes. Molecular markers for *Yr5* and *Yr15* are currently being used to combine these two genes, each of which confers resistance to all *P. striiformis* f. sp. *tritici* races in North America (Chen 2005). The recombination frequency between the markers and resistance genes in Wabe are too large to be used in marker-assisted selection.