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Belayneh Admassu Yimer "Genetic and virulence diversity of *Puccinia graminis* f. sp. *tritici* populations in Ethiopia and stem rust resistance genes in wheat", University of Giessen, 2010

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 in Ethiopia. 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 *Sri 3* 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 270 farmers' fields in four major wheat producing regions (Arsi, Bale, Shewa and Northwest Ethiopia) of Ethiopia were visited and rusted wheat samples were collected. In addition, samples were collected from off-season nursery sites in two agricultural research centers, Debre Zeit and Kulumsa. Sample collection was 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. 152 single pustule isolates were prepared from the samples collected in the four regions. Multiplication of single pustule isolates and race analysis (inoculation onto wheat differential lines, disease scoring and race designation) was done based on the standard procedure in the greenhouse and growth chambers of the National Plant Protection Research Center at Ambo in Ethiopia.

The analysis detected 22 *Pgt* races from the four 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 Ug99 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 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 the stem rust resistance gene *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 prevalent in Ethiopia. These were *Sri 3, Sr36, SrTmp, and Sr30.* Three of these four resistance genes *(Sr]3, 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 for a sustainable control of stem rust in Ethiopia.

Genetic characterization of *Pgt* isolates was done using 48 isolates that were selected based on their virulence spectrum and geographic origin. The total genomic DNA of each isolate was extracted from 20 mg urediospores using the Nucleoplex™ Plant DNA kit. Amplification of DNA was conducted on a GeneAmp® PCR System 9700. The PCR products were denatured and later subjected to capillary electrophoreses in an ABI PRISM® 3100 genetic analyzer for detection of allele sizes. 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. Results showed high genetic diversity within, but low genetic distance among Ethiopian *Pgt* populations. The genie 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 absence of population differentiation based on geographic origin of isolates is a phenomenon that could create a situation where stem rust resistance genes that are deployed to counter stem rust in farmers' fields in Ethiopia can 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 for a sustainable control of stem rust in Ethiopia.

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, Sril, 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 37 wheat genotypes were postulated to have unknown resistance genes, requiring further investigation using additional races to determine their identity. 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 introduce stem rust resistance genes like *Sri 3* that is effective against race Ug99 and other prevalent races into commercial wheat cultivars grown in Ethiopia. Development and deployment of wheat cultivars that contain combined genes through gene pyramiding and/or cultivar mixture is another option to protect wheat from stem rust.

Sri 3 was one of a few stem rust resistance genes that confer resistance against Ug99 that is threatening world wheat production. It is also effective against the majority of *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 to combat Ug99 worldwide. To achieve this, a cross between the resistant cultivar 'Khapstein/9*LMPG' and the susceptible cultivar 'Morocco' was done and 158 F; plants were developed. Results of the phenotypic analysis showed that segregation of F; plants and FS 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 the 10 polymorphic markers, five (barc37, wmc256, bard 07, gwm570 and barcl46) were mapped on the long arm of chromosome 6A. Barc37 and wmc256 were the closest markers that flanked *Sri 3* distally and proximally at distances of 3.0 and 6.0 cM, respectively. The other three markers bare 107, gwm570 and barcl46 were mapped at 7.0, 18.0 and 19.0 cM, respectively. The two closely linked flanking markers could potentially be used for marker assisted selection there by facilitating and increasing the incorporation of this important gene to commercial wheat cultivars via back crossing or incorporation of the gene in newly developed wheat cultivars in the fight against Ug99 in Ethiopia as well as in countries affected and threatened by race Ug99.

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