COLEGIO DE POSTGRADUADOS

INSTITUCIÓN DE ENSEÑANZA E INVESTIGACIÓN EN CIENCIAS AGRÍCOLAS

CAMPUS MONTECILLO

PROGRAMA DE POSTGRADO EN FITOSANIDAD

FITOPATOLOGÍA

"MAPEO DE GENES DE RESISTENCIA A LAS TRES ROYAS DEL TRIGO (*Triticum aestivum* L.) EN LA POBLACIÓN F_6 APAV #1 × KIJIL"

MARICARMEN SANDOVAL SÁNCHEZ

T E S I S

PRESENTADA COMO REQUISITO PARCIAL

PARA OBTENER EL GRADO DE:

DOCTORA EN CIENCIAS

MONTECILLO, TEXCOCO, ESTADO. DE MÉXICO

2019

CARTA DE CONSENTIMIENTO DE USO DE LOS DERECHOS DE AUTOR Y DE LAS REGALIAS COMERCIALES DE PRODUCTOS DE INVESTIGACION

En adición al beneficio ético, moral y académico que he obtenido durante mis estudios en el Colegio de Postgraduados, el que suscribe Maricarmen Sandoval Sánchez, Alumno (a) de esta Institución, estoy de acuerdo en ser participe de las regalías económicas y/o académicas, de procedencia nacional e internacional, que se deriven del trabajo de investigación que realicé en esta institución, bajo la dirección del Profesor Reyna I. Rojas Martínez, por lo que otorgo los derechos de autor de mi tesis "Mapeo de genes de resistencia a las tres royas del trigo (Triticum aestivum en la población F6 Apav #1 x Kijil"

y de los productos de dicha investigación al Colegio de Postgraduados. Las patentes y secretos industriales que se puedan derivar serán registrados a nombre del colegio de Postgraduados y las regalías económicas que se deriven serán distribuidas entre la Institución, El Consejero o Director de Tesis y el que suscribe, de acuerdo a las negociaciones entre las tres partes, por ello me comprometo a no realizar ninguna acción que dañe el proceso de explotación comercial de dichos productos a favor de esta Institución.

Montecillo, Mpio. de Texcoco, Edo. de México, a 14 de Enero de 2019

Firma del
Alumno (a)

Dra. Reyna I. Rojas Martínez
Vo. Bo. del Consejero o Director de Tesis

La presente tesis, titulada: **"Mapeo de genes de resistencia a las tres royas del trigo (*Triticum aestivum* L.) en la población F_6 Apav #1 x Kijil"**, realizada por la alumna **Maricarmen Sandoval Sánchez**, bajo la dirección del Consejo Particular indicado, ha sido aprobada por el mismo y aceptada como requisito parcial para obtener el grado de:

DOCTORA EN CIENCIAS
FITOSANIDAD
FITOPATOLOGÍA

CONSEJO PARTICULAR

CONSEJERA: _____
DRA. REYNA I. ROJAS MARTÍNEZ

DIRECTOR DE TESIS: _____
DR. JULIO HUERTA ESPINO

ASESOR: _____
DR. MANDEEP SINGH RANDHAWA

ASESOR: _____
DR. IGNACIO BENÍTEZ RIQUELME

ASESOR: _____
DR. CRISTIAN NAVA DÍAZ

Montecillo, Texcoco, Estado de México, enero de 2019

MAPEO DE GENES DE RESISTENCIA A LAS TRES ROYAS DEL TRIGO (*Triticum aestivum* L.) EN LA POBLACIÓN F_6 APAV #1 × KIJIL

Maricarmen Sandoval Sánchez, Dra.

Colegio de Postgraduados, 2019

RESUMEN

La línea de trigo harinero (*Triticum aestivum* L.) 'Kijil' desarrollada en el CIMMYT, mostró niveles adecuados de resistencia en planta adulta para roya de la hoja, roya amarilla y roya del tallo en ambientes evaluados en México. La base genética de su resistencia se estudió utilizando 198 líneas recombinantes (RIL) derivadas de la cruza del padre resistente "Kijil" con el padre susceptible "Apav #1". Los padres y las RILs se evaluaron en ensayos de campo realizados en El Batán, Toluca y Ciudad Obregón, México para roya de la hoja (4 ambientes), roya amarilla (6 ambientes) y roya del tallo (2 ambientes) durante 2016 y 2017. Adicionalmente, se realizó un ensayo en invernadero en El Batán para evaluar resistencia a roya del tallo en 2017. La población RIL de Apav #1 × Kijil y los padres fueron genotipeados con marcadores vinculados para la identificación de líneas para presencia o ausencia de *Sr2*/*Yr30* (*gwm533* & *csSr2*), *Lr46*/*Yr29* (*csLV46* & *csLVG22*) y *Lr37*/ *Yr17* (*cslVrga* & *VENTRIUP-LN2*). Al eliminar las líneas heterocigotas, se genotipeó un conjunto seleccionado de 125 RILs negativas a *Lr37/Yr17*, utilizando marcadores DArT-GBS, y se usaron datos genotípicos y fenotípicos para el análisis de QTL. Finalmente, se construyó un mapa genético de 6168.0 cM (1824.2, 3883.7 y 460.1 cM para los genomas A, B y D, respectivamente) utilizando 5,890 marcadores polimórficos (4,338 PAVs, 1,548 SNPs, *gwm533, csSr2, csLV46* y *csLV46G22*). El análisis genético indicó que la resistencia a roya de la hoja está determinada por 3 a 4 genes, al igual que para roya amarilla. A través del mapeo de intervalo compuesto inclusivo (ICIM), se detectaron dos loci de resistencia para roya de la hoja, *QLr.cim-1DS* y *QLr.cim-5AS* en los cromosomas 1DS y 5AS, respectivamente; para roya amarilla, dos loci localizados en el cromosoma 3BS, correspondientes a *Yr30* y *QYr.cim-3BS*. Además, se identificó un locus de resistencia a roya de la hoja y roya amarilla co-localizado en el cromosoma 1BL (correspondiente a *Lr46/Yr29*). Estos QTLs se derivaron de Kijil. Asimismo, se identificaron 3 QTLs derivados de Apav #1 de efectos menores para resistencia a roya de la hoja: *QLr.cim-1AL, QLr.cim-2AL* y *QLr.cim-2BL* en los cromosomas 1AL, 2AL y 2BL, respectivamente. La genética de la resistencia a roya del

tallo se investigó en una subpoblación F_6 de 99 familias derivadas de la cruza Apav #1 × Kijil. El análisis genético indicó que existen cuatro genes de raza específica que confieren resistencia en plántula. Un primer gen se identificó como *Sr38* y los demás se designaron temporalmente como *SrKj;1*, *SrKj22+* y *SrKjX-*, de acuerdo con los tipos de infección registrados. Para corroborar el efecto en planta adulta de los genes de raza específica, se observó que las familias positivas a *Sr38*, mostraron niveles de infección de 0-20 %, mientras que las familias portadoras de los genes *SrKj;1*, *SrKj22+* y *SrKjX-* presentaron rangos de 1-30 %, 30-60 % y 1-30 %, respectivamente. Por otra parte, se determinó que existen tres genes menores que confieren resistencia en planta adulta, entre ellos *Sr2* y *Sr58*. Las familias positivas a *Sr58* mostraron severidades de 30-90 % y sólo una presentó 5 %; aquellas positivas a *Sr2+Sr58* presentaron 60-100 %. La línea de trigo harinero 'Kijil' puede servir como una fuente potencial de resistencia a las tres royas en un programa de mejoramiento, ya que esta resistencia puede transferirse fácilmente a otro germoplasma de trigo a través de la selección asistida por marcadores (MAS).

Palabras clave: *Puccinia triticina*, *Puccinia striiformis* f. sp. *tritici*, *Puccinia graminis* f. sp. *tritici*, QTL, gen pleiotrópico.

MAPPING OF GENES OF RESISTANCE TO THE THREE RUSTS OF BREAD WHEAT (*Triticum aestivum* L.) IN THE F_6 APAV #1 × KIJIL POPULATION

Maricarmen Sandoval Sánchez, Dra.

Colegio de Postgraduados, 2019

ABSTRACT

Bread wheat (*Triticum aestivum* L.) line 'Kijil' developed at CIMMYT showed adequate levels of adult plant resistance to leaf rust, stripe rust and stem rust in Mexican testing environments. The genetic basis of this resistance was revealed using 198 recombinant inbred lines (RILs) derived from the cross of resistant parent 'Kijil' with the susceptible parent 'Apav'. The parents and RILs were phenotyped in the field trials conducted at El Batan, Toluca and Ciudad Obregon, Mexico for leaf rust (4 seasons), yellow rust (6 seasons) and stem rust (2 seasons) during 2016 and 2017. Additionally, one trial was carried out in greenhouse at El Batán for evaluation to stem rust. The Apav × Kijil RIL population and parents were genotyped with gene linked markers for identification of lines for presence or absence of *Sr2*/*Yr30* (*gwm533* & *csSr2*), *Lr46*/*Yr29* (*csLV46* & *csLVG22*) and *Lr37*/*Yr17* (*cslVrga* & *VENTRIUP-LN2*). Upon eliminating heterogenous lines, a selected set of 125 Apav × Kijil RILs not carrying positive allele for *Lr37*/*Yr17*-linked markers (*cslVrga* & *VENTRIUP-LN2*) was genotyped using DArT-GBS markers, and their genotypic and phenotypic data was used for QTL analysis. Finally, a genetic linkage map of 6168.0 cM (1824.2, 3883.7 and 460.1 cM for A, B and D genomes, respectively) was constructed using 5,890 polymorphic markers (4,338 PAVs, 1,548 SNPs, *gwm533*, *csSr2*, *csLV46* and *csLV46G22*). Two leaf rust resistance loci, *QLr.cim-1DS* and *QLr.cim-5AS* on chromosomes 1DS and 5AS, respectively, and a yellow rust resistance locus *QYr.cim-3BS* on chromosome 3BS were detected through inclusive composite interval mapping (ICIM). In addition, a co-located resistance locus to both leaf rust and yellow rust on chromosome 1BL (corresponding to *Lr46/Yr29*), and a yellow rust resistance locus on chromosome 3BS (corresponding to *Yr30*) were also identified. These QTLs were derived from Kijil. Parent Apav-derived 3 minor effect QTLs for leaf rust resistance namely; *QLr.cim-1AL*, *QLr.cim-2AL* and *QLr.cim-2BL* on chromosomes 1AL, 2AL and 2BL, respectively, were also identified. Genetics of resistance to stem rust was investigated in a F_6 population of 99 families derived from a cross between Apav #1 and Kijil.. Genetic analysis indicated that there are four

race-specific genes that confer resistance in seedling stage. A first gene was identified as *Sr38* and the others were designated temporarily as *SrKj;1*, *SrKj22+* and *SrKjX-*, according to the infection types recorded. In order to corroborate the effect of race-specific genes in adult plant, it was observed that families positive to *Sr38*, showed levels of infection of 0-20 %, while families carrying *SrKj;1*, *SrKj22+* and *SrKjX-* genes presented ranges of 1-30 %, 30-60 % and 1-30 %, respectively. On the other hand, it was determined that there are three minor genes that confer resistance in adult plant stage, among them *Sr2* and *Sr58*. Families positive to *Sr58* displayed severities of 30-90 % and only one presented 5 %; those positive for *Sr2+Sr58* presented 60-100 %. Bread wheat line Kijil can serve as a potential source of LR and YR resistance in a breeding program as this resistance can be easily transferred to other wheat germplasm through marker assisted selection (MAS).

Keywords: *Puccinia triticina*, *Puccinia striiformis* f. sp. *tritici*, *Puccinia graminis* f. sp. *tritici*, QTL, pleiotropic gene.

AGRADECIMIENTOS

Al Consejo Nacional de Ciencia y Tecnología (CONACYT) por otorgarme una beca para realizar mis estudios de Doctorado.

Al Colegio de Postgraduados y al programa de Fitosanidad-Fitopatología, por aceptarme y darme la oportunidad de seguir con mi formación profesional.

Al Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) por la oportunidad de trabajar en sus instalaciones y por la financiación del proyecto de investigación.

Al Dr. Ravi P. Singh, gracias por su aceptación, gestión, apoyo, amplia experiencia y asesoramiento para el éxito de este trabajo de investigación.

A mi Consejo Particular, por su apoyo en la realización de esta investigación:

Dra. Reyna I. Rojas Martínez, gracias por su experiencia, conducción y por brindarme su confianza, apoyo incondicional y disponibilidad.

Dr. Julio Huerta Espino, gracias por su disponibilidad, asesoramiento y conducción, y por compartir su extensa experiencia y aportaciones para el éxito de este trabajo de investigación.

Dr. Mandeep Singh Randhawa, thank you so much for your kind help, support, suggestions and wide experience contributed to the present research.

Dr. Ignacio Benítez Riquelme, gracias por aportar su amplia experiencia, por las recomendaciones, revisiones y correcciones durante este trabajo de investigación.

Dr. Cristian Nava Díaz, gracias por su disponibilidad, acertados comentarios, revisiones y correcciones a este trabajo de investigación.

Al Dr. Daniel L. Ochoa Martínez, gracias por su apoyo y disponibilidad como sinodal.

A los asistentes de investigación del CIMMYT por su apoyo amablemente brindado durante mi estancia en CIMMYT: Silverio Muñoz, Violeta Calvo, Martha Hernández, Bacilisa Luna, Cynthia Ortiz, Alberto Vergara y Blanca Minerva González.

A mis papás, José Sergio Sandoval Islas y Ma. del Carmen Sánchez Gálvez y a mi esposo David Hernández Alfonso por su apoyo incondicional y por haberme impulsado y motivado a realizar mis estudios de Doctorado.

A mi hija María Fernanda Vargas Sandoval por su comprensión y amor incondicional.

A todas las personas que de alguna manera intervinieron en la realización del presente trabajo de investigación.

Creciste con esta tesis y a ti te la dedico, María Fernanda

CONTENIDO

LISTA DE CUADROS

CAPÍTULO I

CHAPTER II

LISTA DE FIGURAS

CAPITULO I

CHAPTER II

INTRODUCCIÓN GENERAL

El trigo harinero (*Triticum aestivum* L.) es un cultivo básico importante; entre todos los cereales, es el grano más versátil para la preparación de diversos alimentos, por lo que provee de calorías y proteínas a la población global más que cualquier otro alimento agrícola (más del 20 % de la energía y proteínas dietéticas requeridas mundialmente) (Peña-Bautista *et al*., 2017). Además, el trigo es cultivado en casi todas las regiones del mundo; en 2017, se cosecharon a nivel global 771,718,579 ton de trigo en una superficie de 218,543,071 ha (FAOSTAT, 2018).

En México, los principales estados productores por superficie sembrada bajo condiciones de riego (ciclo Otoño-Invierno) son Sonora (223,373 ha), Guanajuato (50,312 ha), Baja California (46,051 ha), Sinaloa (41,676 ha), Michoacán (39,890 ha) y Jalisco (24,984 ha), mientras que bajo condiciones de temporal (ciclo Primavera-Verano), los principales estados productores son Tlaxcala (20,826 ha), Nuevo León (14,677 ha), Guanajuato (14,466 ha), Oaxaca (11,102 ha), Durango (7,601 ha) y Estado de México (7,355 ha) (SIAP, 2018).

La producción de trigo es afectada por diversos agentes patógenos, entre ellos, las royas. Las royas son hongos pertenecientes al Phylum Basidiomycota, y se conocen tres especies que atacan al trigo: *Puccina graminis* f. sp. *tritici* Erikss. & Hennin., *P. triticina* Erikss. y *P. striiformis* Westend, causantes de roya del tallo, roya de la hoja y roya amarilla, respectivamente. Estos patógenos representan una amenaza significativa en la mayoría de las regiones productoras alrededor del mundo (Basnet *et al*., 2013). En variedades susceptibles, la roya del tallo puede causar pérdidas del rendimiento de más del 40 %, mientras que se han reportado pérdidas del 100 % ocasionadas por roya amarilla y de más del 50 % por roya de la hoja (Hanson *et al*., 1982; Dubin y Brennan, 2009).

Actualmente, la roya amarilla es considerada como la roya principal que afecta la producción de trigo de invierno alrededor del mundo en regiones frías y húmedas (Chen *et al*., 2014). En México, desde el año 2005 esta enfermedad se ha presentado severamente, causando graves pérdidas en el rendimiento, debido a la aparición de una nueva raza, identificada como MEX03.37, la cual se caracteriza por infectar la espiga de variedades que anteriormente eran

resistentes (Huerta-Espino *et al*., 2012). Posteriormente, en 2014 se identificaron los aislamientos MEX14.141 y MEX14.146, los cuales causaron pérdidas de rendimiento de más del 50 % (Solís *et al*., 2016).

Durante los años 2001-2003, se detectó una nueva raza de roya de la hoja denominada BBG/BN, la cual causó pérdidas de USD$32 millones en la producción de trigo duro. Inicialmente se detectó en el Noroeste de México, pero ya se encuentra distribuida en casi todas las zonas trigueras del país (Singh *et al*., 2004).

En México, la roya del tallo está controlada desde que se liberó la variedad Yaqui 50 y otras variedades portadoras del gen *Sr2*, entre otros. Además, no se han observado cambios en las razas de roya del tallo en México en los últimos 40 años y las infecciones naturales son prácticamente inexistentes (Singh *et al*., 2006; Huerta-Espino *et al*., 2011). Sin embargo, la raza Ug99 o TTKSK identificada por primera vez en Uganda, la cual se ha dispersado en algunos países de África y Asia, representa una amenaza significativa para el germoplasma utilizado en México, ya que esta raza es virulenta a los genes que portan dicho germoplasma (Singh *et al.*, 2006; Prasad *et al*., 2016).

Las royas se controlan a través del método cultural, el cual involucra la erradicación de sus hospedantes alternos, así como mediante la utilización de fungicidas cuando se siembran variedades susceptibles. Este último método resulta costoso y no es amigable ambientalmente. La resistencia genética es el método de control de royas más económico y amigable ambientalmente. Este tipo de control involucra la identificación de nuevas fuentes de resistencia genética, así como su caracterización y utilización estratégica. Diversos estudios han revelado que el efecto aditivo de la combinación de tres a cinco genes menores resultan en niveles de infección "cercana a la inmunidad" y en resistencia estable a través de diferentes ambientes (Singh *et al*. 2000).

Hasta ahora, se han catalogado y designado formalmente cerca de 78, 76 y 70 genes de resistencia para roya amarilla, roya de la hoja y roya del tallo, respectivamente (McIntosh *et al*., 2017). Entre éstos, *Lr27*/*Yr30*/*Sr2*/*Pbc1* (localizado en el cromosoma 3BS),

Lr34/*Yr18*/*Sr57*/*Pm38* (7DS), *Lr46*/*Yr29*/*Sr58*/*Pm39* (1BL) y *Lr67*/*Yr46*/*Sr55*/*Pm46* (4DL), confieren resistencia a enfermedades múltiples, como a las tres royas del trigo, así como cenicilla polvorienta (*Blumeria graminis* (DC) Speer f. sp. *tritici* emend. É. J. Marchal), por lo que también se conocen como genes con efecto pleiotrópico (Singh *et al*. 1998; William *et al*., 2003; Mago *et al*., 2011; Herrera-Foessel *et al*., 2014). Además, se han reportado cerca de 80 y 140 loci de carácter cuantitativo (QTLs) que proveen resistencia a roya de la hoja y roya amarilla, respectivamente (Rosewarne *et al*., 2013; Li *et al*., 2014).

La emergencia de nuevas razas virulentas de royas del trigo, representan una amenaza significativa en la producción mundial de trigo por lo que es esencial la identificación y caracterización molecular de nuevos genes/QTLs de resistencia. Comparativamente con el uso de genes de raza específica, la utilización de genes de raza no específica o genes de resistencia de planta adulta, es considerada como el control de royas más efectivo.

La línea avanzada de trigo harinero 'Kijil' (Klein Don Enrique*2/3/ Fret2/Wbll1//Tacupeto F2001), desarrollada por el programa de trigos harineros del Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), ha mostrado niveles adecuados de resistencia de planta adulta a roya de la hoja, roya amarilla y roya del tallo, sin embargo; se desconoce la base genética de esta resistencia, por lo que los objetivos de la presente investigación fueron: 1) determinar el número de genes en Kijil que controlan la resistencia a roya de la hoja, roya amarilla y roya del tallo, 2) identificar loci de carácter cuantitativo (QTLs) para roya de la hoja y roya amarilla y 3) encontrar loci de resistencia de efecto pleiotrópico a roya de la hoja y roya amarilla, utilizando una población F_6 Apav × Kijil de líneas altamente recombinantes (RILs).

LITERATURA CITADA

Basnet B. R., R. P. Singh, S. A. Herrera F., A. M. H. Ibrahim, J. Huerta E., V. Calvo S. and J. C. Rudd. 2013. Genetic analysis of adult plant resistance to yellow rust and leaf rust in common spring wheat Quaiu 3. Plant Disease 97: 728-736.

Chen W., C. Wellings, X. Chen, Z. Kang and T. Liu. 2014. Wheat stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici*. Molecular Plant Pathology 15: 433-446.

Dubin H. J. and J. P. Brennan. 2009. Fighting a "Shifty Enemy": The international collaboration to contain wheat rusts. In: Millions Fed: Proven successes in agricultural development. Spielman, D. J. and R. Pandya-Lorch (eds). Washington, D.C. IFPRI. pp: 19-24.

FAOSTAT (FAO, 2018); http://faostat.fao.org

Hanson, H., N. E. Borlaug, and R. G. Anderson. 1982. Wheat in the Third World. Boulder, Colorado, U.S.A.: Westview Press.

Herrera-Foessel, S. A., Singh, R. P., Lillemo, M., Huerta-Espino, J., Bhavani, S., Sukhwinder, S., Lan, C. X., Calvo-Salazar, V., & Lagudah, E. S. 2014. *Lr67/Yr46* confers adult plant resistance to stem rust and powdery mildew in wheat. Theoretical and Applied Genetics 127: 781-789.

Huerta E. J., H. E. Villaseñor M., E. Espitia R., E. Solís M. and M. Van Ginkel. 2011. The history of wheat breeding in México. In: The Wheat Book V2, History of Wheat Breeding. Alain P. Bonjean, William J. Angus, Maarten van Ginkel (eds.). Londres, Paris, New York. pp: 275-308.

Huerta- Espino J., R. Torres G., M. F. Rodríguez G., H. E. Villaseñor M., S. G. Leyva M., y E. Solís M. 2012. Resistencia a roya amarilla (*Puccinia striiformis* f. sp. *tritici*) en variedades de trigo harinero. Revista Mexicana de Ciencias Agrícolas 5: 879-891.

Li, Z., Lan, C. X., He, Z., Singh, R. P., Rosewarne, G. M., Chen, X., & Xia, X. 2014. Overview and application of QTL for adult plant resistance to leaf rust and powdery mildew in wheat. Crop Science 54: 1907-1925.

Mago, R., Tabe, L., McIntosh, R. A., Pretorius, Z., Kota, R., Paux, E., Wicker, T., Breen, J., Lagudah, E. S., Ellis, J. G., & Spielmeyer, W. 2011. A multiple resistance locus on chromosome arm 3BS in wheat confers resistance to stem rust (*Sr2*), leaf rust (*Lr27*) and powdery mildew. Theoretical and Applied Genetics 123: 615-623.

McIntosh R. A., J. Dubcovsky, W. J. Rogers, C. Morris and X. C. Xia. 2017. Catalogue of gene symbols for wheat: 2017 supplement.

Peña-Bautista, R. J., Hernandez-Espinosa, N., Jones, J. M., Guzman, C. and Braun, H. J. 2017. CIMMYT series on carbohydrates, wheat, grains, and health: wheat-based foods: their global and regional importance in the food supply, nutrition, and health. Cereal Foods World 62: 231-249.

Prasad, P., Bhardwaj, S. C., Khan, H., Gangwar, O. P., Kumar, S. and S. B. Singh. 2016. Ug99: saga, reality and status. Current science 110: 1614-1616.

Rosewarne G. M., Herrera-Foessel, S. A., Singh, R. P., Huerta-Espino, J., Lan, C. X., He, Z. H. 2013. Quantitative trait loci of stripe rust resistance in wheat. Theoretical and Applied Genetics 126: 2427-2449.

Servicio de Información Agroalimentaria y Pesquera (SIAP). 2018. Secretaría de Agricultura y Desarrollo Rural (SADER), México. https://www.gob.mx/siap

Singh, R. P., Mujeeb-Kazi, A., & Huerta-Espino, J. 1998. *Lr46*: A gene conferring slow rusting resistance to leaf rust in wheat. Crop Science 37: 390-395.

Singh R. P., J. Huerta E. and S. Rajaram. 2000. Achieving near-immunity to leaf and stripe rusts in wheat by combining slow rusting resistance genes. Acta Phytopathologica et Entomologica Hungarica 35: 133-139.

Singh, R. P., Huerta-Espino, J., Figueroa, P. L and Pfeiffer, W. 2004. Occurrence and impact of a new leaf rust race on durum wheat in the Nothwestern Mexico from 2001-2003. Plant Disease 88: 703-708.

Singh R. P., Hudson D., Yuen J., Huerta E. J., Kinyua M. G., Wanyera R., Njau P., and Ward R. W. 2006. Current status, likely migration and strategies to mitigate the threat to wheat production from race *Ug99* (TTKS) of stem rust pathogen. CAB reviews: Perspectives in agriculture, veterinary science, nutrition and natural resources 54: 1-13.

Solís-Moya E., J. Huerta-Espino, P. Pérez-Herrera, H. E. Villaseñor-Mir, A. Ramírez-Ramirez y M. de L. de la Cruz-González. 2016. Alondra F2014, nueva variedad de trigo harinero para el Bajío, México. Revista Mexicana de Ciencia Agrícolas 5: 1225-1229.

William, M., Singh, R. P., Huerta-Espino, J., Ortiz-Islas, S., & Hoisington, D. 2003. Molecular marker mapping of leaf rust resistance gene *Lr46* and its association with stripe rust resistance gene *Yr29* in wheat. Phytopathology 93: 153-159.

CAPITULO I. RESISTENCIA PARCIAL Y ESPECÍFICA A ROYA DEL TALLO EN LA LINEA AVANZADA DE TRIGO HARINERO 'KIJIL'

Maricarmen Sandoval-Sánchez[1], Julio Huerta-Espino[2], Ravi P. Singh[3], Mandeep Singh-Randhawa[4], Reyna I. Rojas-Martínez[1*], Ignacio Benitez-Riquelme[5], Cristian Nava-Díaz[1]

[1]Fitosanidad-Fitopatología, [5]Recursos Genéticos y Productividad, Campus Montecillo, Colegio de Postgraduados, Km. 36.5, Carr. México-Texcoco. 56230, Montecillo, Texcoco, Edo. de México. [2]Campo Experimental Valle de México, Instituto de Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Km. 13.5, Carr. Los Reyes-Texcoco. 56250, Coatlinchán, Edo. de México. [3]Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), Km. 45, Carr. México-Veracruz, El Batán, Texcoco, Estado de México. 56237. [4]CIMMYT, ICRAF House, United Nations Avenue, Gigiri, Village Market-00621, Nairobi, Kenya.

* *Autor de correspondencia* (rojas@colpos.mx).

ARTÍCULO ENVIADO A LA REVISTA FITOTECNIA MEXICANA

1.1 RESUMEN

El hongo *Puccina graminis* f. sp. *tritici*, agente causal de la roya del tallo del trigo, puede causar pérdidas del rendimiento de más del 40 % en variedades susceptibles, así como mutar y vencer genes de resistencia, por lo que es necesario identificar nuevas fuentes de resistencia para combatir las razas virulentas del patógeno. La línea avanzada de trigo harinero 'Kijil' ha mostrado niveles altos de resistencia a roya del tallo en plántula y planta adulta, pero se desconoce el número de genes que confieren tal resistencia. La genética de su resistencia se investigó en una población F_6 de 99 familias derivadas de la cruza Apav #1 × Kijil. Los ensayos en invernadero y en campo se llevaron a cabo en El Batán, Texcoco y Ciudad Obregón, Sonora, respectivamente, durante 2016 y 2017. El análisis genético indicó que existe la presencia de cuatro genes de raza específica que confieren resistencia en plántula. Un primer gen se identificó como *Sr38* y los demás se designaron temporalmente como *SrKj;1*, *SrKj22+* y *SrKjX-*, de acuerdo con los tipos de infección registrados. Para corroborar el efecto en planta adulta de los genes de raza específica, se observó que las familias positivas a *Sr38*, mostraron niveles de infección de 0-20 %, mientras que las familias portadoras de los genes *SrKj;1*, *SrKj22+* y *SrKjX-* presentaron rangos de 1-30 %, 30-60 % y 1-30 %, respectivamente. Por otra parte, se determinó que existen tres genes menores que confieren resistencia en planta adulta, entre ellos *Sr2* y *Sr58*. Las familias positivas a *Sr58* mostraron severidades de 30-90 % y sólo una presentó 5 %; aquellas positivas a *Sr2+Sr58* presentaron 60-100 %. De acuerdo con los resultados obtenidos, 'Kijil' puede ser una fuente potencial de resistencia a roya del tallo por conferir altos niveles de resistencia en plántula y planta adulta.

Palabras clave: *Triticum aestivum*, *Puccina graminis* f. sp. *tritici*, resistencia de raza específica, resistencia de raza no específica.

1.2 SUMMARY

The fungus *Puccina graminis* f. sp. *tritici*, causal agent of stem rust of wheat, can cause yield losses of more than 40 % in susceptible varieties, as well as mutate and overcome resistance genes, so it is necessary to identify new sources of resistance to fight the virulent races of the pathogen. Bread wheat advanced line Kijil has shown high levels of resistance to stem rust in seedling and adult plant stages, but the number of genes that confer such resistance is unknown.

Genetics of its resistance was investigated in a F_6 population of 99 families derived from a cross between Apav #1 and Kijil. Greenhouse and field trials were carried out in El Batán, Texcoco and Ciudad Obregón, Sonora, respectively, during 2016 and 2017. Genetic analysis indicated that there are four race-specific genes that confer resistance in seedling stage. A first gene was identified as *Sr38* and the others were designated temporarily as *SrKj;1*, *SrKj22+* and *SrKjX-*, according to the infection types recorded. In order to corroborate the effect of race-specific genes in adult plant, it was observed that families positive to *Sr38*, showed levels of infection of 0-20 %, while families carrying *SrKj;1*, *SrKj22+* and *SrKjX-* genes presented ranges of 1-30 %, 30-60 % and 1-30 %, respectively. On the other hand, it was determined that there are three minor genes that confer resistance in adult plant stage, among them *Sr2* and *Sr58*. Families positive to *Sr58* displayed severities of 30-90 % and only one presented 5 %; those positive for *Sr2+Sr58* presented 60-100 %. According to the obtained results, 'Kijil' can be a potential source of resistance to stem rust by conferring high levels of resistance in seedling and adult plant stages.

Index words: *Triticum aestivum*, *Puccina graminis* f. sp. *tritici*, race-specific resistance, race non-specific resistance.

1.3 INTRODUCCIÓN

La roya del tallo causada por el hongo *Puccina graminis* f. sp. *tritici* Erikss. & Hennin, es una enfermedad importante del trigo harinero (*Triticum aestivum* L.) que representa una amenaza significativa en la mayoría de las regiones productoras alrededor del mundo (Basnet *et al*., 2013). En variedades susceptibles, la enfermedad causa pérdidas del rendimiento de más del 40 % en países como India, Pakistán, Bangladesh, China, Kenia, Etiopía y Brasil (Dubin y Brennan, 2009). Sin embargo, la roya del tallo se ha controlado con éxito durante más de tres décadas en todo el mundo, gracias a los esfuerzos y contribuciones que han realizado diversos profesionales provenientes de distintos países, a través de la incorporación de resistencia al germoplasma de trigo por más de 120 generaciones de mejoramiento genético y la erradicación del hospedante alterno que utiliza este hongo para completar su ciclo (Huerta-Espino *et al*., 2011; Singh *et al*., 2011a).

En México, la incidencia de roya del tallo ha disminuido considerablemente y sólo se encuentra en niveles significativamente bajos desde que se liberó la variedad Yaqui 50 y otras variedades portadoras del gen *Sr2*, entre otros. Además, no se han observado cambios en las razas de roya del tallo en México en los últimos 40 años y las infecciones naturales son prácticamente inexistentes (Singh *et al*., 2006; Huerta-Espino *et al*., 2011).

Se han reportado más de 70 genes de resistencia para roya del tallo (McIntosh *et al*. 2017). La mayoría de éstos, conocidos como genes dominantes únicos con efectos mayores, confieren resistencia expresada desde el estado de plántula y durante todas las etapas de crecimiento, la cual es heredada como un carácter cualitativo y es específica a las diferentes razas del patógeno. Este tipo de resistencia es poco durable, ya que el patógeno puede evolucionar hacia virulencia y vencer estos genes de resistencia que generalmente se usan individualmente. Por otra parte, existen genes que confieren resistencia en planta adulta y son tanto de raza específica como de raza no específica (Johnson, 1981); es decir, son genes cuyos efectos individuales son menores, pero cuando se conjuntan tres o más suman sus efectos y se heredan como si fuera un carácter cuantitativo.

La resistencia parcial o de infección lenta (Niederhauser *et al*., 1954), también se le conoce como resistencia de raza no específica, y generalmente es asociada con la resistencia durable y referida comúnmente como resistencia de planta adulta porque se expresa en plantas adultas como un desarrollo lento de la enfermedad en el campo, comparativamente con un testigo susceptible, a pesar de presentar un tipo de infección compatible (Herrera-Foessel *et al*., 2014). La acumulación de genes que confieren resistencia parcial explota los efectos aditivos (acción génica aditiva) de este tipo de genes. Diversos estudios han revelado que las combinaciones de tres a cinco genes de resistencia resultan en niveles de infección "cercana a la inmunidad" y en resistencia estable a través de diferentes ambientes (Singh *et al*. 2000; Singh *et al*., 2011b). Así mismo, se pueden lograr niveles altos de resistencia a royas cuando un gen de raza específica efectivo se combina con genes de planta adulta (Basnet *et al*., 2015).

Se han catalogado y designado pocos genes de este tipo, debido a sus efectos individuales menores. Los conocidos como *Sr2/Lr27*/*Yr30/Pbc1*, *Sr57*/*Lr34/Yr18/Pm38/Stb1/Ltn1*,

Sr58/*Lr46/Yr29/ Pm39/Ltn2* y *Sr55/Lr67/Yr46/Pm46*, muestran un efecto pleiotrópico, es decir, confieren resistencia a múltiples enfermedades como roya del tallo, roya de la hoja (*Puccinia triticina* Erikss.), roya amarilla (*P. striiformis*. f. sp. *tritici* Westend) y cenicilla polvorienta (*Blumeria graminis* (DC) Speer f. sp. *tritici* emend. É. J. Marchal*)* (Herrera-Foessel *et al*., 2014).

Por otra parte, se han transferido al trigo harinero diversos genes de resistencia a roya del tallo provenientes de ancestros silvestres, tales como *Sr21, Sr22 y Sr35* a partir de *Triticum monococcum* (The, 1973; Kerber and Dyck, 1973; McIntosh *et al*., 1984); *Sr24, Sr25*, *Sr26* y *Sr43* a partir de *Thinopyrum ponticum* (McIntosh *et al*., 1976; Niu *et al*., 2014) ó *Sr38* proveniente de *T. ventricosum* (Bariana and McIntosh, 1993).

El control genético de enfermedades es el método más seguro y amigable ambientalmente para reducir las pérdidas de rendimiento comparado con el control químico. Debido a que las razas virulentas de la roya del tallo continúan representando una amenaza seria en la producción mundial de trigo; es necesario y con propósitos de prevención, identificar y caracterizar, a nivel molecular, nuevos genes de resistencia. En este sentido, la línea avanzada de trigo harinero 'Kijil' (KLEIN DON ENRIQUE*2/3/FRET2/WBLL1//TACUPETO F2001), desarrollada por el programa de trigos harineros del Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), ha mostrado niveles altos de resistencia a roya del tallo en plántula y planta adulta. El objetivo de esta investigación es determinar el número de genes de plántula y planta adulta que están confiriendo la resistencia a roya del tallo en la población F_6 Apav #1 × Kijil.

1.4 MATERIALES Y MÉTODOS

Material vegetal

Se utilizó una población F_6 de 99 familias derivada de la cruza Apav #1 × Kijil. 'Apav #1' es una línea altamente susceptible a la roya del tallo, derivada de la cruza Avocet-*YrA* × Pavon 76. 'Kijil' proviene de la cruza KLEIN DON ENRIQUE*2/3/FRET2/WBLL1//TACUPETO F2001 y muestra niveles altos de resistencia a la raza RTR de roya del tallo en plántula en el invernadero y en planta adulta en campo. La población se desarrolló por el método masal con selección que implica seleccionar al azar un número grande de plantas, las cuales se trillan en

masa (todas juntas) y se toma una muestra para sembrar la siguiente generación. Después, en F_4 se generaron líneas puras mediante la selección de una espiga. La progenie de ésta se cosechó en masa para dar origen a la generación F_6.

Evaluación en plántula

Las 99 familias Apav #1 × Kijil y los progenitores, se evaluaron en plántula bajo condiciones de temperatura controladas en el invernadero de royas del Centro Internacional de Mejoramiento de Maíz y Trigo durante el mes de junio de 2017. Se sembraron 8 semillas por familia en charolas de plástico, así como un juego con 20 líneas diferenciales del Laboratorio de enfermedades de cereales (CDL) de Minnesota (Jin *et al*., 2007). Las plántulas se inocularon 10 días después de la siembra, cuando las plántulas desarrollaron la segunda hoja, a través de aspersiones de urediniosporas de la raza RTR suspendidas en aceite mineral Soltrol 170®. La fórmula de avirulencia/virulencia de RTR es: *Sr7a*, *9e*, *10*, *12*, *13*, *14*, *22*, *23*, *24*, *25*, *26*, *27*, *29*, *30*, *31*, *32*, *33*, *35*, *Dp2*, *H*, *Gt*, *Wld*, *W3560*, *AgI / Sr5*, *6*, *7b*, *8a*, *8b*, *9a*, *9b*, *9d*, *9f*, *9g*, *11*, *15*, *17*, *21*, *28*, *34*, 36, *Pl* (Singh, 1991). Las plántulas se colocaron dentro de una cámara de rocío durante 9 h y 3 h de luz a 20 °C y después de 16 horas se trasladaron al invernadero. Los tipos de infección en las familias y los progenitores, se registraron 14 días después de la inoculación utilizando la escala de 0-4 de Roelfs *et al*. (1992), donde 0, ;, 1, 2 y X se consideran plantas resistentes, mientras que 3 y 4 se consideran plantas susceptibles. Los signos + y – describen si el tamaño de la uredinia es más grande o más pequeña, respectivamente, comparado con el tamaño normal.

Evaluación en campo

Las pruebas de campo se llevaron a cabo en dos ciclos de evaluación en 2015-2016 y 2016-2017 en la Estación Experimental Norman E. Borlaug (CENEB) del CIMMYT en Ciudad Obregón, Sonora. La epidemia artificial de roya del tallo se inició con urediniosporas de la raza RTR suspendidas en aceite mineral Soltrol 170®. La severidad de la enfermedad en los progenitores y en las familias se registró dos y tres veces en el primer y segundo ciclo de evaluación, respectivamente, utilizando la escala modificada de Cobb (Peterson *et al*., 1948). La primera nota se registró cuando el progenitor susceptible Apav #1 mostró 90 % de severidad; posteriormente, las notas se registraron semanalmente hasta que la severidad

alcanzó 100 %. La respuesta del tipo de infección del hospedante se determinó de acuerdo con la escala de Roelfs *et al*. (1992), donde R=resistente o uredinias diminutas rodeadas por tejido necrótico; MR= moderadamente resistente o uredinias pequeñas a moderadas rodeadas por tejido necrótico o clorótico; MS= moderadamente susceptible o uredinias de tamaño moderado sin tejido necrótico o clorótico; y S= susceptible o uredinias grandes sin tejido necrótico o clorótico.

Análisis molecular

El ADN de los progenitores y las familias se extrajo de tejidos foliares de aproximadamente 20 plantas por familia, utilizando el método del bromuro de cetiltrimetilamonio (CTAB) (Dreisigacker et al., 2016). Los progenitores se analizaron previamente con seis marcadores moleculares asociados con genes de resistencia de planta adulta: marcador de repeticiones de secuencia simple (SSR) *Xgwm533* ligado a *Sr2*, marcador de secuencia polimórfica amplificada y cortada (CAPS) *csLV46G22* para *Sr58*; marcador de secuencia de sitio marcado (STS) *csLV34* y PCR competitiva alelo-específica (KASP) (*Lr34SNP*) ligados a *Sr57*; *Lr67SNPTM4* estrechamente relacionado con *Sr55*, y el marcador CAPS *VENTRIUP-LN2* ligado al gen de raza específica *Sr38*. Los marcadores *csLV46G22*, *Xgwm533* y *VENTRIUP-LN2* fueron polimórficos entre los progenitores y se usaron para analizar toda la población utilizando los protocolos de PCR de Helguera *et al*., (2003) y Dreisigacker *et al*., (2016).

Análisis genético

El número de genes en plántula y planta adulta se estimó a través del análisis de segregación Mendeliana (Singh y Rajaram, 1992), en donde las familias se clasificaron en tres categorías fenotípicas: resistentes homocigotas de tipo parental (RHTP), susceptibles homocigotas de tipo parental (SHTP) y OTRAS (familias heterocigotas segregantes con respuestas diferentes a aquellas de los progenitores). Las frecuencias observadas para cada categoría fueron probadas contra las frecuencias esperadas para diferente número de genes utilizando la prueba de X^2.

1.5 RESULTADOS

Evaluación fenotípica en plántula y análisis molecular

El progenitor susceptible 'Apav #1' mostró el tipo de infección 3+, mientras que el progenitor resistente 'Kijil' mostró el tipo de infección ";". Al clasificar las familias en resistentes y susceptibles, se determinó que la resistencia en 'Kijil' está condicionada por cuatro genes de raza específica (Cuadro 1.1). Un primer gen se identificó como *Sr38*, ya que, con base en el análisis molecular, 'Kijil' resultó positivo para el marcador *VENTRIUP-LN2*, asociado con el segmento del cromosoma 2NS/2AS que contiene los genes de resistencia *Sr38*/*Yr17/Lr37*. Para observar la segregación del gen *Sr38*, las 99 familias se analizaron con los marcadores correspondientes. Un total de 37 familias resultaron positivas a los marcadores, de éstas, 32 se agruparon como resistentes y 5 como susceptibles (Cuadro 1.1). Las familias resistentes expresaron los tipos de infección ;, 0;, ;1-, ;1, 2, 22+ y X.

Cuadro 1.1. Número de genes de resistencia de plántula a roya del tallo en la población F_6 Apav #1 × Kijil de 99 familias, estimado a través del análisis de segregación Mendeliana.

	Número de familias			X^2	No. de genes
	Total	**Resistentes**	**Susceptibles**		
Familias F_6	99	76	23	0.01	2
Positivas a *Sr38*	37	32	5*	-	-
Negativas a *Sr38*	62	44	18	0.05	2

*Falsos positivos

Por otra parte, un total de 62 familias resultaron negativas a los marcadores ligados a *Sr38*; de éstas, 44 se agruparon como resistentes y 18 como susceptibles. Las resistentes mostraron los tipos de infección ;1, ;1-, ;, 2, 22+ y X-, y las susceptibles 3+ y 33+. De acuerdo con el análisis de segregación Mendeliana de esas 62 familias negativas a *Sr38*, se observa que existen dos genes que confieren resistencia a roya del tallo en plántula (Cuadro 1.1).

Al reclasificar las 44 familias resistentes y negativas a *Sr38*, se observó que existen familias que agrupan tres tipos de infección consistentes: 25 familias registraron ;1, 12 familias presentaron 22+ y 7 familias X-. De acuerdo con los tipos de infección, los genes se designaron

temporalmente como *SrKj;1*, *SrKj22+* y *SrKjX-*, los cuales corresponden al segundo, tercer y cuarto gen que confieren resistencia a roya del tallo en plántula, de acuerdo con el análisis de segregación Mendeliana. El análisis molecular no identifica a dichos genes.

Efecto de los genes *Sr38*, *SrKj;1*, *SrKj22+* y *SrKjX-* en planta adulta

Para corroborar el efecto en planta adulta del gen *Sr38* en las 32 familias agrupadas como resistentes en plántula, se observó que 22 mostraron un nivel de infección en planta adulta de 0-1 % (R), muy similar a 'Kijil' (1 % R), mientras que las 10 restantes presentaron severidades en un rango de 5-20 % (R-MR). Las familias portadoras de los genes *SrKj;1*, *SrKj22+* y *SrKjX-* presentaron rangos de severidad de 1-30 % (R-MR), 30-60 % (MR) y 1-30 % (R-MR), respectivamente.

Evaluación fenotípica en planta adulta y análisis molecular

De las 99 familias, se identificaron 23 que resultaron susceptibles en plántula, las cuales se utilizaron para realizar el análisis de segregación Mendeliana. En el ciclo 2016-2017, la severidad final de roya del tallo en 'Kijil' fue 1 % (R) y 100 % (S) para 'Apav # 1'. El análisis de segregación Mendeliana indicó que la resistencia a roya del tallo en la población Apav #1 × Kijil, está conferida por tres genes de resistencia de planta adulta (Cuadro 1.2). De las 23 familias, 21 fueron positivas al marcador ligado a *Sr58* y mostraron severidades de 30-90 % (MS-S); una de esas familias presentó 5 % (MS) de infección. Sólo 10 familias, resultaron positivas a los marcadores de *Sr2+Sr58* y el rango de severidad fue 60-100 % (MS-S). Las dos familias restantes fueron negativas a ambos marcadores y presentaron severidades de 90 y 100 % (S).

Cuadro 1.2. Número de genes de resistencia de planta adulta (APR) a roya del tallo en la población F_6 Apav#1 × Kijil, estimado a través del análisis de segregación Mendeliana, durante el ciclo 2016-2017 en Ciudad Obregón, Sonora.

Número de familias			X^2	No. de genes
Total	**Resistentes**	**Susceptibles**		
23	20	3	0.26	3

La distribución de frecuencias de las familias para severidad de roya del tallo se observó de tipo discreta con tendencia a la resistencia, lo cual puede explicarse por la presencia de los genes de raza específica que confieren un nivel alto de protección en la etapa adulta de la planta (Figura 1.1).

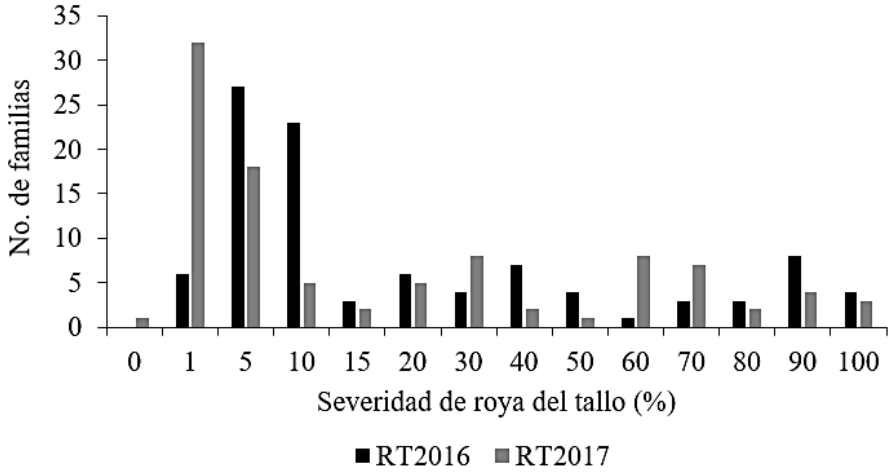


Figura 1.1. Distribuciones de frecuencias de la severidad de roya del tallo en la población F_6 Apav #1 × Kijil de 99 familias en ensayos de campo en Ciudad Obregón, Sonora, durante los ciclos 2015-2016 (RT2016) y 2016-2017 (RT2017).

En el Cuadro 1.3, se puede observar el número de familias positivas y negativas a los marcadores ligados a *Sr2, Sr38* y *Sr58*. No fue posible cuantificar el efecto individual de cada uno de esos genes en planta adulta, ya que las familias portadoras de esos genes también portan otros genes de raza específica.

Cuadro 1.3. Familias Apav #1 × Kijil positivas y negativas a los marcadores asociados con los genes *Sr2, Sr38* y *Sr58* y las combinaciones de *SrKj22+; SrKj;1 y SrKjX-*.

	Marcador	No. de familias
	Sr38	15
	Sr58	8
	Sr2+Sr38	7
	Sr38+Sr58	8
	Sr2+Sr58	9
	Sr2+Sr38+Sr58	7
SrKj22+	*Sr38+Sr58*	1
*SrKj*22+	*Sr2*	1
*SrKj*22+	*Sr58*	2
*SrKj*22+	*Sr2+Sr58*	3
*SrKj*22+	Ninguno	5
SrKj;1	*Sr2*	8
SrKj;1	*Sr58*	4
SrKj;1	*Sr2+Sr58*	7
SrKj;1	Ninguno	6
*SrKj*X-	*Sr2*	1
*SrKj*X-	*Sr58*	1
*SrKj*X-	*Sr2+Sr58*	5
	Ninguno	1

1.6 DISCUSIÓN

El análisis de segregación Mendeliana indicó que la resistencia a la roya del tallo en la población Apav #1 × Kijil en plántula está condicionada por cuatro genes de raza específica, denominados *Sr38, SrKj;1, SrKj22+* y *SrKjX-*. El segmento del cromosoma 2NS/2AS que contiene los genes de resistencia *Sr38/Yr17/Lr37*, fue incorporado al trigo harinero 'VPM1' a partir de *T. ventricosum* (Tausch) Cess. Estos genes de raza específica, confieren resistencia a roya del tallo (*Sr38*), roya amarilla (*Yr17*) y roya de la hoja (*Lr37*) (Maia, 1967; Bariana y McIntosh, 1993; Helguera *et al*., 2003), por lo que ha sido una fuente de resistencia importante

en los programas de mejoramiento; sin embargo, se ha detectado virulencia a *Sr38*, y junto con la virulencia a *Sr24*, *Sr31* y *Sr36*, se han considerado como las más significativas, ya que estos genes han sido incorporados en una amplia gama de variedades de trigo alrededor del mundo, proporcionado resistencia a diferentes razas de *P. graminis* f. sp. *tritici* (Pretorius *et al*., 2000; Jin *et al*., 2008, 2009; Singh *et al*., 2011a).

En el presente estudio, la evaluación fenotípica en plántula indicó que las familias resistentes y positivas a *Sr38*, expresaron los tipos de infección ;, 0;, ;1-, ;1, 2, 22+ y X, mientras que aquellas susceptibles y positivas al marcador, mostraron los tipos de infección 3+ y 33+ C, lo que indica falsos positivos para el marcador. De acuerdo con algunos autores, los tipos de infección que expresa *Sr38* son: X con pústulas más grandes hacia la base de la hoja (McIntosh *et al*.,1995) ;23 (Jin *et al*., 2007), 0; a ;1 para la línea diferencial 'Trident' y 0;, ;, ;1, ;13-, ;13, ;3, ó 3; para las líneas evaluadas putativamente portando *Sr38* (Zhang *et al*., 2014) ó 0; a ;13-C (Turner *et al*., 2016).

Singh *et al*., (2008) postularon *Sr38* en los cultivares 'Andante', 'Prophet' y 'Torfrida' causando los tipos de infección X+ y 3+. También se postuló en combinación con *Sr31* en los cultivares 'Beaufort', 'Lynx' y 'Turpin' produciendo los tipos de infección ;, 0;, 1;-, ;1+, 1- y 12-. En el cultivar 'Chianti' se postularon los genes *Sr5+Sr38* y se registraron 0;, X, y X+.

Zhang *et al*. (2014) reportan que *Sr38* fue el gen de resistencia a roya del tallo más frecuente en los trigos duros y harineros que evaluaron, así mismo mencionan que este gen pudo haber sido pasado por alto en otros estudios debido a que el fenotipo que expresa a menudo es confuso. El marcador *VENTRIUP-LN2* es un marcador de diagnóstico que favorece la detección del segmento del cromosoma 2NS/2AS. Los resultados obtenidos en el presente estudio coinciden dentro del rango de los tipos de infección reportados y se corrobora la efectividad de los marcadores de diagnóstico para *Sr38*.

El gen *Sr38* ha sido postulado individualmente en los cultivares europeos 'Abbot', 'Arche', 'Rapor', 'Reaper', 'Renan' y 'Terza', los cuales al ser inoculados con diversos patotipos de *P. graminis* f. sp. *tritici*, mostraron diferentes tipos de infección, los más comunes fueron: 0;, X=CN, ;CN, X=N, X–CN; y 3+ (Pathan y Park, 2007). Esos mismos cultivares, al ser evaluados

en planta adulta, presentaron coeficientes de infección en un rango de 20-70 %. De acuerdo con los autores, la variación en el nivel de protección conferida por *Sr38* en diferentes cultivares, se puede deber a los efectos del historial genético o a la presencia o ausencia de un gen de planta adulta en esos cultivares.

El gen *Sr38* no provee protección contra el grupo de razas TTTTF o TTKSK, pero es efectivo contra otras razas presentes en Norteamérica, especialmente en la etapa de planta adulta. Zhang *et al*. (2014) reportan que este gen fue el más efectivo en estudios de campo al presentar un promedio de severidad de 5.2 % en las líneas que portaban sólo este gen, lo cual representó menos de la mitad del valor del segundo gen más efectivo, que fue *Sr31* y mostró severidad de 13 %.

Por otra parte, Pathan y Park (2007) postularon la combinación de *Sr31+Sr38* en los cultivares 'Beaufort', 'Caxton', 'Equinox' y 'Hussar', registrando los tipos de infección 0;, ;CN, ;N y ;12=. El tipo de infección 0; posiblemente indica la presencia de uno o más genes adicionales o es el resultado de la interacción entre esos dos genes; sin embargo, se atribuye a *Sr38* ese tipo de infección en presencia de *Sr31*, ya que de manera individual, fue más bajo (;CN ó X=N) que el de *Sr31* (2–2=). En planta adulta, los cuatro cultivares proporcionaron un nivel alto de protección al mostrar coeficientes de infección de 20-40 %, más bajos que aquellos donde el cultivar portaba esos genes de manera individual, sugiriendo un efecto por interacción entre éstos o la presencia de fuentes resistencia adicionales.

En el presente estudio, las líneas resistentes en plántula y positivas a *Sr38*, presentaron un rango de severidad de 0-20 % (R-MR), mientras que las familias portadoras de los genes *SrKj;1*, *SrKj22+* y *SrKjX-* mostraron rangos de severidad de 1-30 % (R-MR), 30-60 % (MR) y 1-30 % (R-MR), respectivamente. A excepción de *SrKj22+*, los otros genes proveen de un alto nivel de resistencia en planta adulta en la población Apav #1 × Kijil.

Por otra parte, el análisis de segregación Mendeliana en planta adulta indicó que la resistencia a roya del tallo en 'Kijil' está conferida por tres genes de resistencia de planta adulta, de los cuales se detectó la presencia de *Sr2* y *Sr58*. Las familias positivas a *Sr58* mostraron

severidades de 30-90 % (MS-S) y sólo una presentó 5 % (MS) de infección; aquellas positivas a *Sr2+Sr58* presentaron un rango de severidad de 60-100 % (MS-S). Los resultados indican que los genes *Sr2* y *Sr58* confieren poca resistencia en las familias Apav #1 × Kijil que los portan y su efecto podría verse enmascarado por la presencia de algún otro gen.

La disminución de la incidencia de roya del tallo a niveles insignificantes en México y en la mayor parte del mundo, repercutió en la disminución de la investigación para buscar fuentes de resistencia contra esta enfermedad. A través de reducidos programas de mejoramiento, se ha seleccionado germoplasma para resistencia a roya del tallo en México desarrollando epidemias artificiales con la raza RTR de *P. graminis* f. sp. *tritici*. De acuerdo con los resultados derivados de la presente investigación, Kijil puede ser una fuente potencial de resistencia a roya del tallo para ser usada en programas de mejoramiento, ya que confiere altos niveles de resistencia en plántula y planta adulta.

1.7 CONCLUSIONES

La resistencia en plántula y planta adulta en la población Apav #1 × Kijil está conferida por cuatro genes de raza específica denominados *Sr38*, *SrKj;1*, *SrKj22+* y *SrKjX-*, mientras que la resistencia en planta adulta está conferida por tres genes de raza no específica, entre ellos los genes *Sr2* y *Sr58*.

1.8 AGRADECIMIENTOS

Los autores agradecen al CIMMYT, Colegio de Postgraduados y CONACYT por el financiamiento y las facilidades concedidas para la realización de este trabajo de investigación.

1.9 BIBLIOGRAFÍA

Bariana H. S. and R.A. McIntosh (1993) Cytogenetic studies in wheat. XV. Location of rust resistance genes in VPM1 and their genetic linkage with other disease resistance genes in chromosome 2A. *Genome* 36: 476-482, https://doi.org/10.1139/g93-065

Basnet B. R., R. P. Singh, S. A. Herrera F., A. M. H. Ibrahim, J. Huerta E., V. Calvo S. and J. C. Rudd (2013) Genetic analysis of adult plant resistance to yellow rust and leaf rust in

common spring wheat Quaiu 3. *Plant Disease* 97: 728-736, https://doi.org/10.1094/PDIS-02-12-0141-RE

Basnet B. R., S. Singh, E. E. Lopez V., J. Huerta E., S. Bhavani, Y. Jin,…R. P. Singh (2015) Molecular mapping and validation of *SrND643*: A new wheat gene for resistance to the stem rust pathogen Ug99 race group. *Phytopathology* 105: 470-476, https://doi.org/10.1094/PHYTO-01-14-0016-R

Dreisigacker, S., D. Sehgal, A. E. Reyes J., B. Luna G., S. Muñoz Z., C. Núñez R.,…S. Mall (Eds.). (2016) CIMMYT wheat molecular genetics: laboratory protocols and applications to wheat breeding. México, D.F.: CIMMYT.

Dubin H. J. and J. P. Brennan (2009) Fighting a "Shifty Enemy": The international collaboration to contain wheat rusts. *In*: Millions Fed: Proven successes in agricultural development. Spielman, D. J. and R. Pandya-Lorch (eds). Washington, D.C. IFPRI. pp: 19-24, http://ebrary.ifpri.org/cdm/ref/collection/p15738coll2/id/130812

Helguera M., I. A. Khan, J. Kolmer, D. Lijavetzky, L. Zhong-qi and J. Dubcovsky (2003) PCR assays for the *Lr37-Yr17-Sr38* cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. *Crop Science* 43: 1839-1847, https://escholarship.org/uc/item/2dk0791m

Herrera F. S. A., R. P. Singh, M. Lillemo, J. Huerta E., S. Bhavani, S. Sukhwinder,…E. S. Lagudah (2014) *Lr67/Yr46* confers adult plant resistance to stem rust and powdery mildew in wheat. *Theoretical and Applied Genetics* 127: 781-789, https://doi.org/10.1007/s00122-013-2256-9

Huerta E. J., H. E. Villaseñor M., E. Espitia R., E. Solís M. and M. Van Ginkel (2011) The history of wheat breeding in México. *In*: The Wheat Book V2, History of Wheat Breeding. Alain P. Bonjean, William J. Angus, Maarten van Ginkel (eds.). Londres, Paris, New York. pp:275-308, https://doi.org 10.1016/j.jcs.2011.10.001.

Jin Y., R. P. Singh, R. W. Ward, R. Wanyera, M. Kinyua, P. Njau,…A. Yahyaoui (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 Disease* 91: 1096-1099, https://doi.org/10.1094/PDIS-91-9-1096

Jin Y., L. J. Szabo, Z. A. Pretorius, R. P. Singh, R. Ward and T. Fetch Jr. (2008) Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici. Plant Disease* 92: 923-926, https://doi.org/10.1094/PDIS-92-6-0923

Jin Y., L. J. Szabo, M. N. Rouse, T. Fetch Jr, Z. A. Pretorius, R. Wanyera and P. Njau (2009) Detection of virulence to resistance gene *Sr36* within the TTKS race lineage of *Puccinia graminis* f. sp. *tritici*. *Plant Disease* 93: 367-370, https://doi.org/10.1094/PDIS-93-4-0367

Johnson, R. (1981) Durable resistance: definition, genetic control and attainment in plant breeding. *Phytopathology* 71: 567-568, https://doi.org/10.1094/Phyto-71-567

Kerber E. R. and P. L. Dyck (1973) Inheritance of stem rust resistance transferred from diploid wheat (*Triticum monococcum*) to tetraploid and hexaploid wheat and chromosome location of the gene involved. *Canadian Journal of Genetics and Cytology* 15: 397-409, https://doi.org/10.1139/g73-050

Maia N. (1967) Obtention de blés tendres résistants au piétin-verse (*Cercosporella herpotrichoïdes*) par croisements interspécifiques. *Comptes rendus de l'Académie d'Agriculture de France* 53: 149-154.

McIntosh R. A., P. L. Dyck and G. J. Green (1976) Inheritance of leaf rust and stem rust resistances in wheat cultivars Agent and Agatha. *Australian Journal of Agricultural Research* 28: 37-45, https://doi.org/10.1071/AR9770037

McIntosh R. A., P. L. Dyck, T. T. The, J. Cusick, D. L. Milne (1984) Cytogenetical studies in wheat XIII. *Sr35*, a third gene from *Triticum monococcum* for resistance to *Puccinia graminis tritici. Zeitschrift für Pflazenzüchtung* 92:1-14.

McIntosh R. A., C. R. Wellings and R. F. Park (1995) Wheat rusts: An atlas of resistance genes. CSIRO Publications, East Melbourne, Australia. pp: 138-139, https://doi.org/10.1007/978-94-011-0083-0

McIntosh R. A., J. Dubcovsky, W. J. Rogers, C. Morris and X. C. Xia (2017) Catalogue of gene symbols for wheat: 2017 supplement.

Niederhauser J. S., Cervantes J. and L. Servin (1954) Late blight in Mexico and its implications. *Phytopathology* 44: 406-408.

Niu Z., D. L. Klindworth, G. Yu, L. Friesen, S. Chao, Y. Jin, X. Cai, J. B. Ohm, J. B. Rasmussen and S. S. Xu (2014) Development and characterization of wheat lines carrying stem rust

resistance gene *Sr43* derived from *Thinopyrum ponticum*. *Theorical and Applied Genetics* 127: 969-980, https://doi.org/10.1007/s00122-014-2272-4.

Pathan A. K. and R. F. Park (2007) Evaluation of seedling and adult plant resistance to stem rust in European wheat cultivars. *Euphytica* 155: 87-105, https://doi.org/10.1007/s10681-006-9308-z

Peterson R. F., A. B. Campbell and A. E. Hannah (1948) A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Canadian Journal of Research* 26: 496-500, https://doi.org/10.1139/cjr48c-033

Pretorius Z. A., R. P. Singh, W. W. Wagoire, and T. S. Payne (2000) Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. *Plant Disease* 84: 203.2, http://dx.doi.org/10.1094/PDIS.2000.84.2.203B

Roelfs A. P., R. P. Singh and E. E. Saari (1992) Rust diseases of wheat: Concepts and methods of disease management. CIMMYT, Mexico, D.F., http://hdl.handle.net/10883/1153

Singh R. P. (1991) Pathogenicity variations of *Puccinia recondita* f. sp. *tritici* and *P. graminis* f. sp. *tritici* in wheat-growing areas of Mexico during 1988 and 1989. *Plant Disease* 75:790-794, http://hdl.handle.net/10883/2280

Singh R. P. and S. Rajaram (1992) Genetics of adult plant resistance to leaf rust in 'Frontana' and three CIMMYT wheats. *Genome* 35: 24-31, https://doi.org/10.1139/g92-004

Singh R. P., J. Huerta E. and S. Rajaram (2000) Achieving near-immunity to leaf and stripe rusts in wheat by combining slow rusting resistance genes. *Acta Phytopathologica et Entomologica Hungarica*. 35: 133-139, https://www.researchgate.net

Singh R. P., D. P. Hodson, Y. Jin, J. Huerta E., M. G. Kinyua, R. Wanyera,…R.W. Ward (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*. 1: 1-13, https://www.researchgate.net

Singh R. P., D. P. Hodson, J. Huerta E., Y. Jin, P. Njau, R. Wanyera…R. W. Ward (2008) Will stem rust destroy the world's wheat crop? *Advances in Agronomy* 98: 271-309, https://doi.org/10.1016/S0065-2113(08)00205-8

Singh R. P., D. P. Hodson, J. Huerta E., Y. Jin, S. Bhavani, P. Njau…V. Govindan (2011a) The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production.

Annual Review of Phytopathology 49:465-481, https://doi.org/10.1146/annurev-phyto-072910-095423

Singh R. P., J. Huerta E., S. Bhavani, S. S. Herrera F., D. Singh, P. K. Singh,…J. Crossa (2011b) Race non-specific resistance to rust diseases in CIMMYT spring wheats. *Euphytica* 179:175-186, https://doi.org/10.1007/s10681-010-0322-9

The T. T. (1973) Chromosome location of genes conditioning stem rust resistance transferred from diploid to hexaploid wheat. *Nature New Biology*: 241: 256, https://doi.org/10.1038/newbio241256a0

Turner M. K., Y. Jin, M. N. Rouse and J. A. Anderson (2016) Stem rust resistance in 'Jagger' winter wheat. *Crop Science* 56: 1-7, https://doi.org/10.2135/cropsci2015.11.0683

Zhang D., R. L. Bowden, J. Yu, B. F. Carver and G. Bai (2014) Association analysis of stem rust resistance in U.S. winter wheat. *PLoS ONE* 9: 1-10, https://doi.org/10.1371/journal.pone.0103747

CHAPTER II. GENETIC AND MOLECULAR DISSECTION OF ADULT PLANT RESISTANCE LOCI TO LEAF RUST AND STRIPE RUST IN BREAD WHEAT (*Triticum aestivum* L.)

Maricarmen Sandoval-Sánchez[1,3], Julio Huerta-Espino[2], Ravi P. Singh[3], Reyna I. Rojas-Martínez[1], Ignacio Benitez-Riquelme[4], Cristian Nava-Díaz[1], Mandeep S. Randhawa[5*].

[1]Colegio de Postgraduados- Fitosanidad, Campus Montecillo, Carretera Mexico-Texcoco Km 36.5, Montecillo, Texcoco 56230, Edo. de México, México

[2]Campo Experimental Valle de México, Instituto de Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). Km. 13.5, Carr. Los Reyes-Texcoco. 56250, Coatlinchán, Estado de México

[3]International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 México D.F., México

[4]Recursos Genéticos y Productividad, Campus Montecillo, Colegio de Postgraduados Km 36.5, Carretera México-Texcoco, Montecillo, Texcoco, Estado de México, 56230

[5]International Maize and Wheat Improvement Center (CIMMYT), ICRAF House, United Nations Avenue, Gigiri, Village Market-00621, Nairobi, Kenya

*Corresponding author: Mandeep S. Randhawa, email: M.Randhawa@cgiar.org

2.1 ABSTRACT

Bread wheat (*Triticum aestivum* L.) line 'Kijil' developed at CIMMYT showed adequate levels of adult plant resistance to both leaf rust (LR) and stripe rust (YR) in Mexican testing environments. The genetic basis of this resistance was revealed using 198 recombinant inbred lines (RILs) derived from the cross of resistant parent 'Kijil' with the susceptible parent 'Apav'. The parents and RILs were phenotyped in the field trials conducted at El Batan, Toluca and Ciudad Obregon, Mexico for LR (4 seasons) and YR (6 seasons) during 2016 and 2017. The Apav × Kijil RIL population and parents were genotyped with gene linked markers for identification of lines for presence or absence of *Sr2*/*Yr30* (*gwm533* & *csSr2*), *Lr46*/*Yr29* (*csLV46* & *csLVG22*) and *Lr37*/*Yr17* (*cslVrga* & *VENTRIUP-LN2*). Upon eliminating heterogenous lines, a selected set of 125 Apav × Kijil RILs not carrying positive allele for *Lr37*/*Yr17*-linked markers (*cslVrga* & *VENTRIUP-LN2*) was genotyped using DArT-GBS markers, and their genotypic and phenotypic data was used for QTL analysis. Finally, a genetic linkage map of 6168.0 cM (1824.2, 3883.7 and 460.1 cM for A, B and D genomes, respectively) was constructed using 5,890 polymorphic markers (4,338 PAVs, 1,548 SNPs, *gwm533*, *csSr2*, *csLV46* and *csLV46G22*). Two LR resistance loci, *QLr.cim-1DS* and *QLr.cim-5AS* on chromosomes 1DS and 5AS, respectively, and a YR resistance locus *QYr.cim-3BS* on chromosome 3BS were detected through inclusive composite interval mapping (ICIM). In addition, a co-located resistance locus to both LR and YR on chromosome 1BL (corresponding to *Lr46/Yr29*), and a YR resistance locus on chromosome 3BS (corresponding to *Yr30*) were also identified. These QTLs were derived from Kijil. Parent Apav-derived 3 minor effect QTLs for LR resistance namely; *QLr.cim-1AL*, *QLr.cim-2AL* and *QLr.cim-2BL* on chromosomes 1AL, 2AL and 2BL, respectively, were also identified. Bread wheat line Kijil can serve as a potential source of LR and YR resistance in a breeding program as this resistance can be easily transferred to other wheat germplasm through marker assisted selection (MAS).

Keywords: DArT-GBS, QTLs, Linked markers, Pleiotropic, Wheat (*Triticum aestivum* L.)

2.2 INTRODUCTION

Rust diseases of wheat, namely; stripe rust (YR) caused by *Puccinia striiformis* f. sp. *tritici* and leaf rust (LR) by *P. triticina*, cause significant yield losses if susceptible cultivars are grown under favorable climatic conditions for rust growth. Hanson et al. (1982) reported estimated yield losses of 100% caused by YR and up to 50% by LR on wheat grown in developing countries under epidemic conditions. It was also indicated that YR is the most destructive in an epidemic whereas LR is more significant endemically (Hanson et al. 1982). There are three methods to control rust diseases; cultural, chemical and resistance breeding. Cultural method involves use of different practices like eradication of alternate hosts (Barberry, and Mahunia spp.) and manual cutting of grasses that act as green bridge for growth of pathogen during the time between two wheat seasons. Chemical method involves spraying of fungicides to control rust diseases. Application of fungicides is not an eco-friendly and cost-effective method especially for the small-scale farmers in the developing countries who cannot afford expensive chemicals. Resistance breeding involving identification of new sources of genetic resistance against rust diseases, their characterization and strategic utilization, is considered as the safest and most effective control measure. Thus, breeding for rust resistant wheat varieties is preferred than cultural control and use of chemicals.

Generally, rust resistance has been classified into two types; as race specific resistance and race non-specific resistance. Race specific resistance expresses at the seedling stage and usually effective at all stages of plant growth, is conferred by major genes and specific to the different races/isolates of the pathogen. It is inherited as a qualitative trait and usually effective for short duration as the pathogen can evolve virulence and overcome the resistance genes if used alone (Johnson, 1981). On the contrary, race non-specific resistance, also known as partial resistance, expresses at post-flowering stage of plant growth as a slow development of disease in the field, compared to susceptible control, despite having a compatible type of infection (Herrera-Foessel et al., 2014). This type is also known as adult plant resistance (APR), conferred by minor genes, usually inherited as a quantitative trait and it is durable due to its race non-specific nature. Single minor gene confers a small proportion of resistance, therefore, combination of few minor genes in essential to have complete resistance. Combinations of 3 to 5 APR genes result in

infection levels "close to immunity" and effect is constant across different environments (Singh *et al*., 2000; Singh *et al*., 2011).

Up to now, around 76 and 78 resistance genes for LR and YR, respectively, have been catalogued and formally designated (McIntosh *et al*., 2017). Out of these, *Lr27*/*Yr30*/*Sr2*/*Pbc1* (located on chromosome 3BS), *Lr34*/*Yr18*/*Sr57*/*Pm38* (7DS), *Lr46*/*Yr29*/*Sr58*/*Pm39* (1BL) and *Lr67*/*Yr46*/*Sr55*/*Pm46* (4DL), conferring resistance to multiple diseases i.e. LR, YR, stem rust and powdery mildew (*Blumeria graminis* f. sp. *tritici*), therefore, these are also known as pleiotropic genes (Dyck 1987, 1991; Hare and McIntosh 1979; Singh et al. 1998; William *et al*., 2003; Mago *et al*., 2011a; Herrera-Foessel *et al*., 2014). Recent identification of genes *Lr68* (Herrera-Foessel *et al*., 2012) and *Sr56* (Bansal et al. 2014) was another addition to the above list of adult plant resistance genes, although their effect on other disease remains unknown. In addition, about 80 and 140 quantitative trait loci (QTLs) that provide resistance to LR and YR, respectively, have been reported (Rosewarne *et al*., 2013; Li *et al*., 2014). Emergence of new races of wheat rust pathogen with added virulence to deployed resistance genes poses a serious threat to world wheat production, therefore, identification and molecular characterization of new sources of resistance genes/QTLs are essential. Compared to use of major genes, deployment of sources APR is considered as the most effective control measures for rust diseases.

Bread wheat line ‘Kijil’ (pedigree: Klein Don Enrique*2/3/ Fret2/Wbll1//Tacupeto F2001), developed at Global Wheat Program of the International Maize and Wheat Improvement Center (CIMMYT), showed adequate levels of APR to LR and YR; however, the genetic basis of this resistance was unknown. Therefore, this study was planned to 1) determine the number of genes in Kijil that control LR and YR resistance 2) identify the genomic loci APR genes to LR and YR, and 3) find the pleiotropic/collocated resistance loci to both rusts, using a recombinant inbred line (RIL) population, Apav × Kijil.

2.3 MATERIAL AND METHODS

Development of F_5-derived F_6 RIL population

One hundred and ninety-eight F_5-derived F_6 RILs derived from the cross of susceptible parent 'Apav' with resistant parent 'Kijil' were used for phenotypic and genotypic analysis in this study. Resistant parent 'Kijil', pedigree: Klein Don Enrique*2/3/Fret2/Wbll1//Tacupeto F2001, shows adequate levels of APR to LR and YR, whereas 'Apav' is a RIL derived from cross of Avocet-*YrA* and Pavon 76 and is highly susceptible to both LR and YR.

The Apav × Kijil RIL population was developed using a modified bulk approach from three F_1 plants that were harvested individually. About 500 seeds of each F_2 populations were sown as 10 m long paired rows on top of 80 cm wide raised beds. One spike of each F_2 plant in each population was harvested in a bag and bulk-threshed. The population was advanced to F_5 through planting, harvesting and threshing in the same way as for the F_2. At the F_5 generation, 100 plants were harvested from each population, threshed individually, and labelled as RIL#3 to 200 with Apav and Kijil as parents 1 and 2, respectively. During population advancement, fungicide was applied to ensure representation of all genotypic classes in each generation. The $F_{5:6}$ RILs along with parents Apav and Kijil were multiplied and finally seed of 198 RILs was used for phenotypic evaluations and a selected set of 125 RILs was genotyping with DArT-GBS markers.

Field evaluations

Rust evaluations were conducted at three experimental stations of CIMMYT in Mexico. The field evaluations of two rusts were carried out in years 2016 and 2017 in Mexico; LR evaluations during seasons 2016 (LR16B) and 2017 (LR17B) at El Batan, Estado de Mexico, and during seasons 2015-16 (LR16Y) and 2016-17 (LR17Y) at Yaqui Valley, Ciudad Obregon, Sonora; YR evaluation with one replication in 2016 (YR16T) and two replications YR17T (R1) and YR17T (R2) in 2017 were conducted at Toluca, Estado de Mexico. Each trait was abbreviated with prefix of type of rust (YR or LR) followed by year, then followed by location where B stands for El Batan, T for Toluca and Y for Ciudad Obregon.

About 60-80 seeds (3g) of the RILs along with parents were sown on raised-bed plots consisting of 0.7 m paired rows with a 0.3 m space between rows in Mexico. A mixture of lines Avocet+*Yr24* and Avocet+*Yr26* were used as spreaders for LR, whereas a combination of wheat lines derived from an Avocet × Attila cross, Morocco and an Avocet for genes *Yr17* and *Yr31* were used as YR spreaders. The spreaders were planted around the experimental area and as hill plots on one side of each experimental plot, in the middle of a 0.3 m pathway.

A mix of *Pt* races MBJ/SP and MCJ/SP (1:1 ratio) was used to inoculate the LR spreaders, whereas a mixture of *Pst* races Mex96.11, Mex08.13 and Mex14.191 was sprayed on the YR spreaders. Inoculum was prepared by suspending the mixtures of races described above (separately for each rust) in light mineral oil Soltrol 170 (Phillips *Petroleum* Company, Borger, TX). Updated avirulence/virulence profiles of LR isolates (MBJ/SP and MCJ/SP) and YR isolates (Mex96.11, Mex08.13 and Mex14.191) were reported in Randhawa *et al*. (2018).

Disease severity and response of parents and RILs was recorded at each evaluation site, using the modified Cobb Scale (Peterson *et al*., 1948). The first note was recorded when the susceptible parent Apav displayed 70-80 % severity; subsequently, notes were recorded at weekly interval until severity reached 90-100 %. Final disease severity (FDS) values and their means (YRM and LRM) over seasons for each location were used during statistical and genetic analysis.

Genetic and statistical analysis

The number of APR genes segregating in the RIL population was estimated through Mendelian segregation analysis (Singh and Rajaram, 1992), where RILs were classified into three phenotypic categories based on disease severity: homozygous parental type resistant (HPTR), homozygous parental type susceptible (HPTS) and lines whose responses were different from those of the parents as OTHER. The observed frequencies for each category were tested against frequencies expected for different number of additive genes using χ^2 analysis. Pearson's correlation coefficient (*r*) among final disease severities (FDS) of LR and YR across different environments was calculated using the software SAS 9.2 (SAS Institute, 2008).

Genotyping

Gene-linked markers

DNA of parental lines and RILs was extracted from leaf tissues of approximately 20 plants per line using the cetyltrimethylammonium bromide (CTAB) method (Dreisigacker *et al*., 2016). Firstly, parental lines were genotyped with molecular markers linked with known slow rusting APR genes to know the presence or absence of these genes. These markers included simple sequence repeats (SSR) marker *gwm533* (Spielmeyer et al., 2003) and cleaved amplified polymorphic sequences (CAPS) marker *csSr2* (Mago *et al*., 2011) linked to *Lr27*/*Yr30*; *cslVrga* (E. Lagudah, CSIRO Canberra, Australia, pers. comm.) & *VENTRIUP-LN2* (Helguera et al. 2003) linked to *Lr37*/*Yr17*; *csLV46* & CAPS marker *csLV46G22* linked to *Lr46*/*Yr29* (E. Lagudah, CSIRO Canberra, Australia, pers. comm.); sequence-tagged site (STS) marker *csLV34* (Lagudah et al. 2006) and kompetitive allele specific polymerase chain reaction (KASP) marker *Lr34SNP* (Lagudah et al., 2009) linked to *Lr34*/*Yr18*; KASP marker *Lr67SNP-TM4* (Moore et al. 2015) linked to *Lr67*/*Yr46*, and STS marker *csGS* (Herrera-Foessel et al. 2012) associated with *Lr68*.

Amplification of CAPS, STS and SSR markers was conducted following the polymerase chain reaction (PCR) conditions of denaturation at 94°C for 5 min, followed by 30 cycles of denaturation 94°C for 1 min, annealing at 56°C for 1 min, extension at 72°C for 1 min, and final extension of 72°C for 5 min. For CAPS marker, *csSr2*, amplified products were digested with the restriction enzyme *Bspe I* at 37 °C for 1 h and the digested PCR products were electrophoresed in 3 % agarose gel to identify digested products of different sizes. On the other hand, amplified products of STS and SSR markers were electrophoresed on 2.5 % agarose gel (Dreisigacker *et al.*, 2016). For amplification of KASP markers, a touchdown PCR profile was used: denaturation at 94°C (7 min); followed by 7 cycles of 94°C for 1 min and 61°C for 1 min (dropping 1°C per cycle); 26 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and final extension of 72°C for 5 min (Randhawa *et al*., 2015). The plates were read with PHERAstar Plus (BMG Labtech, 2010) and KlüsterCaller (LGC, 2014) software to analyze the PCR products.

DArT-GBS markers

The diluted DNA of parents and a set of 125 RILs was adjusted to concentration 50 ng/µL and was sent to the Genetic Analysis Service for Agriculture (SAGA) laboratory at CIMMYT-Mexico for genotyping using DArT-GBS (Diversity Arrays Technology-Genotyping By Sequencing) markers.

Linkage map construction and QTL analysis

Marker data of 125 Apav × Kijil RILs not-carrying positive allele for *Lr37*/*Yr17*-linked markers (*cslVrga* & *VENTRIUP-LN2*) were used for linkage map construction and QTL mapping. JoinMap 4.1 was used to construct a genetic linkage map of polymorphic SNPs and gene linked markers for the RIL population (Van Ooijen 2006). LOD threshold of 10.0 was used to assign markers to groups and generate linkage between markers in each group. Genetic distances between markers were calculated using the Kosambi mapping function (Kosambi 1944). Linkage maps were drawn using Mapchart software (reference).

Inclusive composite interval mapping (ICIM) implemented in IciMapping 4.1 software (Wang et al. 2016), was performed to detect and map QTL providing resistance to YR and LR using final disease severity (FDS) of each experiment for each rust and mean FDS over years (LRM and YRM). The logarithm of odds (LOD) threshold was calculated from 1,000 permutations for each trait to declare significant QTL at the $p = 0.05$ level. The percentages of phenotypic variance explained (PVE) and the additive effect (Add) were obtained through stepwise regression with IciMapping 4.1 software.

2.4 RESULTS

Rust evaluations

Phenotypic data of four LR and six YR evaluations of 198 Apav × Kijil RILs was used to calculate number of segregating APR genes and Pearson's correlation coefficient (r), and to generate frequency distribution graphs for each rust environment.

Leaf rust:

Consistent distribution of LR was observed across all tested seasons. Resistant parent Kijil displayed FDS of 0 to 1% with response of MS whereas susceptible parent Apav was 80 to 100% with response of S, across four LR experiments. Mean LR severity of RILs ranged from 23.3 to 36.7% across four experiments (Table 2.1). Continuous distribution for LR severity of RILs was observed across the four evaluations (Figure 2.1a), indicating that LR resistance in this population is quantitatively (more than 1 or 2 genes) controlled. Mendelian segregation analysis indicated that resistance to LR in the Apav × Kijil population is conferred by three to four APR genes (Table 2.1, Figure 2.1a).

Stripe rust:

Uniform development of YR was observed across all seasons. Respectively, the FDS values of susceptible parent Apav and resistant parent Kijil were ranged from 40-100% (response S) and 0-1% (response MS), respectively. Mean FDS value of RILs ranged from 16.1% to 49.2% across two seasons. The frequency distribution of RILs for YR severity was continuous with representatives from each rust score in the evaluated environments (Figure 2.1b), indicating quantitative inheritance of APR to YR. Mendelian segregation analysis indicated that resistance to YR in the Apav × Kijil population is conferred by three to four APR genes (Table 2.1, Figure 2.1b).

Table 2.1. Estimated number of adult plant resistance (APR) genetic loci to leaf rust and stripe rust in 198 Apav × Kijil recombinant inbred lines (RILs) based on Mendelian segregation analysis.

Response/category	Number of RILs									
	LR16B	LR16Y	LR17B	LR17Y	YR16Y	YR16B	YR17B	YR16T	YR17T-R1	YR17T-R2
Apav	90	80	100	100	50	60	40	100	90	90
Kijil	1	0	1	1	0	1	0	1	0	1
Population mean	23.3	24.9	33.8	36.7	16.1	18.0	18.9	39.0	45.7	49.2
Range	1-100	0-90	0-100	0-100	0-50	0-60	0-80	1-100	0-100	0-90
HPTR[a]	25	9	30	29	32	25	22	17	10	13
HPTS[b]	9	9	14	10	14	11	28	16	13	29
OTHERS[c]	159	178	152	157	152	157	146	160	172	154
Missing	5	2	2	2	0	5	2	5	3	2
Total	198	198	198	198	198	198	198	198	198	198
P-value[d]	0.02	0.97	0.04	0.01	0.03	0.07	0.15	0.46	0.45	0.04
Number of genes	3	4	3	3	3	3	3	3	4	3

[a]Homozygous parental type susceptible (HPTR)

[b]Homozygous parental type resistant (HPTS)

[c]Lines with responses different from the parents (OTHERS)

[d]*P* value is for the $\chi 2$ test. The expected ratio of RILs grouped under HPTS, HPTR, and OTHER are 0.103:0.103:0.794 and 0.048:0.048:0.904 for segregation of 3and 4 independently inherited genes, respectively, in a F_5-derived F_6 generation

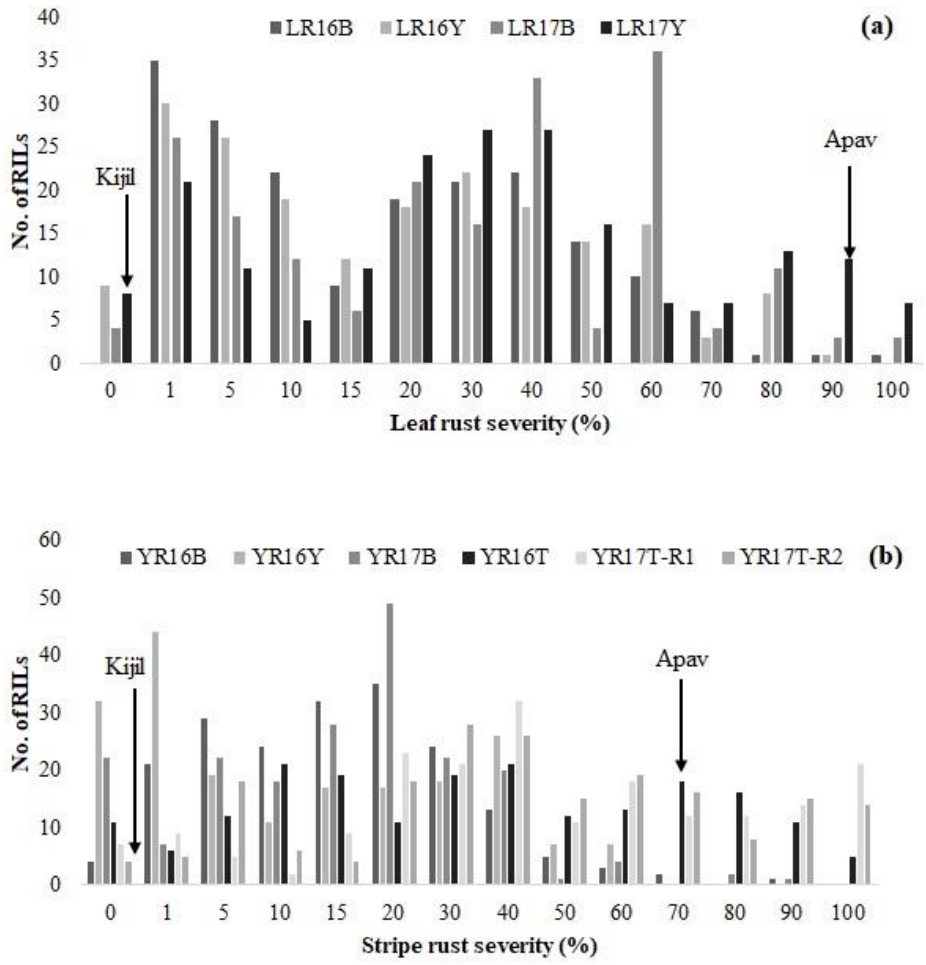


Figure 2.1. Frequency distributions of the 198 Apav × Kijil recombinant inbred lines (RILs) for (a) final leaf rust (LR) severity in field trials at Ciudad Obregon during 2015-2016 (LR16Y) and 2016-2017 (LR17Y), at El Batan in 2016 (LR16B) and 2017 (LR17B), and for (b) final stripe rust (YR) severity at El Batan in 2016 (YR16B) and 2017 (YR17B), at Ciudad Obregon in 2016 (YR16Y), Toluca in 2016 (YR16T) and 2017 (YR17T-R1 & YR17T-R2). Mean values for the parents Apav and Kijil, are indicated by arrows.

Correlation

High and significant correlation among LR severities of Apav × Kijil RILs across four years of evaluation was observed with Pearson correlation coefficient (*r*) values ranged from 0.8-0.9 at $p < 0.0001$. Similarly, significant correlation (r= 0.54-0.92, $p < 0.0001$) was also observed for YR among all tested environments. Moreover, significant correlations (r=0.44-0.77, $p < 0.0001$) were observed between LR and YR severities across all tested environments (Table 2.2).

Gene linked markers

The resistant parent Kijil displayed positive alleles for markers *gwm533* & *csSr2* linked to *Lr27*/*Yr30*/*Sr2*, and *csLV46G22* linked to *Lr46*/*Yr29*/*Sr58*, whereas corresponding negative alleles were amplified in the susceptible parent Apav. Dominant markers *cslVrga* & *VENTRIUP-LN2* amplified *Lr37*/*Yr17* linked positive allele in resistant parent Kijil whereas no amplified product was obtained in the susceptible parent Apav. The markers *csLV34* & *Lr34SNP* linked to *Lr34*/*Yr18*/*Sr57*, and *Lr67SNP-TM4* linked to *Lr67*/*Yr46*/*Sr55* amplified negative alleles in both parents Kijil and Apav indicating no differences between them at respective loci whereas *Lr68*-linked marker *csGS* (dominant marker) showed null allele (no amplified product) in both parents.

Polymorphic markers *gwm533* & *csSr2* linked to *Lr27*/*Yr30*/*Sr2*, and *csLV46* & *csLV46G22* (*Lr46*/*Yr29*/*Sr58*) were used to genotype the 125 Apav × Kijil RIL population. The calculated χ^2 and *P*-values for segregation ratio (1:1) for homozygous positive allele versus homozygous negative allele for marker *gwm533* were χ^2=3.81 (non-significant), *P*=0.05; *csSr2* were χ^2=2.12 (non-significant) and *P*=0.15; *csLV46* were χ^2=4.23 (non-significant) and *P*=0.04; and *csLV46G22* were χ^2=4.40 (non-significant) and *P*=0.07, respectively (Table 2.3).

Table 2.2. Pearson's correlation coefficient among final disease severities of Apav × Kijil recombinant inbred lines (RILs) in leaf rust (LR16B, LR16Y, LR17B and LR17Y) and stripe rust (YR16B, YR16Y, YR16T, YR17B, YR1-17T and YR2-17T) experiments.

Trait	LR16B	LR16Y	LR17B	LR17Y	YR16B	YR16Y	YR16T	YR17B	YR1-17T	YR2-17T
LR16B	1.00	0.85**	0.80**	0.86**	0.50**	0.51**	0.76**	0.46**	0.66**	0.68**
LR16Y	0.85**	1.00	0.82**	0.90**	0.46**	0.54**	0.72**	0.44**	0.65**	0.69**
LR17B	0.80**	0.82**	1.00	0.87**	0.55**	0.56**	0.75**	0.54**	0.74**	0.72**
LR17Y	0.86**	0.90**	0.87**	1.00	0.46**	0.53**	0.76**	0.53**	0.75**	0.77**
YR16B	0.50**	0.46**	0.55**	0.46**	1.00	0.67**	0.64**	0.59**	0.56**	0.54**
YR16Y	0.51**	0.54**	0.56**	0.53**	0.67**	1.00	0.74**	0.60**	0.64**	0.67**
YR16T	0.76**	0.72**	0.75**	0.76**	0.64**	0.74**	1.00	0.62**	0.81**	0.84**
YR17B	0.46**	0.44**	0.54**	0.53**	0.59**	0.60**	0.62**	1.00	0.71**	0.69**
YR1-17T	0.66**	0.65**	0.74**	0.75**	0.56**	0.64**	0.81**	0.71**	1.00	0.92**
YR2-17T	0.68**	0.69**	0.72**	0.77**	0.54**	0.67**	0.84**	0.69**	0.92**	1.00

***P* <0.0001

Table 2.3 Frequency of positive and negative alleles of markers linked to genes *Lr27*/*Yr30* and *Lr46*/*Yr29* among 125 Apav × Kijil recombinant inbred lines (RILs)

Marker	Gene(s)	Observed number of RILs[a]			χ^{2b}	*P*-value
		a	b	h		
gwm533	*Lr27*/*Yr30*	90	49	6	3.81	0.05
csSr2	*Lr27*/*Yr30*	63	51	11	2.12	0.15
csLV46	*Lr46*/*Yr29*	74	51	-	4.23	0.04
csLV46G22	*Lr46*/*Yr29*	70	50	5	4.40	0.07

[a] 'a'= homozygous for positive allele; 'b' = homozygous for negative allele; 'h' = heterozygous

[b] χ^2 values calculated for 1:1 segregation for co-dominant and dominant markers; heterozygous RILs were not included in calculation χ^2 values

Linkage map construction

One hundred and twenty-five Apav × Kijil RILs, Apav and Kijil were genotyped using 64,877 DArT-GBS markers (37,925 PAVs and 26,952 SNPs). Here, PAVs and SNPs means presence-absence variations and single nucleotide polymorphisms, respectively. Out of these, 29,537 markers (17,801 PAVs and 11,726 SNPs) were found polymorphic between parents. Markers with >10% missing data, segregation distortion ratio (1:1; $P<0.0001$), and without chromosomal information were excluded from analysis. Besides, redundant markers were removed using *bin* command in IciMapping 4.1 software. As a result, 5,890 markers (4,338 PAVs, 1,548 SNPs, *gwm533*, *csSr2*, *csLV46* and *csLV46G22*) with known chromosome locations were considered for QTL analysis. Finally, genotypic data of 5,890 DArT-GBS markers of a set of selected 125 RILs (not carrying positive alleles of markers linked to *Lr37*/*Yr17*), was used to construct a genetic linkage map using Joinmap 4.1 software (Van Ooijen, 2006). The constructed genetic linkage map contained 62 linkage groups over 21 chromosomes with total genetic distance of 6168.0 cM spanning 1824.2, 3883.7 and 460.1 cM in the A, B and D genome, respectively.

Kijil derived APR loci

Overall, six genetic loci (3 each for LR and YR resistance) including a previously known locus *Lr46*/*Yr29* and *Yr30* contributed by resistant parent Kijil were detected through ICIM. These genetic loci are described below:

QLr.cim-1BL/*QYr.cim-1BL*

A pleiotropic APR locus that conferred resistance to LR and YR was detected. This QTL was located on chromosome 1BL, flanked by markers *1271458* and *1092668* at 78.0-83.0cM. This QTL corresponded to location of a known pleiotropic APR gene *Lr46*/*Yr29* and was detected over four LR environments (LR16B, LR16Y, LR17B, LR17Y) as well as LRM, and over six YR environments (YR16B, YR16T, YR16Y, YR17B, YR17T-R1, YR17T-R2 as well as YRM, explained 31.9 to 57.6% of LR severity variation and 25.9 to 70.2% of YR severity variation. This QTL was most consistent among the detected QTLs. The LOD value of this QTL ranged from 17.3 to 58.3 (Table 2.4, Figure 2.2a).

QLr.cim-1DS

A QTL for LR resistance, located on chromosome 1DS, was mapped between markers *1254814* and *1099289* at 4.0 to 9.0cM. This QTL was temporarily designated as *QLr.cim-1DS* and showed its effect over four LR experiments LR16B, LR16Y, LR17B, LR17Y and LRM, and it explained 7.4 to 12.8 % of LR variation. The LOD value of this QTL ranged from 10.9 to 23.9 (Table 2.4, Figure 2.2b).

QYr.cim-3BS.1

This QTL, located on chromosome 3BS, was flanked by markers *1003349* and *csSr2* from 456.0 to 481.0cM, corresponded to location of previously known gene *Yr30*. Therefore, this QTL was considered as *Yr30* locus. This QTL was detected in environments YR16T, YR16Y, YR17T-R2 and YRM, and it explained 3.8 to 27.6% of YR variation. The LOD value of this QTL ranged from 4.4 to 27.7 (Table 2.4, Figure 2.2c).

QYr.cim-3BS.2

This QTL was located from 589.0 to 610.0cM on chromosome 3BS and flanked by markers *1127838* and *1281916*. This QTL was detected in two environments YR17T-R1 and YRM, and it explained 5.2 to 23.8% of YR variation. The LOD value of this QTL ranged from 5.4 to 31.7 (Table 2.4, Figure 2.2d).

QLr.cim-5AS

The QTL *QLr.cim-5AS*, was mapped between markers *1084271* and *1228740* at position of 11.0 to 12.0cM on chromosome 5AS. It was detected in environments LR16Y and LR16B, and it explained 0.5 to 4.4% of the LR variation. The LOD value of this QTL ranged from 4.2 to 9.2 (Table 2.4, Figure 2.2e).

Apav derived APR loci

Three genetic loci for LR resistance, contributed by susceptible parent Apav, were detected through ICIM. Details of these loci are given below:

QLr.cim-1AL

This QTL, temporarily designated as *QLr.cim-1AL*, was located on chromosome 1AL. It was flanked by markers *4404988* and *3024600* at position 141.0cM. This QTL was detected in one LR environment (LR17Y) and mean of LR over years (LRM), and it explained 13.1 to 19.4% of the LR severity variation. LOD value of this QTL ranged from 26.9 to 27.8 for two LR experiments.

QLr.cim-2AL

The QTL, temporarily designated as *QLr.cim-2AL*, was located on long arm of chromosome 2A, flanked between markers *4990504* and *2263981-48:C>G* at position 191.0 to 202.0cM. This locus was detected in 2 LR environments (LR16Y and LR16B) and explained 3.9 to 4.0% of LR variation with LOD value ranged 4.0 to 9.9.

QLr.cim-2BL

Third QTL, temporarily named as *QLr.cim-2BL* was located on chromosome 2BL, flanked by markers *5333025* and *7920174*. It was detected in two LR environments (LR16B and LR16Y) and explained 13.2 to 17.9% of the LR variation. LOD value of this QTL ranged from 11.1 to 29.3.

Table 2.4 Position and effects of quantitative trait loci for adult plant resistance to leaf rust (LR) and stripe rust (YR) based on final disease severity and its mean over all environments (LRM and YRM) in Apav × Kijil recombinant inbred line (RIL) population using inclusive composite interval mapping.

QTL[a]	Trait	Position[b]	Marker		LOD[c]	PVE(%)[d]	Add[e]	Resistance source
			Left	Right				
QLr.cim-1BL	LR16B	83.0	3938056	1092668	26.8	45.0	15.2	Kijil
	LR16Y	78.0	1271458	1080616	40.0	31.9	17.4	Kijil
	LR17B	78.0	1271458	1080616	34.4	57.6	22.7	Kijil
	LR17Y	78.0	1271458	1080616	46.3	49.9	25.4	Kijil
	LRM	83.0	3938056	1092668	58.3	57.3	22.5	Kijil
QYr.cim-1BL	YR16B	78.0	1271458	1080616	22.3	32.8	11.8	Kijil
	YR16T	78.0	1271458	1080616	43.1	70.2	25.2	Kijil
	YR16Y	78.0	1271458	1080616	26.2	25.9	10.7	Kijil
	YR17B	78.0	1271458	1080616	17.4	33.6	10.1	Kijil
	YR17T-R1	78.0	1271458	1080616	30.8	51.9	18.7	Kijil
	YR17T-R2	83.0	3938056	1092668	26.3	54.1	19.2	Kijil
	YRM	83.0	3938056	1092668	41.9	38.8	15.4	Kijil
QLr.cim-1DS	LR16B	6.0	3029942	1095833	10.9	12.7	7.9	Kijil
	LR16Y	6.0	3029942	1095833	23.9	12.8	10.9	Kijil
	LR17B	9.0	1095833	1099289	11.3	12.2	10.6	Kijil
	LR17Y	4.0	1254814	3029942	20.9	12.4	12.5	Kijil

	LRM	9.0	1095833	1099289	18.3	7.4	8.2	Kijil
QYr.cim-3BS.1	YR16T	470.0	5411317	4408574	8.8	6.5	7.6	Kijil
	YR16Y	456.0	1003349	3941921	27.7	27.6	10.9	Kijil
	YR17T-R2	481.0	2254240	*csSr2*	4.4	5.7	6.2	Kijil
	YRM	470.0	5411317	4408574	8.2	3.8	4.8	Kijil
QYr.cim-3BS.2	YR17T-R1	610.0	1015856	1281916	5.4	5.2	5.8	Kijil
	YRM	589.0	1127838	4397579	31.7	23.8	11.9	Kijil
QLr.cim-5AS	LR16Y	11.0	1084271	3534287	7.9	0.5	7.0	Kijil
	LR16B	12.0	3534287	1228740	4.2	4.4	4.6	Kijil
QLr.cim-1AL	LR17Y	141.0	4404988	3024600	27.8	19.4	-15.6	Apav
	LRM	141.0	4404988	3024600	26.9	13.1	-10.6	Apav
QLr.cim-2AL	LR16Y	191.0	4990504	3028275	9.9	4.0	-6.1	Apav
	LR16B	202.0	4991330	2263981-48:C>G	4.0	3.9	-4.0	Apav
QLr.cim-2BL	LR16B	897.0	5333025	7920174	11.1	13.2	-8.1	Apav
	LR16Y	897.0	5333025	7920174	29.3	17.9	-12.9	Apav

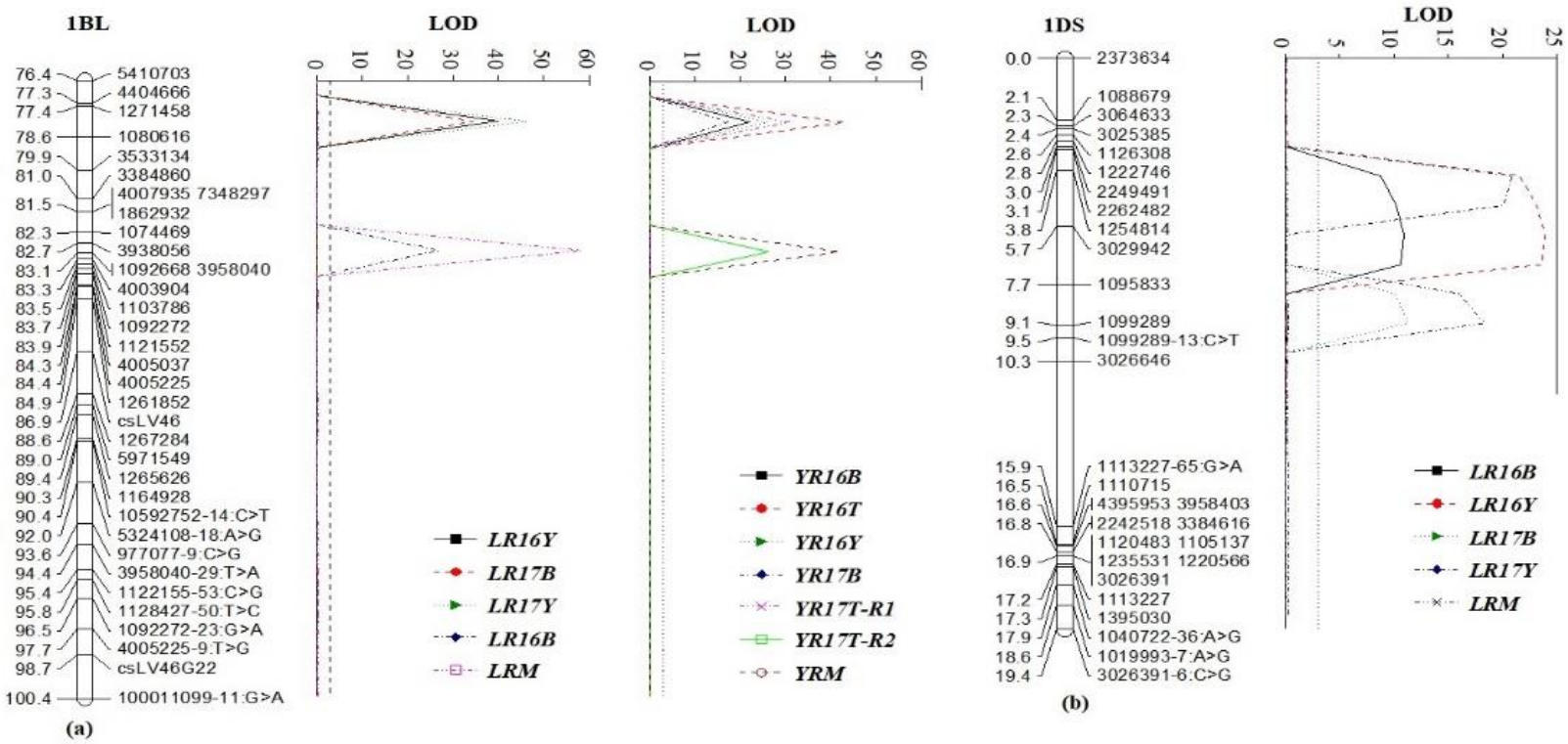
[a]QTL extending across the same confidence intervals was assigned with the same symbol

[b]Peak position in centi-Morgans from the first linked marker of the relevant linkage group

[c]Logarithm of odds (LOD) score based on 1,000 permutations

[d]PVE is the proportion of phenotypic variance explained by the QTL

[e]Additive effect of phenotypic variance for each QTL


1BL
LOD
LOD
76.4 5410703
77.3 4404666
77.4 1271458
78.6 1080616
79.9 3533134
81.0 3384860
4007935 7348297
81.5 1862932
82.3 1074469
82.7 3938056
83.1 1092668 3958040
83.3 4003904
83.5 1103786
83.7 1092272
83.9 1121552
84.3 4005037
84.4 4005225
84.9 1261852
86.9 csLV46
88.6 1267284
89.0 5971549
89.4 1265626
90.3 1164928
90.4 10592752-14:C>T
92.0 5324108-18:A>G
93.6 977077-9:C>G
94.4 3958040-29:T>A
95.4 1122155-53:C>G
95.8 1128427-50:T>C
96.5 1092272-23:G>A
97.7 4005225-9:T>G
98.7 csLV46G22
100.4 100011099-11:G>A
LR16Y
LR17B
LR17Y
LR16B
LRM
YR16B
YR16T
YR16Y
YR17B
YR17T-R1
YR17T-R2
YRM
(a)
1DS
LOD
0.0 2373634
2.1 1088679
2.3 3064633
2.4 3025385
2.6 1126308
2.8 1222746
3.0 2249491
3.1 2262482
3.8 1254814
5.7 3029942
7.7 1095833
9.1 1099289
9.5 1099289-13:C>T
10.3 3026646
15.9 1113227-65:G>A
16.5 1110715
16.6 4395953 3958403
16.8 2242518 3384616
1120483 1105137
16.9 1235531 1220566
3026391
17.2 1113227
17.3 1395030
17.9 1040722-36:A>G
18.6 1019993-7:A>G
19.4 3026391-6:C>G
LR16B
LR16Y
LR17B
LR17Y
LRM
(b)

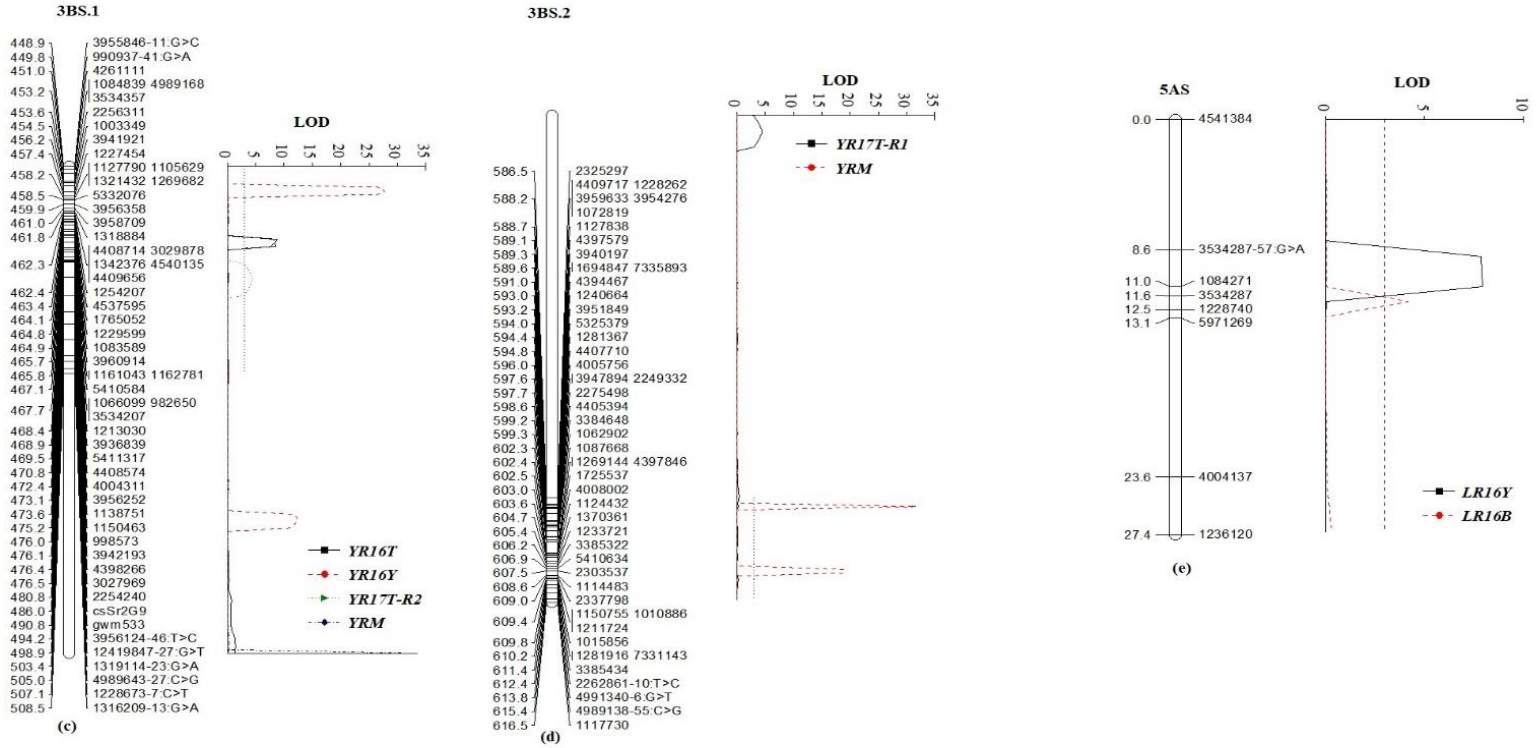


Figure 2.2. Illustrations of quantitative trait loci (QTL) for adult plant resistance (APR) to leaf rust on chromosomes 1BL (a), 1DS (b) and 5AS (e), and for stripe rust resistance on chromosomes 1BL (a), and 3BS (c, d), respectively, identified by using IciMapping 4.0 in the Apav × Kijil RIL population. The LOD thresholds were detected based on 1,000 permutations. Positions (in cM) of the molecular markers along chromosomes are shown on the vertical axes. The phenotypic data for leaf rust tested in four environments (LR16Y, LR16B, LR17Y and LR17B); for stripe rust phenotypic data evaluated in six environments (YR16B, YR17B, YR16Y, YR16T, YR17T-R1 and YR17T-R2); and and the mean of final disease severity over the tested environments (LRM and YRM).

2.5 DISCUSSION

In this study, genetic basis of APR to LR and YR in bread wheat line Kijil was dissected using combination of phenotyping tests for LR & YR, and genotypic data generated using next generation genotyping technologies viz. DArT-GBS. Representation of each phenotypic class in all rust experiments, indicated continuous variation for resistance to both rusts in Apav × Kijil RIL population. Appropriate rust testing methodologies and environment are crucial for evaluation of populations targeted for mapping of APR genes. Moreover, the high-throughput genotyping platform (DArT-GBS in this study) has added advantages of being low-cost technology, whole genome coverage, volume of polymorphic loci without the need for prior sequence information and their codominance behavior (Schouten et al. 2012).

Estimated number of APR genes segregating in the Apav × Kijil RIL population using conventional method complemented the mapping results. Mendelian genetic analyses suggested the resistance to both rusts is controlled by 3 to 4 APR genes in the RIL population. Through ICIM, two QTLs for LR resistance, two QTLs for YR resistance and one co-located QTL for LR-YR resistance were detected in this population. All of these QTLs were contributed by resistant parent Kijil. In addition, 3 LR QTLs derived from susceptible parent Apav were also detected. The estimated number of genes usually represents the minimum number of polygenic loci segregating in a population, as variable effects of different QTLs on phenotypes are a common phenomenon in wheat genotypes and each estimated gene does not necessarily contribute equally to the phenotype (Lan *et al*. 2015). The estimated number of genes segregating in the Apav × Kijil population is equivalent to the number of detected QTLs. Results of this study correspond with the number of genes with additive effects required to be combined to achieve near immune response (Singh *et al*., 2000). Significant correlations observed between LR and YR severities (r= 0.44-0.77, $p< 0.0001$) in the tested environments, attributed to the presence of common pleiotropic APR genes such as *Lr46*/*Yr29*.

The QTL located on chromosome 1BL, corresponds to the same location as of pleiotropic APR gene *Lr46*/*Yr29*, as the flanking markers *1092668* and *1271458* are located on the long arm of the chromosome 1B according to wheat consensus map version 4 (https:/www.diversityarrays.com/technology-and-resources/genetic-maps/). This locus confers

resistance to LR, YR and SR and has been widely used in CIMMYT germplasm (Singh *et al*., 1998; Singh *et al*., 2013; Herrera‐Foessel *et al*., 2014). This locus also co-segregates with *Ltn2*, a gene for leaf tip necrosis (Rosewarne *et al*., 2006) and *Pm39*, conferring resistance to powdery mildew (Lillemo *et al*., 2008). This locus has been reported in several mapping studies conducted using CIMMYT germplasm under different environmental conditions, explaining 7 to 74.5% and 8 to 66% of LR and YR severity variation, respectively (Basnet *et al*. 2013; Calvo-Salazar *et al*. 2015; Lan *et al*. 2014; Lan *et al*. 2015; Ponce *et al*., 2018a, Ponce *et al*., 2018b; Ren *et al*., 2017; Rosewarne *et al*. 2008; Rosewarne *et al*. 2012). In the present study, this locus explained 31.9 to 57.6% and 25.9 to 70.2% of LR and YR severity variation, respectively.

The second QTL *QLr.cim-1DS*, was located on chromosome 1DS and detected in all LR experiments, explaining 7.4 to 12.8% of LR variation. Markers *1254814* and *1099289* flanking this QTL are located on short arm of chromosome 1D according to wheat consensus map version 4. Recent mapping studies also identified contribution of locus on chromosome 1DS to LR resistance. Calvo-Salazar *et al*. (2015) reported QTL, *QLr.cim-1DS*, flanked by markers *wPt-3738* and *wPt-4471*, and explained 6-21% of LR severity variation. Basnet *et al*. (2014) also detected QTL, *QLr.tam-1D*, in common wheat Quaiu 3, flanked by DArT markers *wPt-666067* and *wPt-667180*, explained 24.5-35.2% of LR phenotypic variation. As reported by Basnet et al. (2014), QTL *QLr.tam-1D* could be a moderately effective race specific gene *Lr42* on chromosome 1DS as this QTL is located within 1cM distance of *Lr42*-linked marker *Xwmc432* (Sun *et al*., 2010). Other race specific genes located on chromosome 1DS are *Lr21* (Huang *et al*., 2003) and *Lr60* (Hiebert *et al*., 2008). Thus, *QLr.cim-1DS* could be one of the previously identified QTL/gene.

The third QTL located on chromosome 3BS corresponded to the APR gene *Yr30*, as flanked by markers *1003349* and *csSr2*. The marker *csSr2* is a closely linked marker for marker assisted selection of *Lr27*/*Yr30*/*Sr2* linked marker (Mago *et al*., 2011a). Pleiotropic gene *Lr27*/*Yr30*/*Sr2* provides APR to LR, YR and SR, and is also associated with pseudo black chaff trait (Mago *et al*., 2011a). *Yr30*, is an important APR locus in wheat germplasm worldwide and has been reported in other mapping populations under different environmental conditions, explaining 3.3-50% of YR severity variation (Singh et al. 2000; Spielmeyer et al. 2003; Suenaga et al.

2003; Hayden et al. 2004; William et al. 2006; Dedryver et al. 2009; Rosewarne et al. 2012; Basnet et al. 2014). In the present study, *Yr30* explained 3.8-27.6% of the YR severity variation. The fourth QTL, *QYr.cim-3BS*, detected in YR17T-R1 and YRM, explained 5.2 to 23.8% of YR variation. In addition to APR gene *Yr30*, chromosome 3BS also carries genes *Yr4* linked to marker *barc75* (Bansal et al. 2010), *Yr57* linked to marker *BS00062676* (Randhawa et al. 2015), *Yr58* linked to markers *sun476* and *sun533* (Chhetri et al. 2016), and *Yrwh2* flanked by markers *Xwmc540* and *Xgwm566* (Zhou et al. 2014). According to comparative mapping distances, *Yr57* maps more than 5 cM distal to the marker *gwm533*, linked to *Sr2* (Randhawa et al. 2015), suggesting that *Yr57* and *Sr2* can be combined. Moreover, map position indicated that *Yr58* and *Sr2* are 5.3 cM apart and can be recombined, whereas *Yr57* maps 5.9 cM distal to *Yr58* (Chhetri et al. 2016).

The fifth QTL *QLr.cim-5AS*, detected in environments LR16Y and LR16B, explained 0.5 to 4.4% of LR variation. Singh et al. (2009), identified *QLr.pbi-5AS* derived from the wheat cultivar Beaver. This QTL was flanked by markers *wPt1931-wPt8756*, explaining 11.2% of LR phenotypic variance. In addition, Messmer *et al*. (2000) reported in cultivar Forno one QTL on chromosome 5AS, mapped between markers *Xpsr945a-Xglk424* and explained 7.7% of LR variation. Moreover, Li et al. (2017) identified *QLr.hwwg5AS* in cultivar Ning7840 explaining 7.5% of LR severity variation.

Three QTLs for LR resistance located on chromosomes 1AL, 2AL and 2BL contributed by parent Apav were identified. The QTL *QLr.cim-1AL*, was mapped on chromosome 1AL, explaining 13.1 to 19.4% of LR severity variation. Zhou *et al*. (2014) identified the first LR QTL on chromosome 1AL, designated as *QLr.hebau-1AL*, derived from the wheat cultivar Shanghai 3/Catbird. This QTL was flanked by SSR markers *Xbarc213* and *Xcfa2219*, explaining 5.3 to 8.2% of LR phenotypic variance. In addition, Gao *et al*. (2016) reported the QTL *QLr.umn-1AL*, mapped to position 149.8 cM on chromosome 1AL for both field BLUE (best linear unbiased estimates) and seedling race mixture traits evaluated on a genome wide association study (GWAS). This QTL, along with *QTLs QLr.umn-4AS* and *Q.Lr.cimmyt-4BL*, together explained 12% of phenotypic variation.

The LR resistance QTL *QLr.cim-2AL* was located on chromosome 2AL and explained 3.9 to 4% of LR variation. The QTL *QLr.hebau-2AL*, derived from Chinese Spring, was detected in the marker interval *wmc181-BS00057060_51*, explaining 4.8-6.6% of LR phenotypic variance (Zhang *et al*., 2017). According to Li *et al*., (2014), this locus has been reported in other mapping populations: *QLr.cimmyt-2AL*, flanked by markers *wPt-4419* and *wPt-8226* explained 5.8-7.2% of LR severity variation (Rosewarne et al., 2012); *QLr.sfr-2AL* was mapped between *cfa2263c* and *sfr.BE590525* explaining 9.5-12% of LR phenotypic variance (Schnurbusch *et al*., 2004) and *QLr.ubo-2A*, detected in the marker interval *wPt-386-310911*, explained 18.6-30% of LR severity variation (Maccaferri *et al*., 2008). The QTL *QLr.cim-2BL* was located on the long arm of chromosome 2B explaining 13.2 to 17.9% of LR variation. *QLr.sfrs-2BL* was derived from the variety Oberkulmer and flanked by markers *Xpsr924* and *Xglk699a*, explaining 7.2% of variance for LR severity (Messmer *et al*., 2000). *QLr.osu-2B*, was flanked by makers *Xagc.tgc135* and *Xcatg.atgc60*, explaining 18.8% of LR severity variance (Xu *et al*., 2005a), while *QLrlp.osu-2B* was located in the interval of markers *Xcag.cgat70-Xcatg.atgc60* and explained 16.2% of variance for LR severity (Xu *et al*., 2005b). Both loci were located on the long arm of chromosome 2B and were derived from wheat line CI 13227 (Li *et al*., 2014). Buerstmayr *et al*., (2014) detected the QTL *QLr.ifa-2BL* in cultivar Capo, in the interval markers *wPt-6643-wPt-2425*, explaining 3.9 to 11% of LR severity variance. In addition, *QLr.cim-2BL* was detected on cultivar Kenya Kongoni, located in the interval of markers *wPt-6174* and *wPt-8548* and explained 6-21% of variance for LR severity (Calvo-Salazar *et al*., 2015). Moreover, Ren et al. (2017) identified *QLr.cim-2BL* in Avocet, mapped between markers 1237388 and 1081780_35:C>T, explaining 4.1-5.6% of LR variation. Those DArTseq markers have been mapped near DArT-array marker *wPt-5736*. The QTL identified in the present study is located close to marker *wPt-5736*, suggesting it is likely located close to *QLr.cim-2BL* in Avocet.

In conclusion, Kijil is a promising source of APR to combat rust disease through wheat breeding. With the availability of markers linked with identified LR and YR resistance loci, the resistance from Kijil can be easily transferred to other wheat germplasm through marker assisted selection (MAS). Furthermore, these resistance loci dissected from Kijil can be

combined with other APR/minor gene(s) to generate potential wheat cultivars with improved resistance to both rusts.

2.6 ACKNOWLEDGEMENTS

First author is thankful to Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico for providing scholarship to pursue PhD studies at Colegio de Postgraduados, Montecillo, Texcoco, and International Maize and Wheat Improvement Center (CIMMYT) for providing facilities and research support. Authors acknowledge the financial support provided by the Bill and Melinda Gates foundation (BMFG) and Department of International Development, UK (DFID) through the Durable Rust Resistance in Wheat (DRRW) and the Delivering Genetic Gains in Wheat (DGGW) projects (Grant No. OPPGD1389) and Grain Research and Development Corporation of Australia (GRDC).

2.7 REFERENCES

Bansal, U. K., Hayden, M. J., Gill, M. B., & Bariana, H. S. (2010) Chromosomal location of an uncharacterised stripe rust resistance gene in wheat. *Euphytica 171*, 121-127. doi: 10.1007/s10681-009-0007-4

Bansal, U., Bariana, H., Wong, D., Randhawa, M., Wicker, T., Hayden, M., and Keller, B. 2014. Molecular mapping of an adult plant stem rust resistance gene *Sr56* in winter wheat cultivar Arina. Theor. Appl. Genet. 127:1441-1448.

Basnet, B. R., Singh, R. P., Herrera-Foessel, S. A., Ibrahim, A. M. H., Huerta-Espino, J., Calvo-Salazar, V. & Rudd., J. (2013). Genetic analysis of adult plant resistance to yellow rust and leaf rust in common spring wheat Quaiu#3. *Plant Disease 97*, 728-736. doi: 10.1094/PDIS-02-12-0141-RE

Basnet, B. R., Singh, R. P., Ibrahim, A. M. H., Herrera-Foessel, S. A., Huerta-Espino, J., Lan, C. X., & Rudd, J. C. (2014) Characterization of *Yr54* and other genes associated with adult plant resistance to yellow rust and leaf rust in common wheat Quaiu 3. *Molecular Breeding 3*3, 385-399. doi: 10.1007/s11032-013-9957-2

Bjarko, M. E. & Line, R. F. (1988) Heritability and number of genes controlling leaf rust resistance in four cultivars of wheat. *Phytopathology 78*, 457-461. doi: 10.1094/Phyto-78-457

Buerstmayr, M., Matiasch, L., Masher, F., Vida, G., Ittu, M., Robert, O., Holdgate, S., Flath, K., Neumayer, A., & Buerstmayr, H. (2014) Mapping of quantitative adult plant field resistance to leaf and stripe rust in two European winter wheat populations reveals co-location of three QTL conferring resistance to both pathogens. *Theoretical and Applied Genetics 127*, 2011-2028. doi: 10.1007/s00122-014-2357-0

Calvo-Salazar, V., Singh, R. P., Huerta-Espino, J., Cruz-Izquierdo, S., Lobato-Ortiz, R., Sandoval-Islas, J. S., Vargas-Hernández, M., Germán, S., Silva, P., Basnet, B. R., Lan, C. X. & Herrera-Foessel, S. A. (2015). Genetic analysis of resistance to leaf rust and yellow rust in spring wheat cultivar Kenya Kongoni. *Plant Disease 99*, 1153-1160. doi: 10.1094/PDIS-07-14-0718-RE

Chhetri, M., Bariana, H., Kandiah, P., & Bansal, U. (2016) *Yr58*: a new stripe rust resistance gene and its interaction with *Yr46* for enhanced resistance. *Phytopathology 106*, 1530-1534. doi: 10.1094/PHYTO-04-16-0182-R

Dedryver, F., Paillard, S., Mallard, S., Robert, O., Trotter, M., Nègre, S., Verplancke, G., & Jahier, J. (2009). Characterization of genetic components involved in durable resistance to stripe rust in the bread wheat 'Renan'. *Phytopathology 99*, 968-973. doi:10.1094/PHYTO-99-8-0968

Dreisigacker, S., Sehgal, D., Reyes-Jaimez, A. E., Luna-Garrido, B., Muñoz-Zavala, S., Núñez-Ríos, C., Mollins, J., & Mall, S. (Eds.) (2016) CIMMYT wheat molecular genetics: laboratory protocols and applications to wheat breeding. México, D.F.: CIMMYT. 142 p.

Dyck, P. L. 1987. The association of a gene for leaf rust resistance with the chromosome 7D suppressor of stem rust resistance in common wheat. Genome. 29:467-469.

Dyck, P. L. 1991. Genetics of adult-plant leaf rust resistance in Chinese Spring and sturdy wheats. Crop Sci. 31:309-311.

Gao, L., Turner, M. K., Chao, S., Kolmer, J., & Anderson, J. A. (2016) Genome wide association study of seedling and adult plant leaf rust resistance in elite spring wheat breeding lines. *PLoSONE11 2*, e0148671. doi:10.1371/journal. pone.0148671

Hare, R. A., and McIntosh, R. A. 1979. Genetic and cytogenetic studies of durable adult-plant resistances in Hope and related cultivars to wheat rusts. Z Planzenzuchtung. 83:350-367.

Hayden, M. J., Kuchel, H., & Chalmers, K. J. (2004) Sequence tagged microsatellites for the *Xgwm533* locus provide new diagnostic markers to select for the presence of stem rust resistance gene *Sr2* in bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics 109*, 1641-1747. doi: 10.1007/s00122-004-1787-5

Herrera-Foessel, S. A., Singh, R. P., Huerta-Espino, J., Rosewarne, G. M., Periyannan, S. K., Viccars, L., Calvo-Salazar, V., Lan, C. X., & Lagudah, E. S. (2012) *Lr68*: A new gene conferring slow rusting resistance to leaf rust in wheat. *Theoretical and Applied Genetics 124*, 1475-1486. doi: 10.1007/s00122-012-1802-1

Herrera-Foessel, S. A., Singh, R. P., Huerta-Espino, J., Rosewarne, G. M., Periyannan, S. K., Viccars, L., Calvo-Salazar, V., Lan, C. X., and Lagudah, E. S. 2012. *Lr68*: A new gene conferring slow rusting resistance to leaf rust in wheat. Theor. Appl. Genet. 124:1475-1486.

Herrera-Foessel, S. A., Singh, R. P., Lillemo, M., Huerta-Espino, J., Bhavani, S., Sukhwinder, S., Lan, C. X., Calvo-Salazar, V., & Lagudah, E. S. (2014) *Lr67/Yr46* confers adult plant resistance to stem rust and powdery mildew in wheat. *Theoretical and Applied Genetics 127*: 781-789. doi:10.1007/s00122-013-2256-9

Hiebert, C. W., Thomas, J. B., McCallum, B. D., & Somers, D. J. (2008) Genetic mapping of the wheat leaf rust resistance gene *Lr60* (*LrW2*). *Crop Science 48*, 1020-1026. doi: 10.2135/cropsci2007.08.0480

Huang, L., Brooks, S. A., Li, W., Fellers, J. P., Trick, H. N., & Gill, B. S. (2003) Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploidy genome of bread wheat. *Genetics 164*, 655-664.

Huerta-Espino, J., Villaseñor-Mir, H. E., Rodríguez-García, M. F., & Singh, R. P. (2015) Emerging new virulence gene combinations in the Mexican *Pst* population. In: The BGRI technical workshop, September 17-20, Sydney Australia.

Johnson, R. (1981) Durable resistance: definition, genetic control and attainment in plant breeding. *Phytopathology 71*, 567-568. doi: 10.1094/Phyto-71-567

Lagudah, E. S., McFadden, H., Singh, R. P., Huerta-Espino, J., Bariana, H. S., & Spielmeyer, W. (2006) Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. *Theoretical and Applied Genetics 114*, 21-30. doi: 10.1007/s00122-006-0406-z

Lagudah, E. S., Krattinger, S. G., Herrera-Foessel, S., Singh, R. P., Huerta-Espino, J., Spielmeyer, W., Brown-Guedira, G., Selter, L. L., & Keller, B. (2009) Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. *Theoretical and Applied Genetics 119*, 889-898. doi: 10.1007/s00122-009-1097-z

Lan, C. X., Rosewarne, G. M., Singh, R. P., Herrera-Foessel, S. A., Huerta-Espino, J., Basnet, B. R., Zhang, Y. & Yang E. (2014) QTL characterization of resistance to leaf rust and stripe rust in the spring wheat line Francolin#1. *Molecular Breeding 34*, 789-803. doi:10.1007/s11032-014-0075-6

Lan, C. X., Zhang, Y. L., Herrera-Foessel, S. A., Basnet, B. R., Huerta-Espino, J., Lagudah, E. S. & Singh, R. P. (2015) Identification and characterization of pleiotropic and co-located resistance loci to leaf rust and stripe rust in bread wheat cultivar Sujata. *Theoretical and Applied Genetics 128*, 549-561. doi: 10.1007/s00122-015-2454-8

LGC. (2014) KlusterCaller version 3.4.1.36. LGC. https://www. lgcgroup.com/cn/our-science/genomics-solutions/genotyping/genotyping-software/klustercaller/#.WimnoqGVVaZ.

Li, Z., Lan, C. X., He, Z., Singh, R. P., Rosewarne, G. M., Chen, X., & Xia, X. (2014) Overview and application of QTL for adult plant resistance to leaf rust and powdery mildew in wheat. *Crop Science 54*, 1907-1925. doi: 10.2135/cropsci2014.02.0162

Li, C., Wang, Z., Li, C., Bowden, R., Bai, G., Su, Z., & Carver, B. F. (2017) Mapping of quantitative trait loci for leaf rust resistance in the wheat population Ning7840 x Clark. *Plant Disease 101*, 1974-1979. doi: 10.1094/PDIS-12-16-1743-RE

Lillemo, M., Asalf, B., Singh, R. P., Huerta-Espino, J., Chen, X.M., He, Z. H. & Bjørnstad, A. (2008) The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. *Theoretical and Applied Genetics 116*, 1155-1166. doi: 10.1007/s00122-008-0743-1

Maccaferri, M., Mantovani, P., Tuberosa, R., De Ambrogio, E., Giuliani, S., Demontis, A., Massi, A. & Sanguineti, M. C. (2008). A major QTL for durable leaf rust resistance widely exploited in durum wheat breeding programs maps on the distal region of chromosome arm 7BL. *Theoretical and Applied Genetics 117*, 1225-1240. doi: 10.1007/s00122-008-0857-5

Mago, R., Tabe, L., McIntosh, R. A., Pretorius, Z., Kota, R., Paux, E., Wicker, T., Breen, J., Lagudah, E. S., Ellis, J. G., & Spielmeyer, W. (2011a) A multiple resistance locus on chromosome arm 3BS in wheat confers resistance to stem rust (*Sr2*), leaf rust (*Lr27*) and powdery mildew. *Theoretical and Applied Genetics 123*, 615-623. doi: 10.1007/s00122-011-1611-y

Mago, R., Brown-Guedira, G., Dreisigacker, S., Breen, J., Jin, Y., Singh, R., Appels, R., Lagudah E. S., Ellis, J., & Spielmeyer, W. (2011b) An accurate DNA marker assay for stem rust resistance gene *Sr2* in Wheat. *Theoretical and Applied Genetics 122*, 735-744. doi: 10.1007/s00122-010-1482-7

McIntosh, R. A., Dubcovsky, J., Rogers, W. J., Morris, C. & Xia, X. C. (2017) Catalogue of gene symbols for wheat: 2017 supplement.

Messmer, M. M., Seyfarth, R., Keller, M., Schachermayr, G., Winzeler, M., Zanetti, S., Feuillet, C. & Keller, B. (2000) Genetic analysis of durable leaf rust resistance in winter wheat. *Theoretical and Applied Genetics 100*, 419-431. doi: 10.1007/s001220050055

Moore, J. W., Herrera-Foessel, S., Lan, C., Schnippenkoetter, W., Ayliffe, M., Huerta-Espino, J., Lillemo, M., Viccars, L., Milne, R., Periyannan, S., Kong, X., Spielmeyer, W., Talbot, M., Bariana, H., Patrick, J. W., Dodds, P., Singh, R., & Lagudah, E. (2015) A recently hexose transporter variant confers resistance to multiple pathogens in wheat. *Nature Genetics 47*, 1494-1498. doi: 10.1038/ng.3439

Peterson, R. F., Campbell, A. B. & Hannah, A. E. (1948) A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Canadian Journal of Research 26*, 496-500. doi: 10.1139/cjr48c-033

Ponce-Molina, L. J., Huerta-Espino, J., Singh, R. P., Basnet, B. R., Lagudah, E., Aguilar-Rincón, V. H., Alvarado, G., Lobato-Ortiz, R., García-Zavala, J. & Lan, C. (2018a.) Characterization of adult plant resistance to leaf rust and stripe rust in indian wheat cultivar 'New Pusa 876'. *Crop Science 58*, 630-638. doi: 10.2135/cropsci2017.06.0396

Ponce-Molina, L. J., Huerta-Espino, J., Singh, R. P., Basnet, B. R., Alvarado, G., Lobato-Ortiz, R., García-Zavala, J., Randhawa, M. S. & Lan, C. (2018b.) Characterization of leaf rust and stripe rust resistance in spring wheat 'Chilero'. *Plant Disease 102*, 421-427. doi: 10.1094/PDIS-11-16-1545-RE

Randhawa, M. S., Bansal, U. K., Mago, R., & Bariana, H. S. (2015) Mapping of a new stripe rust resistance locus *Yr57* on chromosome 3BS of wheat. *Molecular Breeding 35*, 65. doi: 10.1007/s11032-015-0270-0

Ren, Y., Singh, R. P., Basnet, B. R., Lan, C. X., Huerta-Espino, J., Lagudah, E. S. & Ponce-Molina, L. J. (2017) Identification and mapping of adult plant resistance loci to leaf rust and stripe rust in common wheat cultivar Kundan. *Plant Disease 101*, 456-463. doi: 10.1094/PDIS-06-16-0890-RE

Rosewarne, G. M., Singh, R. P., Huerta-Espino, J., William, H. M., Bouchet, S., Cloutier, S., McFadden, H., & Lagudah, E. S. (2006) Leaf tip necrosis, molecular markers and β1-proteasome subunits associated with the slow rusting resistance genes *Lr46/Yr29*. *Theoretical and Applied Genetics 112*, 500-508. doi: 10.1007/s00122-005-0153-6

Rosewarne, G. M., Singh, R. P., Huerta-Espino, J., & Rebetzke, G. J. (2008) Quantitative trait loci for slow-rusting resistance in wheat to leaf rust and stripe rust identified with multi-environment analysis. *Theoretical and Applied Genetics 116*, 1027-1034. doi: 10.1007/s00122-008-0736-0

Rosewarne, G. M., Singh, R. P., Huerta-Espino, J., Herrera-Foessel, S. A., Forrest, K. L., Hayden, M. J. & Rebetzke, G. J. (2012) Analysis of leaf and stripe rust severities reveals pathotype changes and multiple minor QTLs associated with resistance in an Avocet × Pastor wheat population. *Theoretical and Applied Genetics 124*, 1283-1294. doi: 10.1007/s00122-012-1786-x

Rosewarne, G. M., Herrera-Foessel, S. A., Singh, R. P., Huerta-Espino, J., Lan, C. X., & He. Z. H. (2013) Quantitative trait loci of stripe rust resistance in wheat. *Theoretical and Applied Genetics 126*, 2427-2449. doi: 10.1007/s00122-013-2159-9

SAS Institute. 2008. The SAS system for Windows. Release 9.2. SAS Inst., Cary, NC.

Schouten, H. J., Weg, W. E., Carling, J., Khan, S. A., McKay, S. J., Kaauwen, M. P. W., Wittenberg, A. H. J., Koehorst-van Putten, H. J. J., Noordijk, Y., Gao, Z., Rees, D. J. G., Van Dyk, M. M., Jaccoud, D., Considine, M. J. & Kilian, A. 2012. Diversity arrays technology (DArT) markers in apple for genetic linkage maps. *Molecular breeding 29*, 645-660. doi: 10.1007/s11032-011-9579-5

Schnurbusch, T., Paillard, S., Schori, A., Messmer, M., Schachermayr, G., Winzeler, M., & Keller, B. (2004) Dissection of quantitative and durable leaf rust resistance in Swiss

winter wheat reveals a major resistance QTL in the *Lr34* chromosomal region. *Theoretical and Applied Genetics 108*, 477-484. doi: 10.1007/s00122-003-1444-4

Singh, R. P. & Rajaram, S. (1992) Genetics of adult plant resistance to leaf rust in 'Frontana' and three CIMMYT wheats. *Genome 35*, 24-31. doi: 10.1139/g92-004

Singh, R. P., Mujeeb-Kazi, A., & Huerta-Espino, J. (1998) *Lr46*: A gene conferring slow-rusting resistance to leaf rust in wheat. *Crop Science 37*, 390-395. doi: 10.1094/PHYTO.1998.88.9.890.

Singh, R. P., Huerta-Espino, J. & Rajaram, S. (2000) Achieving near-immunity to leaf and stripe rusts in wheat by combining slow rusting resistance genes. *Acta Phytopathologica et Entomologica Hungarica 35*, 133-139.

Singh, D., Simmonds, J., Park, R. F., Bariana, H. S. & Snape, J. W. (2009) Inheritance and QTL mapping of leaf rust resistance in the European winter wheat cultivar 'Beaver'. *Euphytica 169*, 253-261. doi:10.1007/s10681-009-9959-7

Singh, R. P., Huerta-Espino, J., Bhavani, S., Herrera-Foessel, S. A., Singh, D., Singh, P. K., Velu, G., Mason, R. E., Jin, Y., Njau, P. & Crossa, J. (2011) Race non-specific resistance to rust diseases in CIMMYT spring wheats. *Euphytica 179*, 175-186. doi: 10.1007/s10681-010-0322-9

Singh, R. P., Herrera-Foessel, S. A., Huerta-Espino, J., Lan, C. X., Basnet, B. R., Bhavani, S., & Lagudah, E. S. (2013) Pleiotropic gene *Lr46/Yr29/Pm39/Ltn2* confers slow rusting, adult plant resistance to wheat stem rust fungus. *In*: Proceedings Borlaug Global rust Initiative, 2013 Technical Workshop, 19-22 August, New Delhi, India, p 17.1

Spielmeyer, W., Sharp, P. J., & Lagudah, E. S. (2003) Identification and validation of markers linked to broad-spectrum stem rust resistance gene *Sr2* in wheat (*Triticum aestivum* L.). *Crop Science 43*, 333-336. doi: 10.2135/cropsci2003.3330

Suenaga, K., Singh, R. P., Huerta-Espino, J., & William, H. M. (2003) Microsatellite markers for genes *Lr34/Yr18* and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. *Phytopathology 93*, 881-890. doi: 10.1094/PHYTO.2003.93.7.881.

Sun, Y., Wang, J., Crouch, J. H., & Xu, Y. (2010) Efficiency of selective genotyping for genetic analysis of complex traits and potential applications in crop improvement. *Molecular Breeding 26*, 493-511. doi:10.1007/s11032-010-9390-8

Van Ooijen, J. W. 2006. Join Map 4, software for the calculation of genetic linkage maps in

experimental population. Plant Research International, Wageningen, The Netherlands.

William, M., Singh, R. P., Huerta-Espino, J., Ortiz-Islas, S., & Hoisington, D. (2003) Molecular marker mapping of leaf rust resistance gene *Lr46* and its association with stripe rust resistance gene *Yr29* in wheat. *Phytopathology 93*, 153-159. doi: 10.1094/PHYTO.2003.93.2.153.

William, H. M., Singh, R. P., Huerta-Espino, J., Palacios, G., & Suenaga, K. (2006) Characterization of genetic loci conferring adult plant resistance to leaf rust and stripe rust in spring wheat. *Genome 49*, 977-990. doi: 10.1139/g06-052

Xu, X., Bai, G., Carver, B. F., Shaner, G. E., & Hunger, R. M. (2005a). Molecular characterization of slow leaf-rusting resistance in wheat. *Crop Science 45*, 758-765. doi: 10.2135/cropsci2005.0758

Xu, X., Bai, G., Carver, B. F., Shaner, G. E., & Hunger, R. M. (2005b) Mapping of QTLs prolonging the latent period of *Puccinia triticina* infection in wheat. *Theoretical and Applied Genetics 110*, 244-251. doi:10.1007/s00122-004-1819-1

Zhang, P., Yin, G., Zhou, Y., Qi, A., Gao, F., Xia, X., He, Z., Li, Z., & Liu, D. (2017) QTL mapping of adult-plant resistance to leaf rust in the wheat cross Zhou 8425B/Chinese Spring using high-density SNP markers. *Frontiers in Plant Science 8*, 793. doi: 10.3389/fpls.2017.00793

Zhou, Y., Ren, Y., Lillemo, M., Yao, Z., Zhang, P., Xia, X,, He, Z., Li, Z., Liu, D. (2014) QTL mapping of adult-plant resistance to leaf rust in a RIL population derived from a cross of wheat cultivars Shanghai3/Catbird and Naxos. *Theoretical and Applied Genetics 127*, 1873-1883. doi: 10.1007/s00122-014-2346-3

Singh, R. P., Mujeeb-Kazi, A., and Huerta-Espino, J. 1998. *Lr46*: a gene conferring slow rusting resistance to leaf rust in wheat. Phytopathology. 88:890-894.

CONCLUSIONES GENERALES

El análisis genético indicó que la resistencia a roya de la hoja en la población Apav #1 × Kijil está determinada por 3 a 4 genes. El mismo número de genes condiciona la resistencia para roya amarilla.

Se detectaron cinco loci de resistencia derivados del progenitor Kijil:
Dos QTLs de resistencia para roya de la hoja denominados *QLr.cim-1DS* y *QLr.cim-5AS* localizados en los cromosomas 1DS y 5AS, respectivamente.
Dos QTLs de resistencia para roya amarilla localizados en el cromosoma 3BS, correspondientes a *Yr30* y *QYr.cim-3BS*.
Un QTL de resistencia a roya de la hoja y roya amarilla co-localizado en el cromosoma 1BL (correspondiente a *Lr46/Yr29*).

Se detectaron 3 QTLs derivados del progenitor Apav #1 de efectos menores para resistencia a roya de la hoja, denominados *QLr.cim-1AL, QLr.cim-2AL* y *QLr.cim-2BL*, localizados en los cromosomas 1AL, 2AL y 2BL, respectivamente.

El análisis genético indicó que existen cuatro genes de raza específica que confieren resistencia para roya del tallo en plántula. Un primer gen se identificó como *Sr38* y los demás se designaron temporalmente como *SrKj;1*, *SrKj22+* y *SrKjX-*, de acuerdo con los tipos de infección registrados.

En planta adulta, las familias positivas a *Sr38* mostraron niveles de infección de 0-20 %.
Las familias portadoras de los genes *SrKj;1*, *SrKj22+* y *SrKjX-* presentaron rangos de severidad de 1-30 %, 30-60 % y 1-30 %, respectivamente.

Se determinó que existen tres genes menores que confieren resistencia en planta adulta, entre ellos *Sr2* y *Sr58*.
Las familias positivas a *Sr58* mostraron severidades de 30-90 % y sólo una presentó 5 %;
Las familias positivas a *Sr2+Sr58* presentaron 60-100 % de severidad

La línea de trigo harinero ‘Kijil’ puede servir como una fuente potencial de resistencia a las tres royas en un programa de mejoramiento.

La resistencia de ‘Kijil’ puede transferirse fácilmente a otro germoplasma de trigo a través de la selección asistida por marcadores (MAS).