MOLECULAR MARKER ANALYSIS OF STEM RUST RESISTANCE GENES IN SOME IRANIAN WHEAT GENOTYPES

H. JAVADI, A. DADKHODAIE, B. HEIDARI

Department of Plant Production and Genetics, School of Agriculture, Shiraz University, Shiraz, Iran, 71441-65186

E-mail: dadkhodaie@shirazu.ac.ir

Stem rust caused by Puccinia graminis f. sp. tritici is a destructive wheat disease worldwide, traditionally controlled by the Sr31 resistance gene for many years until the virulent strain; Ug99 emerged in 1999. The new pathotype threatened the global wheat production and was later detected in Hamedan and Lorestan provinces of Iran. To tackle this disease, it is necessary to find new sources of resistance against the Ug99 race and its variants. Ninety-five Iranian wheat genotypes were analyzed for the presence of the stem rust resistance genes; Sr2, Sr22, Sr24, Sr25 and Sr31 with the help of several CAPS, STS and SSR markers. Seeds of the tested genotypes and Thatcher as negative control and five isolines as positive controls were sown in pots in a greenhouse. After DNA extraction, polymerase chain reaction (PCR) was performed using primers for the corresponding markers. The Iag95 marker demonstrated the presence of Sr31 in 10 genotypes. Nine genotypes showing the Gb associated band, carried Sr25. J09, the linked marker with Sr24, detected this gene in only two genotypes. No genotype showed the bands for markers linked to Sr2 or Sr22 genes. The combined presence of Sr24 and Sr31 was identified in six genotypes. So far, neither Ug99 nor its variants have virulence for Sr2, Sr22 and Sr25 suggesting they could be transferred from donor sources to suitable lines for commercial uses.

***Key words**: Molecular Markers; Resistance Genes; Stem Rust; Wheat.*

АНАЛІЗ ГЕНІВ РЕЗИСТЕНТНОСТІ ДЕКІЛЬКОХ ІРАНСЬКИХ ГЕНОТИПІВ ПШЕНИЦІ ДО СТЕБЛОВОЇ ІРЖІ ЗА ДОПОМОГОЮ МОЛЕКУЛЯРНИХ МАРКЕРІВ

Стеблова іржа, викликана *Puccinia graminis* f. sp. *tritici*, – це шкідливе захворювання пшениці, поширене у всьому світі та впродовж багатьох років контрольоване геном резистентності *Sr31* до вірулентного штаму; Ug99 виник у 1999 р. Новий патотип загрожував глобальному виробництву пшениці і згодом був виявлений у таких провінціях Ірану, як Хамедан і Лорестан. Боротьба з цим захворюванням вимагає знаходження нових джерел резистентності до раси Ug99 і його варіантів. Дев'яносто п'ять генотипів іранської пшениці було проаналізовано на наявність генів резистентності до стеблової іржі – *Sr2*, *Sr22*, *Sr24*, *Sr25* та *Sr31* за використання декількох маркерів CAPS, STS і SSR. Зерно рослин з перевіреними генотипами і сорту Тетчер, що слугувало в якості негативного контролю, і п'яти ізоліній – в якості позитивного контролю, посіяли у тепличні посудини. Після екстракції ДНК провели полімеразно-ланцюгову реакцію (ПЛР) за використання праймерів для відповідних маркерів. Маркер *Iag95* продемонстрував наявність *Sr31* у 10 генотипах. Дев'ять генотипів з виявленою *Gb*-асоційованою смугою, були носіями *Sr25. J09,* маркер, пов'язаний з *Sr24*, виявив цей ген лише у двох генотипах. Жоден генотип не продемонстрував смуги щодо маркерів, пов'язаних з генами *Sr2* або *Sr22.* Комбіновану присутність *Sr24* і *Sr31* було ідентифіковано у шести генотипах. Наразі ні Ug99, ні його варіанти не мають вірулентності щодо *Sr2*, *Sr22* і *Sr25,* тому припускаємо, що їх можна перенести від донорів до ліній, придатних для комерційного використання.

Ключові слова: молекулярні маркери, гени резистентності, стеблова іржа, пшениця.

REFERENCES

Afshari F. (2012) Genetic pathogenicity of the disease caused stem rust (*Puccinia graminis* f. sp. *tritici*) and the response of wheat genotypes to the disease, Iran. J Plant Prot Sci. 43:357–365

Anderson JA. (2003) Plant genomics and its impact on wheat breeding. *In* Plant Molecular Breeding 184–215

Bariana HS, Brown GN, Bansal UK, Miah H, Standen GE, Lu M. (2007) Breeding triple rust resistant wheat cultivars for Australia using conventional and marker-assisted selection technologies. Aust J Agric Res. doi: 10.1071/AR07124

Bashir S. (2019) Studies on Molecular Determinants of Stem Rust in Wheat (*Triticum aestivum*), Doctoral dissertation, Government College University, Faisalabad.

Bashir S, Bukhari SA, Mahmood-ur-Rahman MA. (2019) Homology modeling, structure and active site prediction of stem rust resistant gene *Sr22* in wheat cultivars. Pure Appl Biol. doi: 10.19045/bspab.2018.700216

Bhavani S, Singh RP, Argillier O et al. (2011) Mapping durable adult plant stem rust resistance to the race Ug99 group in six CIMMYT wheats to Ug99 group of races. Borlaug Global Rust Initiative 2011 Technical Workshop 43–53

Brown GN. (1997) The inheritance and expression of leaf chlorosis associated with gene *Sr2* for adult plant resistance to wheat stem rust. Euphytica doi: 10.1023/A:1002985326256

Bukhari SA, Mahmood-Ur-Rahman, Shamshari WA, Bashir S. (2018) Homology modeling, structure and active site prediction of stem rust resistant protein in wheat. Mol Biol 7:411–414

Chelkowski J, Golka L, and Stepien L. (2003) Application of STS markers for leaf rust resistance genes in near isogenic lines of spring wheat cv. Thatcher. J Appl Genet 44:323–338

Dadkhodaie NA, Singh D, Park RF. (2011) Characterisation of resistance to leaf rust in an international bread wheat nursery. J Plant Pathol doi: 10.5554/jpp.v93i3.3645

Dhaliwal AS, MacRitchie FJ. (1990) Contributions of protein fractions to dough handling properties of wheat-rye translocation cultivars. J Cereal Sci. doi: 10.1016/S0733-5210(09)80093-3

Ellis JG, Lagudah E, Spielmeyer W, Dodds P. (2014) The past, present and future of breeding rust resistant wheat. Front Plant Sci. doi: 10.3389/fpls.2014.00641

Gerechter-Amitai ZK, Wahl I, Vardi A, Zohary D. (1971) Transfer of stem rust seedling resistance from wild diploid einkorn to tetraploid durum wheat by means of a triploid hybrid bridge. Euphytica 20:281–285

Ghazvini H, Sarhangi M. (2016) Study on presence of stem rust resistance gene *Sr2* in the Iranian varieties and elite wheat lines by using molecular markers. Crop Biotech 14:27–42

Gultyaeva EI, Kanyuka IA, Alpateva NV et al. (2009) Molecular approaches in identifying leaf rust resistance genes in Russian wheat varieties. Russ Agric Sci. doi: 10.3103/S1068367409050085

Hale IL, Mamuya I, Singh D. (2013) *Sr31*-virulent races (TTKSK, TTKST, and TTTSK) of the wheat stem rust pathogen *Puccinia graminis* f. sp. *tritici* are present in Tanzania. Plant Dis. doi: 10.1094/PDIS-06-12-0604-PDN

Harder DE, Dunsmore KM. (1991) Incidence and virulence of *Puccinia graminis* f. sp. *tritici* on wheat and barley in Canada. Can J Plant Pathol. doi: 10.1080/07060669109500922

Hare RA, McIntosh RA. (1979) Genetic and cytogenetic studies of durable adult-plant resistances in 'Hope' and related cultivars to wheat rusts. Z. Pflanzenzuchtung 83:350–367

Jin Y, Singh RP, Ward RW et al. (2007) Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis. doi: 10.1094/PDIS-91-9-1096

Jin Y, Szabo LJ, Pretorius ZA, Singh RP (2008) Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis doi: 10.1094/PDIS-92-6-0923

Khan RR, Bariana HS, Dholakia BB, Naik SV et al. (2005) Molecular mapping of stem and leaf rust resistance in wheat. Theor Appl Genet doi: 10.1007/s00122-005-0005-4

Khlestkina EK, Pestsova EG, Salina E, Röder MS et al. (2002) Genetic mapping and tagging of wheat genes using RAPD, STS, and SSR markers. Cell Mol Biol Lett. 7:795–802

Knott DR. (1980) Mutation of a gene for yellow pigment linked to *Lr19* in wheat. Can J Genet Cytol. 22:651–654

Korzun V, Hackauf B, Wortmann H, Wilde P, Wehling P. (2012) Development of conserved ortholog set markers linked to the restorer gene *Rfp1* in rye. Mol Breed. doi: 10.1007/s11032-012-9736-5

Lagudah ES. (2011) Molecular genetics of race non-specific rust resistance in wheat. *Euphytica* doi: 10.1007/s10681-010-0336-3

Lelley T, Eder C, Grausgruber H. (2004) Influence of 1BL.1RS wheat-rye chromosome translocation on genotype by environment interaction. J Cereal Sci. doi: 10.1016/j.jcs.2003.11.003

Leonard KJ, Szabo LJ. (2005) Stem rust of small grains and grasses caused by *Puccinia graminis*. Mol Plant Pathol doi: 10.1111/j.1364-3703.2005.00273.x

Li HJ, Conner RL, Murray TD. (2008) Resistance to soilborne diseases of wheat: Contributions from the wheatgrasses *Thinopyrum intermedium* and *Th. Ponticum*. Can J Plant Sci doi: 10.4141/CJPS07002

Li HJ, Wang XM. (2009) *Thinopyrum ponticum* and *Th. intermedium*: The promising source of resistance to fungal and viral diseases of wheat. J Genet Genomics doi: 10.1016/S1673-8527(08)60147-2

Lukaszewski AJ. (2000) Manipulation of the 1RS.1BL translocation in wheat by induced homoeologous recombination. Crop Sci. doi:10.2135/cropsci2000.401216x

Mago R, Brown-Guedira G, Dreisigacker S, Breen J et al. (2011) An accurate DNA marker assay for stem rust resistance gene. Theor Appl Genet doi: 10.1007/s00122-010-1482-7

Mago R, Miah H, Lawrence GJ, Wellings CR et al. (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 Gene. doi: 10.1007/s00122-005-0098-9

Mago R, Spielmeyer W, Lawrence GJ, Lagudah ES. (2002) Identification and mapping of molecular markers linked to rust resistance genes located on chromosome 1RS of rye using wheat-rye translocation

lines. Theor Appl Genet doi: 10.1007/s00122-002-0879-3

Malav AK, Kuideep I, Chandrawat KS. (2016) Gene Pyramiding: An Overview, Int J Curr Res Biosci Plant Biol doi: 10.20546/ijcrbp.2016.307.004

Martin DL, Stewart BG. (1990) Dough stickiness in rye-derived wheat cultivars. Euphytica doi: 10.1007/BF00022895

McFadden ESA. (1930) Successful transfer of emmer characters to vulgare wheat. J Am Soc Agron. 22: 1020–1034

McIntosh RA, Dubcovsky J, Rogers WJ et al. (2018) Catalogue of gene symbols for wheat, 12th International Wheat Genetics Symposium held in Yokohama, Japan.

McIntosh RA, Park RF, Wellings CR. (1995) Wheat Rusts. In: McIntosh RA (ed) An Atlas of Resistance Genes, CSIRO Publications, Melbourne, Australia

Mohammadi M, Torkamaneh D, Patpour M. (2013) Seedling stage resistance of Iranian bread wheat germplasm to race Ug99 of *Puccinia graminis* f. sp. *tritici*, Plant Dis doi: 10.1094/PDIS-02-12-0138-RE

Najafian G, Amin H, Afshari F et al. (2010) Sivand, a new bread wheat cultivar, resistant to stem rust (race Ug99) with good bread making quality for cultivation under irrigated conditions of temperate regions of Iran. J Seed Plant Improv 26: 285–288

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. doi: 10.1094/PDIS-93-3-0317B

Nisha R, Sivasamy M, Gajalakshmi K (2015) Pyramiding of stem rust resistance genes to develop durable and multiple disease resistant wheat varieties through marker aided selection. Int J Ext Res 5:1–9

Njau PN, Jin Y, Huerta-Espino J, Keller B, Singh RP. (2010) Identification and evaluation of sources of resistance to stem rust race Ug99 in wheat. Plant Dis doi: 10.1094/PDIS-94-4-0413

Oliver RP. (2014) A reassessment of the risk of rust fungi developing resistance to fungicides. Pest Manage Sci doi: 10.1002/ps.3767

Olson EL, Brown-Guedira G, Marshall DS, Jin Y et al. (2010) Genotyping of US wheat germplasm for presence of stem rust resistance genes. Crop Sci doi: 10.2135/cropsci2009.04.0218

Paull JG, Pallotta MA, Langridge P. The TT (1994) RFLP markers associated with *Sr22* and recombination between chromosome 7A of bread wheat and the diploid species *Triticum boeoticum*. Theor Appl Genet doi: 10.1007/BF00224536

Periyannan SK, Bansal UK, Bariana HS, Pumphrey M, Lagudah ES. (2011) A robust molecular marker for the detection of shortened introgressed segment carrying the stem rust resistance gene *Sr22* in common wheat. Theor Appl Genet. doi: 10.1007/s00122-010-1417-3

Prasha M, Bhardwaj SC, Jain SK, Sharma YP, Mishra B. (2008) Gene deployment: Indian experience. International Conference on Wheat Stem Rust Ug99 threat to food security, Held at NASC.

Pretorius ZA, Singh RP, Wagoire WW, Payne TS. (2002) Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Plant Dis doi: 10.1094/PDIS.2000.84.2.203B

Prins R, Groenewald JZ, Marais GF, Snape JW, Koebner RMD. (2001) AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat. Theor Appl Genet doi: 10.1007/PL00002918

Purnhauser L, Byna L, Láng L. (2011) Occurrence of 1BL. 1RS wheat-rye chromosome translocation and of *Sr36/Pm6* resistance gene cluster in wheat cultivars registered in Hungary. Euphytica doi: 10.1007/s10681-010-0312-y

Rajaram S, Mann CH, Ortiz-Ferrara G, Mujeeb-Kazi A. (1983) Adaptation, stability and high yield potential of certain 1B/1R CIMMYT wheats, Wheat Genet Symp 613–621

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

Rosewarne G, Bonnett D, Rebetzke G, Lonergan P, Larkin PJ. (2015) The potential of *Lr19* and *Bdv2* translocations to improve yield and disease resistance in the high rainfall wheat zones of Australia. Agronomy doi: 10.3390/agronomy5010055

Saghai-Maroof M, Soliman KM, Jorgensen RA, Allard RW. (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci doi: 10.1073/pnas.81.24.8014

Schachermayr GM, Messmer MM, Feuillet C, Winzeler H et al. (1995) Identification of molecular markers linked to the *Agropyron elongatum*-derived leaf rust resistance gene *Lr24* in wheat. Theor Appl Genet doi: 10.1007/BF00222911

Sebesta EE, Wood EA. (1978) Transfer of greenbug resistance from rye to wheat with X-rays. Agronomy 70:61–62

Sedlacek T, Marik P, Chrpova J. (2010) Development of CAPS marker for identification of *rym4* and *rym5* alleles conferring resistance to the barley yellow mosaic virus complex in barley. Czech J Genet Plant Breed doi: 10.17221/7/2010-CJGPB

Sharma RK, Singh PK, Joshi AK et al. (2013) Protecting South Asian wheat production from stem rust (Ug99) epidemic. J Phytopathol. doi:10.1111/jph.12070

Sheen SJ, Ebeltoft DC, Smith GS. (1968) Association

and inheritance of «Black Chaff» and stem rust reactions in Conley wheat crosses 1. Crop Sci doi: 10.2135/cropsci1968.0011183X000800040025x

Sheikh FA, Razvi SM, Malik AA. (2017) Role of wild relatives in imparting disease resistance to rusts of wheat. Int J Pure Appl Biosci doi: 10.5958/2229-4473.2017.00009.X

Singh K, Ghai M, Garg M, Chhuneja P et al. (2007) An integrated molecular linkage map of diploid wheat based on a *Triticum boeoticum* × *T. monococcum* RIL population. Theor Appl Genet doi: 10.1007/s00122-007-0543-z

Singh RP, Hodson DP, Huerta-Espino J, Jin Y et al. (2008) Will stem rust destroy the world's wheat crop? Adv Agron doi: 10.1016/S0065-2113(08)00205-8

Singh RP, Hodson DP, Huerta-Espino J, Jin Y et al. (2011) The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annu Rev Phytopathol doi: 10.1146/an-nurev-phyto-072910-095423

Singh RP, Hodson DP, Jin Y et al. (2015) Emergence and spread of new races of wheat stem rust fungus: Continued threat to food security and prospects of genetic control. Phytopathology doi: 10.1094/PHYTO-01-15-0030-FI

Singh RP, Hodson DP, Jin Y, Huerta-Espino J et al. (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. Perspect Agric 1:1–13

Singh RP, Huerta-Espino J, Pfeiffer W, Figueroa-Lopez P. (2004) Occurrence and impact of a new leaf rust race on durum wheat in northwestern Mexico. Plant Dis doi: 10.1094/PDIS.2004.88.7.703

Singh RP, Huerta-Espino J, Rajaram S, Crossa J. (1998) Agronomic effects from chromosome translocations 7DL.7Ag and 1BL.1RS in spring wheat. Crop Sci doi: 10.2135/cropsci1998.0011183X003800010005x

Smith EL, Schlehuber AM, Young HC, Edwards LH. (1968) Registration of Agent Wheat. Crop Sci. 8(4): 511–512

Sumíková T, Hanzalova A. (2010) Multiplex PCR assay to detect rust resistance genes *Lr26* and *Lr37* in wheat. Czech J Genet Plant Breed. doi: 10.17221/32/2010-CJGPB

The TT, Gupta RB, Dyck PL, Appels R, Hohmann U, McIntosh RA. (1992) Characterization of stem rust resistant derivatives of wheat cultivar Amigo. Euphytica doi: 10.1007/BF00025256

Todorovska E, Christov N, Christova P, Vassilev D. (2009) Biotic stress resistance in wheat-breeding and genomic selection implications. Biotechnology doi: 10.2478/V10133-009-0006-6

Watson IW, Singh D. (1952) The future of rust resistant wheat in Australia. J Aust Inst Agric Sci 28:190–197

Yu LX, Chao S, Singh RP, Sorrells ME. (2017) Identification and validation of single nucleotide polymorphic markers linked to Ug99 stem rust resistance in spring. PloS One doi:10.1371/journal.pone.0171963

Yu LX, Liu S, Anderson JA, Singh RP, Jin Y et al. (2010) Haplotype diversity of stem rust resistance loci in uncharacterized wheat lines. Mol Breed. doi: 10.1007/s11032-010-9403-7

Received February 23, 2020
Received March 15, 2020
Accepted September 18, 2021