

**UNIVERSIDAD DE LA REPÚBLICA
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**ESTRATEGIAS DE MANEJO CON FUNGICIDAS Y RESISTENCIA GENÉTICA PARA EL
CONTROL DE ROYA DE TALLO DE TRIGO**

por

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RESUMEN

La roya de tallo (RT) causada por *Puccinia graminis* f.sp. *tritici* fue la enfermedad del cultivo de trigo más devastadora a nivel mundial hasta la década de 1980 por poder causar pérdidas totales en condiciones favorables en cultivares susceptibles. Posteriormente, el control mediante resistencia hospedera determinó el control de la RT durante décadas. Sin embargo, la amenaza de la migración de las razas africanas (Ug99+) virulentas sobre los principales genes de resistencia utilizados en América del Sur (*Sr24* y *Sr31*) y la re-emergencia de la RT a nivel epidémico en Uruguay debido a la gran proporción de área sembrada con cultivares susceptibles, requiere desarrollar herramientas para el manejo de esta enfermedad. Los objetivos del presente trabajo fueron ajustar el manejo de la RT con fungicidas de manera de contar con una herramienta de control disponible en el corto plazo, y estudiar la base genética de la resistencia de dos materiales altamente resistentes a razas locales y Ug99+. Se llevaron a cabo ensayos de campo durante 2015 y 2016 en INIA La Estanzuela (LE) y se determinó que la aplicación de fungicidas en el momento de la aparición de los primeros síntomas redujo significativamente ($p \leq 0.05$) la severidad de RT, AUDPC e incrementó el rendimiento de grano, el peso hectolítrico y el peso de mil granos, en comparación con el tratamiento sin fungicidas y aplicaciones a Z71 en un cultivar susceptible y en uno moderadamente susceptible. Resultados preliminares indicaron que la combinación de principios activos probados jugó un rol secundario para el manejo de RT. El análisis de QTL con información genotípica (GBS) y datos fenotípicos recolectados a campo e invernáculo en dos localidades en Uruguay y en Kenia, demostraron la presencia de al menos cuatro QTLs de resistencia a RT en el cultivar INIA Tero, y tres QTL en la línea BR23//CEP19/PF85490. En ambos materiales el principal QTL confirmando resistencia en plántula y planta adulta a razas locales y Ug99+ se ubicó en el cromosoma 2B, aunque no fue posible determinar si es el mismo gen en ambos materiales. Este *locus* (o *loci*) puede corresponder a los genes *Sr28* o *Sr9h* ya reportados, un nuevo alelo del gen *Sr9* o un nuevo gen *Sr*. Estudios adicionales son necesarios para determinar la identidad de estos genes para evitar baja diversidad genética de la resistencia a RT en el Programa de Mejoramiento de trigo.

Palabras clave: roya de tallo, fungicidas, resistencia genética, mapeo de QTL

STRATEGIES OF MANAGEMENT WITH FUNGICIDES AND GENETIC RESISTANCE TO CONTROL WHEAT STEM RUST

SUMMARY

Stem rust (SR) was the most devastating wheat disease worldwide as it could cause total grain yield losses under favorable conditions in susceptible varieties. The threat of migration of African races (Ug99+), virulent to the principal resistance genes used in South America (*Sr24* and *Sr31*) and the re-emergence of SR at epidemic levels in Uruguay due to a high proportion of the area planted to susceptible cultivars, requires developing tools to manage SR. The objective of this study was to adjust SR management with fungicide in order to have a control tool available in the short-term, and to study the genetic base of the resistance of two highly resistant materials to local races and Ug99. Field trials were carried out during 2015 and 2016 at INIA La Estanzuela (LE) and it was determined that fungicide application at disease onset significantly reduced ($p \leq 0.05$) SR severity, AUDPC and increased grain yield, test weight and thousand-kernel weight as compared to the non-treated and applications at ZGS71 in a susceptible and in a moderately susceptible cultivar. Preliminary results indicated that active ingredient combinations tested played a secondary role in SR management. QTL analysis with genotypic information from GBS and phenotypic data collected in field and greenhouse at two Uruguayan locations and at Kenya demonstrated the presence of at least four QTLs for SR resistance in cv. INIA Tero and three in line BR23//CEP19/PF85490. In both materials, the main QTL conferring resistance in seedlings and adult plants to local races and Ug99+ is located on chromosome 2B, although it was not possible to determine if it is the same gene on both materials. This locus or loci might correspond to the reported genes *Sr28*, *Sr9h*, a new *Sr9* allele or a new *Sr* gene. Additional studies are necessary to determine the identity of these genes to avoid low genetic diversity of SR resistance in the wheat breeding program.

Keywords: stem rust, fungicides, genetic resistance, QTL mapping.

1. INTRODUCCIÓN

El trigo (*Triticum aestivum* L.) es uno de los cultivos más importantes a nivel mundial con 222 millones de hectáreas sembradas en 2016 y una producción total de 752 millones de toneladas (FAOSTAT, 2016). En Uruguay, es el cultivo de invierno más importante, habiendo aumentado su superficie desde el año 2007 paralelamente a la expansión del cultivo de soja. Desde entonces ocupó un promedio de 422 mil hectáreas anuales, con un rendimiento promedio que supera los 3000 kg/ha (DIEA, 2017).

La roya del tallo de trigo (RT), causada por *Puccinia graminis* f.sp *tritici* Erik. & E. Henn., fue considerada la enfermedad más destructiva de este cultivo en el país y en la región en la primera mitad del siglo XX. Aunque presentaba epidemias más esporádicas que la roya de la hoja (causada por *Puccinia triticina*), los daños provocados eran muy elevados, pudiendo llegar a pérdidas totales del cultivo cuando ocurrían epidemias severas (Germán y Verges, 2007). Debido al uso generalizado de cultivares con resistencia genética a RT, esta enfermedad no causó epidemias importantes por más de tres décadas (Singh *et al.*, 2011). Por este motivo, la elección de los cultivares por parte de los productores y técnicos se centró en otros aspectos, como mayor rendimiento y calidad de grano, sin considerar su comportamiento frente a RT. La elección de cultivares sin tener en cuenta su comportamiento frente a RT determinó que para el año 2015, más del 40% del área del cultivo en Uruguay estuviera sembrada con materiales susceptibles a esta enfermedad (Silvia Germán, com. pers., 15 de setiembre de 2015). Esta situación, agravada además por el uso de los mismos cultivares susceptibles en gran proporción del área de Argentina (Germán *et al.*, 2011), determinó la re-emergencia de la RT a nivel epidémico a partir del año 2014.

La resistencia genética es la mejor medida para el control de RT. Los genes más importantes que confieren resistencia en los cultivares utilizados en la región son *Sr24* y *Sr31* (Germán y Verges, 2007; Germán *et al.*, 2011). En el año 1998 en Uganda, fue detectada una nueva raza del patógeno *P. graminis*, denominada TTKSK (Ug99), que junto con variantes de esta raza comprendidas en el linaje Ug99+, son virulentas sobre los genes *Sr31*, *Sr24* y otros genes de resistencia importantes, y virulentas frente a más del 90% de los cultivares de trigo a nivel mundial (Singh *et al.*, 2011, Jin *et al.*, 2008). El linaje de RT Ug99+ originario de Uganda se dispersó a Kenia, Ethiopia, Sudán, Eritrea, Yemen, Iran, Mozambique, Zimbabwe, Tanzania, Sudáfrica y Egipto, y se prevé que pueda llegar a Asia central. La eventual migración de Ug99+ al resto de las regiones productoras de trigo que poseen condiciones climáticas favorables para la enfermedad implica una gran amenaza para la producción mundial de trigo, y para la seguridad alimentaria (Singh *et al.*, 2011).

Frente a esta situación, con el objetivo de realizar una alerta global e informar sobre las posibles consecuencias de la expansión de las razas Ug99+, el Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) y el Centro Internacional para la Investigación Agropecuaria en Áreas Secas (ICARDA), organizaron un consorcio internacional denominado “Borlaug Global Rust Initiative” (BGRI). Dentro de las actividades planificadas por la BGRI, se estableció un sitio de pruebas *in situ* (en Kenia) para evaluar la resistencia a Ug99+ de cultivares de trigo de todo el mundo. A nivel nacional desde la Estación Experimental Alberto Boerger del Instituto Nacional de Investigación Agropecuaria (INIA) se está monitoreando la situación de la enfermedad en los cultivos comerciales y verificando las razas de *P. graminis* presentes en Uruguay, de manera de identificar la posible incursión de razas exóticas que presentan un riesgo para la producción de trigo del país (Germán y Verges, 2007; Germán *et al.*, 2011), así como también se envían anualmente a Kenia materiales comerciales y experimentales para generar información de su comportamiento frente a Ug99+.

En este contexto, donde se prevé un incremento de temperaturas medias en la región debido al cambio global que podría favorecer el desarrollo de la enfermedad, la presencia de un área importante sembrada de cultivares susceptibles, y la posible incursión de razas exóticas de *P. graminis*, los objetivos de este trabajo fueron (i) ajustar el manejo de la RT de trigo con fungicidas de manera de disponer de una herramienta de control en el corto plazo; y (ii) estudiar la base genética de resistencia a razas locales y Ug99+ de dos líneas de trigo utilizadas como fuentes de resistencia a RT en el Programa de Mejoramiento Genético de Trigo INIA, mediante análisis de QTL (del inglés “*Quantitative Trait Loci*”) de forma de determinar si poseen resistencia diferente, basada en genes reportados o novedosos para disponer de información relevante para el mejoramiento por resistencia a razas locales de RT y el mejoramiento anticipatorio por resistencia a Ug99+.

El trabajo sobre manejo de RT de trigo con fungicidas será publicado en la revista “*Crop Protection*”, y el trabajo sobre el estudio de la base genética de la resistencia a RT será publicado en la revista “*Theoretical Applied Genetics*”. Dichos capítulos en esta tesis tendrán el formato requerido por estas revistas.

1.1. LA ESPECIE *Triticum aestivum* L.

El trigo (*Triticum aestivum* L.) es una especie alohexaploide ($2n = 6X = 42$, genoma AABBDD), producto de la hibridación de *Triticum turgidum* (genoma AABB) y *Triticum tauschii* (genoma DD). A su vez, *Triticum turgidum* es producto de la hibridación de *Triticum urartu* (genoma AA) y *Aegilops speltoides* (genoma BB) (Dvořák *et al.*, 1993; Dvořák *et al.*, 1988). A pesar de ser poliploide, el trigo se comporta como diploide ya que el gen *Ph1* ubicado en el brazo largo del cromosoma 5B, que evita la aparición entre cromosomas homeólogos durante la meiosis, asegurando que el apareamiento y la recombinación ocurra sólo entre cromosomas homólogos (Sears, 1976). El trigo posee un genoma complejo, de gran

tamaño (16Gb), alto número de regiones repetidas (80%) y un 21 % de sus genes duplicados. Estas características genómicas y el hecho de que sus variables agronómicas estén influenciadas por genes provenientes de tres genomas distintos, determina que sea difícil desarrollar mapas genéticos y el estudio de caracteres de herencia compleja (Akhunov *et al.*, 2003; Dubcovsky *et al.*, 1996). Otra característica importante del trigo es que, al ser autógeno, presenta altos niveles de homocigocis, con un flujo génico menor al 1% (Gustafson *et al.*, 2005).

1.2. EL CULTIVO DE TRIGO

El trigo es uno de los principales cultivos a nivel mundial explicado por su gran importancia en la alimentación humana. Es sólo superado por el arroz en área sembrada y en ser fuente de calorías para los países subdesarrollados. Actualmente es sembrado en 125 países del mundo y ocupa un total de 222 millones de hectáreas, con una producción de 752 millones de toneladas en 2016 (USDA, 2017). Los principales productores a nivel mundial son China, India, EEUU y Rusia, con una producción de 122, 93,5, 58 y 52 millones de toneladas respectivamente (FAOSTAT, 2016).

El trigo en Uruguay es el cultivo de invierno más importante, ocupando en 2016 un 49,9% del área sembrada con cultivos de invierno (DIEA, 2017). A partir del año 2007 tuvo un incremento en área y producción, explicado por la realización de la rotación trigo/soja. Pasó de 154 mil ha y producir 454 mil toneladas en la zafra 2005 a 593 mil ha y 2016 mil toneladas en la zafra 2011 (DIEA, 2017). Luego de esta zafra, el área sembrada disminuyó paulatinamente debido a la baja del precio del commodity, aumento de costos de producción y condiciones climáticas adversas para la producción de cultivos de invierno. El área sembrada en 2016 fue de 215 mil ha con una producción de 757 mil toneladas. El rendimiento promedio en el país en los últimos años superó los 3000 kg/ha, a excepción de la zafra 2012, cuando la fusariosis de la espiga determinó rendimientos muy bajos (DIEA, 2017).

1.3. ROYA DE TALLO DE TRIGO

La roya de tallo (RT), causada por el patógeno *Puccinia graminis* f.sp *tritici* Erik. & E. Henn., (Pgt) fue la enfermedad del trigo más temida a nivel mundial. En epidemias severas se han registrado pérdidas de rendimiento de grano del 100% (Germán y Verges, 2007), provocando que un cultivo aparentemente sano tres semanas antes de la cosecha, quedara reducido a un conjunto de tallos quebrados y granos chuzos (Roelfs *et al.*, 1992).

1.3.1. Patógeno y epidemiología

Pgt es un hongo biótrofo, (sobrevive sólo sobre tejido vivo del hospedero), que se propaga mediante urediniosporas (esporas asexuales) transportadas por el viento a largas distancias hasta de 2000 km, en un lapso de 90 días o menos (Roelfs *et al.*, 1992). Se ha registrado diseminación máxima a distancias de hasta 8000 km en al menos tres instancias. El inóculo inicial para que ocurra la enfermedad puede ser local (endógeno) originado en plantas voluntarias que sobreviven durante el verano, o transportado por el viento y depositado por la lluvia (exógeno). La temperatura óptima para la germinación de las esporas en el caso de RT es mayor a la de roya de la hoja (25 °C y 20 °C respectivamente), y requiere un período de rocío más prolongado (de 6 a 8 horas). Si las esporas se secan se interrumpe la germinación de forma irreversible. Las pústulas (uredinios) se observan sobre la superficie de las hojas y tallos, y también sobre las vainas foliares, espigas, glumas, aristas y hasta en los granos. En la mayoría de las regiones del mundo, el ciclo biológico de Pgt está constituido por generaciones continuas de uredinios sobre los hospederos primarios (trigo, cebada y triticale). Rara vez ocurre el ciclo sexual (producción de aeciosporas sobre su hospedante alternativo *Berberis vulgaris*) donde se generan nuevas combinaciones de genes de virulencia y de agresividad en el patógeno. No se ha documentado la ocurrencia del ciclo sexual en Uruguay y la

región, donde Pgt desarrolló la estrategia de reproducción asexual que le permite mantener los genes de virulencia en combinados (Roelfs *et al.*, 1992).

1.3.2. Severidad y daño de la enfermedad

La RT, por desarrollarse a mayores temperaturas que otras enfermedades, es más frecuente en la fase reproductiva del cultivo de trigo, causando mayores daños en variedades que se siembran o maduran tardíamente (Roelfs, 1985; Roelfs *et al.*, 1992). Las mayores pérdidas de rendimiento ocurren cuando la planta es infectada tempranamente en su ciclo de vida. Infecciones antes de la espizagón pueden causar epidemias severas durante la fase inicial de llenado de grano, provocando grandes pérdidas de rendimiento. Infecciones muy severas de la enfermedad pueden detener el crecimiento de las plantas, o incluso matarlas, por reducir el área fotosintéticamente activa, causar la pérdida de nutrientes y agua, y destruir el sistema de transporte de la planta. Las restricciones en el crecimiento frecuentemente resultan en tallos débiles que se rompen y en granos pequeños y chuzos (Roelfs, 1985).

Greaney (1936) determinó que por cada 10% de incremento de la severidad hay una reducción del rendimiento de un 5,4% en trigos primaverales. Kingsolver y sus colaboradores (1959) encontraron diferencias en las pérdidas de rendimiento según la etapa de desarrollo del cultivo en la que aparece la RT, siendo las pérdidas mayores cuanto antes en el ciclo ocurrieran (98% en embuche y 73% en llenado de grano final). En el sur de Australia, Mayfield (1985) determinó una relación donde la prevención del incremento en 1% de la severidad de RT en el pedúnculo de las plantas, determinó una reducción de 2% en las pérdidas de rendimiento en grano.

En las epidemias más severas ocurridas en Europa, se detectó una pérdida de rendimiento del 9 al 33% en 1951 en Escandinavia y de 5 a 20% en 1932 en el este y centro de Europa. En Minnesota y North Dakota (EEUU), ocho de los 40 años

comprendidos entre 1920 y 1960 tuvieron pérdidas de rendimiento mayores a 10% por RT. En cinco de esos años, las pérdidas superaron el 20%, y en la epidemia más severa (ocurrida en 1935) las pérdidas de rendimiento fueron mayores al 50% (Leonard y Szabo, 2005). En Uruguay, las pérdidas en rendimiento alcanzaron 61% en cultivares susceptibles (Castro *et al.* 2016), habiéndose registrado pérdidas totales en epidemias muy severas (Silvia Germán, com. pers., 15 de setiembre de 2016).

1.3.3. Diversidad del patógeno

La población de Pgt está compuesta por razas que difieren entre sí en su combinación de avirulencia/virulencia frente a genes mayores presentes en el hospedero. Las poblaciones son muy diversas a nivel mundial, existiendo más de 300 razas reportadas en todo el mundo (Mehta, 2014). Los mecanismos más importantes de variación del patógeno son la ocurrencia de mutaciones, la migración de razas dentro de las zonas epidemiológicas y la recombinación sexual en el hospedero alternativo (Roelfs *et al.*, 1992).

En el Cono Sur de América existen dos zonas epidemiológicas para las royas del trigo, la zona oeste (Chile) y la zona este (Argentina, Brasil, Paraguay y Uruguay), separada por la Cordillera de los Andes. Si bien los Andes no permiten el movimiento de razas entre las zonas, se han encontrado razas comunes entre ambas zonas lo que sugiere la existencia de una conexión entre las mismas (Saari y Prescott, 1985; Germán *et al.*, 2007). Desde la década del 50 se han identificado más de 30 razas de Pgt en Argentina y Brasil (Germán y Verges, 2007). En Uruguay, se comenzó a trabajar más recientemente, habiéndose identificado 13 razas desde el año 1996 al 2017 (Silvia Germán, com. pers., 1 de setiembre de 2018). Los genes de resistencia *Sr24* y *Sr31* del set diferencial fueron los únicos resistentes a todas las razas identificadas (Germán, 2012).

1.3.4. Raza Ug99

En el año 1998 fue detectada en Uganda por primera vez la raza TTKSK (Ug99), la primera raza reportada virulenta sobre el gen de resistencia *Sr31* (Pretorius *et al.*, 2000). Además, es virulenta a la mayoría de los genes originalmente presentes en trigo y a *Sr38*, introducido al trigo desde *Triticum ventricosum* (Singh *et al.*, 2011). Estos genes están presentes en varios cultivares europeos y australianos, y en una alta proporción del germoplasma CIMMYT (Singh *et al.*, 2008). En el NE de África, en el 2005, se detectaron variantes de la raza Ug99, con virulencia adicional sobre el gen *Sr24* (Jin *et al.*, 2008). La mayor parte de las variedades de trigo utilizadas a nivel mundial, regional y nacional son susceptibles a Ug99 y a las once razas derivadas de la misma (Ug99+), y por lo tanto, representan una gran amenaza para la producción de trigo (Singh *et al.*, 2011; Germán y Verges, 2007).

Ante esta situación se creó la “Borlaug Global Rust Initiative” (BGRI), para realizar una alerta global por la amenaza de las royas para la producción mundial de trigo. Esta organización es un consorcio internacional formada por el Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) y el Centro Internacional para la Investigación Agropecuaria en Áreas Secas (ICARDA). Los objetivos principales de la BGRI son: reducir la vulnerabilidad del trigo a las royas, facilitar las asociaciones internacionales para contener la amenaza de las royas y mejorar la productividad mundial para resistir las amenazas globales a la seguridad del trigo. Los componentes principales de la BGRI incluyen la formación de una comunidad de instituciones, investigadores y otras organizaciones relacionadas al cultivo de trigo para contar con sistemas de monitoreo y vigilancia de razas de royas a nivel mundial, descubrir nuevos genes de resistencia relevantes para la producción, mejorar técnicas de pruebas de variedades, multiplicación y adopción de nuevas variedades, entrenamiento y desarrollo de capital humano científico, entendimiento de los distintos tipos de resistencia genética a royas y aumentar el nivel de inversiones y coordinación en la investigación. Dentro de las actividades

realizadas por la BGRI, se instaló una plataforma de fenotipado de RT en Kenia de manera que todos los mejoradores a nivel mundial pudieran enviar sus materiales para ser caracterizados *in situ* frente a Ug99+.

1.4. MEDIDAS DE MANEJO PARA EL CONTROL DE ROYA DE TALLO EN TRIGO

La protección de los cultivos puede ser realizada por control químico, cultural y/o resistencia genética. De estos métodos, la resistencia genética es la mayormente preferible ya que no significa un gasto adicional para el productor ni complejidad en el manejo del cultivo, y contribuye a una producción sustentable, amigable con el ambiente.

1.4.1. Control con fungicidas

Si bien la resistencia genética es el método más eficiente y sustentable de manejar la RT, es una tecnología disponible en el mediano y largo plazo, ya que el desarrollo de cultivares resistentes es un proceso que insume varios años. Por este motivo, desarrollar estrategias de control de la enfermedad con fungicidas es fundamental para contar con un manejo disponible en el corto plazo. Además, las pérdidas potenciales causadas por RT en trigo y su consecuente importancia económica, claramente demuestran la necesidad de utilizar fungicidas como medida de control en caso de que el nivel de infección lo justifique (Germán *et al.*, 2011). El criterio para determinar la aplicación de fungicidas es dinámico y debe ser basado según el comportamiento sanitario del cultivar, rendimiento potencial del cultivo, estado vegetativo y niveles de infección del cultivo (Germán *et al.*, 2011).

1.4.1.1. Principios activos disponibles

Los fungicidas disponibles para el control de RT en Uruguay incluyen al menos uno de los siguientes grupos: triazoles, estrobilurinas y carboxamidas. Los triazoles son inhibidores de la síntesis de ergosterol (lípidos esteroidales, constituyente estructural de la membrana celular fúngica) y pertenecientes al subgrupo de los

inhibidores de la demetilación-C14 (DMI). Son sistémicos, con una penetración rápida y distribución acropétala (vía xilema). Las estrobilurinas son inhibidores de la respiración, interfieren el intercambio de electrones en un punto determinado (complejo del citocromo bc1 en las mitocondrias fúngicas), y se denominan inhibidores QoI (=Quinone Outside Inhibitors) por su sitio de acción (Oliver and Hewitt, 2014). Las estrobilurinas impiden la germinación de las esporas y el desarrollo inicial de la enfermedad. Son epistémicos, penetran en forma translaminar por difusión en la fase gaseosa, tienen distribución uniforme en la hoja y dentro de ésta hay difusión mínima. Las carboxamidas también son inhibidores de la respiración, aunque concretamente actúan bloqueando sitios de enlace de la ubiquinona (inhiben a la enzima succinato dehidrogenasa (SDHI) en el ciclo de Krebs, complejo II). Suprimen el crecimiento del tubo germinativo y la formación de apresorios. Pueden tener acción protectora, translaminar o sistémica.

1.4.1.2. Control de roya de tallo con fungicidas

La información sobre estrategias para el control de RT con fungicidas a nivel local, regional y mundial es limitada (Pereyra y Germán, 2012). Los fungicidas son capaces de reducir significativamente ($P < 0.05$) la severidad de RT, el área bajo la curva de progreso de la RT (AUDPC), y aumentar el rendimiento en grano y peso de mil granos (Macharia *et al.*, 2013; Tedesse *et al.*, 2010; Wanyera *et al.*, 2009; Loughman *et al.*, 2005; Mayfield, 1985). Se ha reportado que los fungicidas con mayor eficiencia de control entre los evaluados fueron azoxistrobin + ciproconazol (AmistarXtra 280 SC®), tebuconazol (Folicur 250 EC®, Orius 25 EW®), tebuconazol + triadimenol (Silvacur 375 EC®) y prothioconazol + tebuconazol (Prosaro 250 EC®) (Macharia *et al.*, 2013; Wanyera *et al.*, 2009; Loughman *et al.*, 2005). A su vez, se han reportado menores eficiencias de control con productos como propiconazol, flutriafol (Impact 250 SC®) (Loughman *et al.*, 2005), trifloxistrobin + propiconazol (Stratego 250 EC®), hexaconazol (Cotaf 5 EC®), epoxiconazol + carbendazim (Swing 250 EC®), cyproconazol + propiconazol (Artea 220 EC®) y epoxiconazol +

carbendazim (Soprano C 250 EC®) (Wanyera *et al.*, 2009). En Argentina, ensayos realizados aplicando tebuconazol (Brach y Cracogna, 2016) y azoxistrobin + epoxiconazol (Alberione *et al.*, 2017) lograron controlar la enfermedad y disminuir la pérdida de rendimiento en comparación con el testigo sin aplicación de fungicida.

En cultivares susceptibles, fueron necesarias aplicaciones semanales de fungicidas para lograr un control total de RT (Tadesse *et al.*, 2010). Tanto en Australia como en Etiopía, las aplicaciones más eficientes fueron realizadas antes de que la infección sea visible o a muy bajos niveles de infección, y no se aconseja la aplicación de fungicidas cuando la enfermedad está avanzada o en infecciones tardías como en grano lechoso (ZGS 77) (Tadesse *et al.*, 2010; Loughman *et al.*, 2005). En Argentina, la doble aplicación de fungicidas fue la que determinó rendimientos en grano significativamente mayores, y las aplicaciones tardías no se diferenciaron del testigo sin aplicación de fungicidas (Alberione *et al.*, 2017). No hay trabajos publicados sobre tecnología de aplicación que, principalmente en cultivos densos, debe asegurar la llegada del producto hasta los puntos de infección en los tallos y vainas.

A nivel nacional, las mezclas de triazoles y estrobilurinas tienen mayor eficiencia de control general para todas las enfermedades foliares del cultivo (Pereyra y Germán, 2012). Sin embargo, el uso de estrobilurinas para el control de RT puede ser riesgoso al desarrollarse la enfermedad cercana al período crítico para el desarrollo de fusariosis de la espiga (causado por una o más especies del género *Fusarium*). Se ha reportado que la aplicación de estrobilurinas, si bien controla el desarrollo de la fusariosis de la espiga, incrementa la cantidad de toxina deoxinivalenol (DON) en grano (Díaz y Pereyra, 2011).

1.4.2. Resistencia genética a roya del tallo en trigo

La resistencia genética es la mejor medida para controlar las royas, ya que mediante el uso de cultivares resistentes se logra no sólo reducir los daños causados por la enfermedad, sino también, frenar su diseminación (Johnson, 1981).

La resistencia a RT puede ser clasificada en dos categorías descritas como resistencia de plántula (ASR, "*all stage resistance*") que se expresa durante todo el ciclo del cultivo y resistencia de planta adulta (APR, "*adult plant resistance*"). La ASR se expresa en todas las etapas fisiológicas de la planta. Está caracterizada por ser monogénica y oligogénica, basada en genes de herencia cualitativa, dominantes y de efecto mayor, que siguen la relación "gen por gen" entre patógeno y hospedero descrita por Flor (1956), donde cada gen en el hospedero confiere resistencia únicamente a razas del patógeno que contienen el gen de avirulencia complementario. Es por lo tanto una resistencia raza-específica, y su mecanismo se basa en la reacción de hipersensibilidad (Ayliffe *et al.*, 2008). En la mayoría de los casos, esta resistencia no ha sido durable ya que puede dejar de ser efectiva por la aparición de razas del patógeno con virulencia específica. La APR, en contraste, se expresa mejor en estadios post-plántula. Se basa en genes de herencia cuantitativa de efecto menor, que confieren resistencia parcial o de enroyamiento lento (asociado con periodos de latencia largos, pocas y pequeñas uredinias y reducida producción de esporas). Es necesaria la acumulación de 4 o 5 genes menores para obtener altos niveles de resistencia cercana a la inmunidad (Bhavani *et al.* 2011, Singh *et al.* 2000). La APR es mayormente raza no-específica y es considerada duradera.

A la fecha, se han reportado más de 60 genes de resistencia a RT (Yu *et al.* 2014, McIntosh *et al.* 2014, McIntosh *et al.* 1995). La mayoría de estos confieren resistencia desde el estado de plántula, sólo cinco confieren APR y en trabajos recientes se han reportado muchos QTLs asociados a resistencia a RT (Yu *et al.* 2014). Desde la aparición de Ug99+, se han realizado grandes esfuerzos para

afrentar la amenaza de la posible dispersión de estas razas, incluyendo trabajos para identificar nuevos genes de resistencia a RT para uso para el mejoramiento genético.

1.5. MÉTODOS PARA ESTUDIAR LA BASE GENÉTICA DE LA RESISTENCIA A ENFERMEDADES

Existen varios métodos para estudiar la base genética de la resistencia a enfermedades. En el caso de las royas, los métodos más utilizados son: estudios de herencia, postulación de genes y métodos basados en genética molecular, donde se incluyen los marcadores moleculares y distintas formas de realizar asociaciones entre fenotipo y genotipo.

1.5.1. Estudio de herencia

Los estudios de herencia convencionales se utilizaron previamente a los estudios moleculares para identificar el número de genes de resistencia y tipo de dominancia presentes en materiales de trigo. Se requiere realizar un cruzamiento entre la fuente de resistencia y una línea susceptible. Si la resistencia a estudiar es monogénica, en la generación F_1 se puede determinar la dominancia de la resistencia evaluando los fenotipos. En base a la proporción de plantas F_2 resistentes en relación a la proporción de plantas susceptibles se puede inferir el número de genes de resistencias presentes en la población segregante. También se pueden estudiar las proporciones de segregación de una población BC_1F_1 (retrocruza 1 F_1) utilizando al padre susceptible como recurrente. Este tipo de población provee un background genético más uniforme, muy conveniente en estudios de campo (Roelf *et al.*, 1992).

1.5.2. Postulación de genes

El objetivo de la postulación de genes es identificar los genes de resistencia (Roelf *et al.*, 1992). Esta metodología está basada en la comparación del tipo de infección (TI) de un material de trigo cuyos genes de resistencia se desconocen, con el TI de líneas isogénicas que contienen un solo gen de resistencia. Se debe utilizar un conjunto de razas de *P. graminis* que difieran en su combinación de avirulencia/virulencia. Se parte del conocimiento de la relación hospedero/patógeno que opera en este sistema, donde los genes de resistencia y los genes de avirulencia del patógeno actúan de forma complementaria de acuerdo a la teoría “gen por gen” formulada por Flor (1956). Para cada gen que condiciona resistencia en la planta hospedera, existe un gen específico que condiciona avirulencia en el patógeno. En función de las combinaciones de genes presentes en el patógeno y en el huésped, la interacción puede determinar una reacción de incompatibilidad o de compatibilidad. Alcanza con la presencia de un único gen de resistencia en el hospedero, con el correspondiente gen de avirulencia en el patógeno, para que la reacción sea incompatible. Una reacción de susceptibilidad en el material de trigo estudiado indica que no tiene ninguno de los genes de resistencia para los cuales el patógeno es avirulento. Cuando el material tiene el mismo patrón de TI que una línea isogénica específica, se postula la presencia de ese gen. La genealogía de la línea evaluada puede también ayudar a realizar una postulación cuando se conoce la resistencia de los progenitores (Roelfs *et al.*, 1992). Este método tiene como ventajas que se puede efectuar rápidamente (4 semanas) y que es bastante preciso y fácil cuando existen pocos genes. Sin embargo, si bien se demuestra en forma concluyente la ausencia de un gen de resistencia, la presencia de un gen específico se puede postular pero no confirmar. Además se necesita una colección de aislamientos del patógeno con combinaciones de avirulencia/virulencia diversas, lo que implica un buen manejo del inóculo y de razas.

1.5.3. Confirmación de la identidad de genes de resistencia

El test de alelismo tiene como objetivo confirmar la identidad de los genes presentes en determinada variedad. Para esto, es necesario cruzar dicha variedad con otro material que contenga el gen de resistencia postulado y estudiar la segregación en una población F2 con un alto número de individuos, utilizando una raza avirulenta sobre el gen postulado. La ausencia de plantas susceptibles en dicha generación confirma la identidad del gen de resistencia en estudio (Roelfs *et al.*, 1992). Otro mecanismo para determinar la identidad de los genes de resistencia es el uso de marcadores moleculares perfectos, que se encuentran dentro de la secuencia del mismo gen de resistencia. La obtención de estos marcadores se logra con la aplicación de herramientas biotecnológicas de clonación y secuenciación sobre líneas mutantes para el gen de resistencia en cuestión. Sin embargo, no son muchos los genes *Sr* para los cuales se ha logrado obtener marcadores moleculares perfectos. En la actualidad, varios grupos de investigación se encuentran avocados a aumentar el número de marcadores disponibles (Mago *et al.*, 2013; Ejaz *et al.*, 2012; Mago *et al.*, 2009; Liu *et al.*, 2010; Tsilo *et al.*, 2008; Tsilo *et al.*, 2007; Mago *et al.*, 2005).

1.5.4. Marcadores moleculares

Los marcadores moleculares son una importante herramienta para el mejoramiento genético vegetal que permite detectar la variabilidad genética disponible en una colección de germoplasma. La variabilidad genética detectada ha sido muy útil para el entendimiento de la dinámica del genoma. El uso de marcadores moleculares ha determinado un aumento de eficiencia en el mejoramiento genético (Varshney, 2010). Muchos de estos marcadores moleculares desarrollados hasta el presente están asociados a importantes caracteres agronómicos, como estreses abióticos y bióticos, y se encuentran disponibles para su uso en el mejoramiento a través de la selección asistida por marcadores

moleculares (MAS). Hasta hace muy poco tiempo, los marcadores moleculares más utilizados fueron los RFLP (polimorfismo en el largo de los fragmentos de restricción), AFLPs (polimorfismo en el largo de los fragmentos amplificados) y SSR (microsatélites o secuencias simples repetidas). Si bien su aplicación es muy efectiva para identificar los efectos de genes que codifican características simples, su uso para caracteres complejos, producto de la interacción de muchos genes, no es tan eficiente. Además, requieren de alta inversión en tiempo y recursos, ya sea para su desarrollo como para su aplicación, lo que determina que la caracterización de poblaciones con un alto número de individuos no sea muy conveniente (Gupta *et al.*, 2008).

Más recientemente los SNP (polimorfismo en una sola base) fueron creados en base a la automatización de la generación de información de la secuencia del ADN (secuenciación masiva), y la identificación de polimorfismos de una sola base automática mediante algoritmos informáticos. Así, se convirtieron en los marcadores más utilizados por su abundancia y distribución uniforme a lo largo del genoma (Gupta *et al.*, 2008). Actualmente hay más de 30 métodos disponibles para realizar el genotipado con SNPs que permiten el análisis de muchas muestras a un costo cada vez más alcanzable. El estándar de información de los marcadores necesario según el objetivo del estudio a realizar, determina cuál de los diferentes métodos de secuenciación a utilizar. Al genotipar para realizar MAS o mapeo de QTL, donde las líneas a secuenciar tienen un limitado número de polimorfismos y los genotipos de los progenitores son conocidos, el genotipado de alta cobertura, como lo es el GBS (“genotyping-by-sequencing”) es suficiente para inferir ligamientos, siempre que se cuente con un genoma de referencia disponible (Gupta *et al.*, 2008).

1.5.5. Métodos para el estudio de asociación entre genotipo y fenotipo

La mayoría de los caracteres de interés agronómico, como rendimiento, calidad y resistencia a algunas enfermedades, están controlados por múltiples genes de efecto aditivo. Algunos de estos genes, a su vez, están ligados entre sí o tienen efectos pleiotrópicos. El conocimiento de su posición y efecto es importante para utilizarlos eficientemente en los programas de mejoramiento genético vegetal. A través del estudio de la segregación entre marcadores genéticos y el valor fenotípico en poblaciones amplias o biparentales, es posible detectar y localizar los *loci* que afectan estos caracteres cuantitativos o QTL (Asins *et al.*, 2010). Específicamente, lo que se busca es identificar la acción, interacción, número y ubicación precisa de dichas regiones (Miles y Wayne, 2008).

1.5.5.1. Mapeo de QTL en poblaciones biparentales

El mapeo de QTL basado en poblaciones biparentales es un método estadístico que combina la información fenotípica (medida del carácter) e información genética (presencia o ausencia de marcadores moleculares en los individuos fenotipados, así como la posición relativa en el genoma de esos marcadores moleculares), con el objetivo de explicar la base genética de la variación para caracteres complejos o cuantitativos (Asins *et al.*, 2010). Los insumos para realizar este análisis son: disponer de una población biparental, información genotípica y fenotípica de la misma, y construcción de un mapa de ligamiento (Collard *et al.*, 2005).

La población debe ser creada a partir de la hibridación de dos líneas parentales que difieran entre sí en uno o más caracteres cuantitativos de interés (Broman, 2001). El tamaño de la población generalmente varía entre 50 a 250 individuos, aunque pueden requerirse poblaciones más grandes para un mapeo de alta resolución (Collard *et al.*, 2005). Pueden crearse distintos tipos de poblaciones

biparentales para este estudio. Dentro de las más utilizadas están las poblaciones F2 (derivadas de híbridos F1), poblaciones retrocruzas (derivados de la retrocruza de la F1 con uno de los padres), líneas recombinantes endocriadas (RILs, líneas altamente homocigotas con combinaciones únicas de segmentos cromosómicos de los progenitores) y poblaciones de haploides duplicados (DH) (Abdurakhmonov y Abdugarimov, 2008). Las ventajas de las poblaciones RILs es que son altamente homocigotas, conllevan alto número de recombinaciones (en cada evento meiótico ocurre una recombinación) y, al ser líneas estabilizadas, pueden ser multiplicadas y utilizadas en múltiples localidades y años.

A partir del estudio de segregación entre los marcadores genéticos de la población creada, se realiza la construcción de un mapa de ligamiento. Éste indica la posición y la distancia genética entre marcadores a lo largo de los cromosomas (Broman, 2001). Para su construcción, se parte del principio que genes y/o marcadores segregan en función de la recombinación cromosómica que ocurre durante la meiosis. Genes o marcadores que están cercanos físicamente o estrechamente ligados serán transmitidos juntos a la progenie más frecuentemente que aquellos que estén localizados distantemente. La frecuencia de genotipos recombinantes puede ser usada para calcular la frecuencia de recombinación y así inferir la distancia genética entre marcadores. Marcadores o genes que tienen una frecuencia de recombinación de 50% son descritos como “no-ligados” y se asume que están posicionados distantes en un cromosoma o en diferentes cromosomas.

El mapeo de QTL se basa en detectar asociación entre el fenotipo y genotipo (Asins *et al.*, 2010). Marcadores que están genéticamente ligados a un QTL que influencia el carácter de interés, segregarán más frecuentemente con valores fenotípicos altos o bajos, mientras que marcadores que no están ligados, no mostrarán asociación significativa con el fenotipo (Miles and Wayne, 2008). Existen varios métodos estadísticos para detectar QTLs. Entre ellos, los más ampliamente utilizados son: análisis de marcador individual (SMA, single-marker analysis),

mapeo por intervalo simple (SIM, simple interval mapping) y mapeo por intervalo compuesto (CIM, composite interval mapping) (Collard *et al.*, 2005).

SMA es el método más simple que incluye el análisis de cada marcador por separado con pruebas *t*, análisis de varianza (ANOVA), análisis de Kruskal-Wallis o/y regresión lineal con el valor fenotípico. Este método no requiere necesariamente de la construcción de un mapa de ligamiento y tiene como principal desventaja que, en mapas poco saturados, la recombinación que ocurre entre los marcadores mejor asociados y el QTL determina que haya dificultad para detectar dichos QTL y/o se subestima el efecto de los mismos. El método SIM utiliza el mapa de ligamiento y analiza los intervalos formados por cada par de marcadores ligados a lo largo de todo el cromosoma, simultáneamente (Broman, 2001). A su vez, se crean “pseudomarcadores” entre marcadores simultáneos, y en función de la recombinación entre ellos se le asigna el valor más probable a cada línea. El uso del mapa de ligamiento compensa la recombinación que puede existir entre marcadores y QTLs y es considerado estadísticamente más poderoso que el método SMA. En la actualidad, el método más utilizado es el CIM, que realiza el mapeo por intervalos e incluye cofactores dentro del modelo. Para cada intervalo se define una “ventana” donde actúan cofactores que equivalen a los efectos de QTLs encontrados fuera de dicha ventana, lo que permite estimar y eliminar las interacciones del *background* genético. Los resultados estadísticos de SIM y CIM son presentados utilizando el logaritmo de odds (LOD) o estadístico de la razón de verosimilitud (LRS) ($LRS = 4.6 \times LOD$) (Liu, 1998 citado por Collard *et al.*, 2005). La posición más probable del mapa donde se encuentra el QTL es aquella que tenga el valor LOD más alto. Una vez que el o los QTLs han sido identificados, se pueden generar mapas densos para esa región cromosómica y fenotipado de poblaciones más grandes que permitan el mapeo fino, y finalmente el secuenciado de los amplicones más asociados a la resistencia para asociar dichos QTL a genes candidatos (Miles and Wayne, 2008).

Si bien el mapeo de QTLs es muy utilizado para identificar la base genética de la resistencia a enfermedades, el hecho de necesitar una población segregante de una cruce definida limita la base genética del carácter de interés a las diferencias entre los parentales, siendo ésta muchas veces poco representativa del germoplasma en uso en los programas de mejoramiento (Mather *et al.*, 1997; Kraakman *et al.*, 2004).

1.5.5.2. Mapeo asociativo

El mapeo asociativo (MA) surgió como alternativa al mapeo de QTL basado en poblaciones biparentales. La técnica fue desarrollada para investigación en humanos, donde la creación de poblaciones segregantes balanceadas presentaba dificultades de diversos tipos (Cardon y Bell, 2001). El MA consiste en la identificación de QTLs que codifican para caracteres cuantitativos de interés, mediante el estudio del desequilibrio de ligamiento (grado de asociación no aleatoria entre alelos de distintos *loci*) entre los marcadores moleculares y dichos valores fenotípicos, pero en un panel de germoplasma amplio, no limitado a una población biparental (Zhu *et al.*, 2008). Algunas de las ventajas que ofrece el MA son una mayor resolución de los mapas genéticos creados (explicado por una mayor historia de recombinación en la población utilizada), menor tiempo de investigación necesario (no se necesita crear la población partiendo de cruzamientos dirigidos y se puede utilizar la información histórica de un programa de mejoramiento, por ejemplo) y un mayor número de alelos en estudio (en poblaciones biparentales hay únicamente dos alelos posibles para cada *locus*) (Yu y Buckler, 2006). Sin embargo, el MA tiene poder limitado para detectar variantes alélicas presentes en baja proporción, como puede ser el caso de algunos alelos de resistencia a enfermedades (Brachi *et al.*, 2011) o *loci* con múltiples variantes alélicas (Zhang *et al.*, 2012).

Se han realizado grandes esfuerzos utilizando estas valiosas herramientas moleculares en todo el mundo para identificar la base genética de la resistencia a RT desde la aparición de la raza Ug99 (Gao *et al.*, 2017; Babiker *et al.*, 2016; Li *et al.*, 2016; Guerrero-Chavez *et al.*, 2015; Chen *et al.*, 2015; Lopez-Vera *et al.*, 2014; Singh *et al.*, 2013; Haile *et al.*, 2012; Hiebert *et al.*, 2010; Kaur *et al.*, 2009) y volcar dicho conocimiento en el mejoramiento por resistencia.

1.6. MEJORAMIENTO GENÉTICO POR RESISTENCIA A ROYA DEL TALLO

El uso generalizado del gen *Sr31* de resistencia a RT, ubicado en la translocación *1BL.1RS* del centeno, contribuyó al control mundial de la enfermedad durante muchos años (Singh *et al.*, 2000). La ausencia de RT durante un largo período de tiempo ha disminuido las oportunidades de seleccionar por resistencia y también la prioridad que los programas de mejoramiento han asignado a esta característica (Germán *et al.*, 2007). Sin embargo, la emergencia y diseminación de Ug99+ determinó una inminente amenaza para la producción mundial de trigo y una importante necesidad de desarrollar cultivares con resistencia duradera a RT. La combinación de varios genes, ya sean genes ASR, APR o la combinación de ambos, en variedades adaptadas, en comparación con la creación de variedades con un único gen de resistencia, es considerada un método más eficiente para generar resistencia genética más durable y combatir el avance de las nuevas razas (Haile and Röder, 2013).

Se han descrito cinco genes que confieren APR (*Sr2*, *Sr55*, *Sr56*, *Sr57* y *Sr58*), los cuales poseen un alto valor para la producción de trigo porque las regiones genómicas donde se localizan, también confieren resistencia a otras enfermedades relevantes para el cultivo (*Sr2/Yr30*, *Yr* resistencia a roya estriada, *Sr57/Lr34/Yr18/Pm38* *Lr* resistencia a roya de la hoja, *Pm* resistencia a oídio, *Sr58/Lr46/Yr29/Pm39*, respectivamente) (Singh *et al.*, 2011). La utilización de APR en los programas de mejoramiento genético es difícil debido a la necesidad de

acumular cuatro a cinco genes y/o QTLs para determinar niveles de resistencia adecuados (Singh *et al.*, 2011). Además, no es posible identificarlos en presencia de resistencia conferida por genes mayores como lo son el *Sr31* y *Sr24*, los cuales están ampliamente utilizados en el germoplasma de Uruguay.

Otra estrategia de mejoramiento por resistencia a RT es diversificar la base genética de la resistencia en el cultivo, con la introducción de genes de herencia cualitativa. Para esto es necesario incorporar genes efectivos ASR en combinaciones de al menos dos, para incrementar la duración de la resistencia. Cuando se acumulan dos o más genes mayores (ASR) efectivos en un mismo cultivar, el patógeno requiere dos o más mutaciones a virulencia simultáneas para quebrar la resistencia (Mundt, 2004; Singh *et al.*, 2000). Esto disminuye considerablemente la probabilidad de que aparezcan razas virulentas. En el caso del mejoramiento utilizando genes mayores, la selección fenotípica es muy efectiva para identificar genotipos resistentes. Sin embargo, no permite discriminar entre presencia de uno o más genes de resistencia efectivos frente a la población local. Identificar la presencia de varios genes de resistencia mayores efectivos acumulados es ahora posible con el uso de marcadores moleculares.

1.7. MEJORAMIENTO GENÉTICO DE TRIGO EN URUGUAY

El mejoramiento genético de trigo en Uruguay tuvo sus comienzos en 1912 en Toledo, y en 1913 continuó en Bañado de Medina, Cerro Largo. En 1914 se trasladó al departamento de Colonia, donde se instaló La Estanzuela, Semillero Nacional, dirigido por el Dr. Alberto Boerger. Desde su fundación, La Estanzuela centró sus esfuerzos fundamentalmente en la creación y difusión de mejores variedades de los principales cultivos del país, desarrollando nuevos cultivares adaptados y proporcionándole a los productores semilla de alta calidad (Germán y Luizzi, 2018). Durante este período a la actualidad, se han liberado más de 65 cultivares de trigo con alto impacto en la producción nacional. Los objetivos de mejoramiento se

fueron incorporando con los cambios en las prácticas de manejo del cultivo y cambios en los patógenos. Los más importantes son rendimiento y calidad molinera e industrial, combinada con caracteres que aseguren la expresión de los anteriores: menor altura, resistencia a vuelco, desgrane, brotado en la espiga y resistencia a las enfermedades prevalentes (roya de la hoja, septoriosis de la hoja, mancha amarilla, fusariosis de la espiga, roya de tallo) y también considerando enfermedades poco prevalentes (oídio y roya estriada).

A partir de la aparición de la raza Ug99 y desde el resurgimiento de la RT a nivel epidémico en Uruguay en 2014, el mejoramiento genético por resistencia a RT se priorizó como un objetivo relevante dentro del Programa Nacional de Mejoramiento Genético de Trigo. En 2005 se comenzaron a evaluar en Kenia líneas avanzadas para determinar su resistencia a Ug99+. El cultivar INIA Tero, liberado por INIA en el año 2005 y la línea BR23//CEP19/PF85490 (EMBRAPA-CNPTrigo, Brasil) fueron resistentes a RT en Uruguay y en la plataforma de fenotipado en Kenia, y están siendo ampliamente utilizados para el desarrollo de nuevas variedades en el PMGT-INIA. Sin embargo, se desconoce la base genética que confiere la resistencia a RT, la cual es fundamental para diseñar estrategias de mejoramiento genético por resistencia. Disponer de esta información permitirá ser más eficiente en el proceso de mejoramiento, evitando redundancia y facilitando la identificación de nuevas fuentes a ser utilizadas para ampliar la diversidad genética de la resistencia.

2. FUNGICIDE STRATEGIES TO MANAGE WHEAT STEM RUST

2.1. SUMMARY

Stem rust (SR) was considered one of the most devastating diseases worldwide as it could cause complete grain yield losses under favorable conditions in susceptible varieties. The threat of the spread of African races (Ug99+), virulent over the principal genes conferring resistance in South America (*Sr24* and *Sr31*) and the re-emergence at epidemic levels of SR in Uruguay due to the high proportion of susceptible cultivars used by farmers, determined the need to develop tools for SR management. The objective of this study was to develop an efficient SR management based on foliar fungicide, in order to have a short-term available tool under Uruguayan productive conditions. Results from field trials carried out during 2015 and 2016 at INIA La Estanzuela (LE) indicated that fungicide application at disease onset was the main factor to manage SR with fungicides in a susceptible and moderately susceptible cultivar. Thirty-eight percent grain yield increase was achieved when fungicides were sprayed at disease onset rather than applying them at watery-milky grain ripening stage. Additionally, applying fungicides early in the SR epidemic resulted in 26% greater test weight and 63% higher thousand-kernels weight values than a late application. From plots that were sprayed late in the growing cycle, 94% of the grain samples had grain residue of the active ingredients metconazole and/or epoxiconazole. However, concentrations were lower than the maximum residue limit established by the European Union. The use of TTJ60 nozzles in a susceptible cultivar lead to lower SR severity levels at crop maturity as compared to hallow cone nozzles. Preliminary results indicated that active ingredient combinations play a secondary role in SR management, yet, more research is warranted.

Keywords: wheat, stem rust, foliar fungicides, grain yield

2.2. INTRODUCTION

Bread wheat (*Triticum aestivum*) is one of the most important crops worldwide and in South America, where 8.6 million ha approximately a year are cultivated in Uruguay, Argentina, Brazil, Chile and Paraguay (FAOSTAT 2016). In the Southern Cone of America, cereal diseases are one of the main constraints to achieve higher grain yields (Germán *et al.* 2007).

Wheat stem rust (SR) caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. and E.Henn. (Pgt), was considered one of the most devastating diseases worldwide as it could cause grain yield losses up to 100% in susceptible varieties under favorable conditions (Roelfs *et al.* 1985, Leonard and Szabo, 2005). SR was successfully controlled over 30 years, as a result of significant efforts to breed for SR resistance (Singh *et al.* 2008). However, the appearance of Pgt race Ug99 (TTKSK) in Uganda in 1998 posed a new threat for wheat crop (Pretorius *et al.* 2000). Ug99 is widely known because it is virulent to the SR resistance gene *Sr31*, which was extensively used by CIMMYT and provided protection against all prevalent Pgt races worldwide (Jin *et al.* 2008). Ug99 is also virulent to most *Sr* genes of wheat origin (Singh *et al.* 2011), and to more than 90% of the current wheat varieties worldwide (Singh *et al.* 2015). Several variants of this race have been identified, including some with additional virulence to *Sr24* and *Sr36* (Jin *et al.* 2008, 2009), which together with the original Ug99 and other variants, have been designated as the Ug99 lineage group (Ug99+). The evolution and spread of Ug99+ out of Eastern Africa is one of the foremost threats to wheat production and food security (Singh *et al.* 2011).

In Southern Cone of America, *Sr24* and *Sr31* are still effective and are currently the most important genes conferring resistance to SR. By 2008, the area grown with cultivars carrying these genes exceeded half of the total wheat area (Germán *et al.* 2009). Since early 2000's, high yielding European cultivars susceptible to SR have been introduced and the total acreage with SR susceptible cultivars has significantly

increased in Argentina and Uruguay. This situation led to the re-emergence of SR at epidemic levels since 2014 in Uruguay and Argentina (Campos *et al.* 2014).

Stem rust will likely increase in importance in the future due to several reasons. The high proportion of the grown area with susceptible cultivars entails a significant area where the pathogen can over-winter on volunteer plants and provide primary inoculum for the next season. Subsequently, secondary infections may develop during the growing season, leading to a significant production of inoculum and fast development of SR epidemics. Global warming favors SR development, since *P. graminis* is adapted to relatively high temperatures compared to other rust diseases. The potential spread of Ug99+ represents a significant threat to wheat production in the Southern Cone region, since most wheat cultivars, which are currently resistant would become susceptible. Therefore, strategies to manage SR in wheat need to be developed in order to minimize yield losses caused by the disease.

The use of resistant varieties is considered the most economical, efficient and environmentally safe method to control SR. Medium to long term efforts pursuing the development of adapted lines resistant to local and Ug99+ races are currently ongoing. However, the optimization of strategies based on the use of foliar fungicides to manage SR in the short term is also required. Since the appearance of Ug99, many studies to adjust wheat SR control with fungicides were carried out mainly in Africa (Macharia *et al.* 2013, Tedesse *et al.* 2010, Wanyera *et al.* 2009). However, conditions for SR epidemics during the wheat growing season and production technologies available in Uruguay and South America are different from those in other regions. Therefore, the evaluation of application strategies and available active ingredient efficacies is necessary for efficient SR control in Uruguay. In order to optimize SR management using foliar fungicides under Uruguayan conditions, the effect of two spraying times and two nozzle types were studied on a susceptible and a moderately susceptible cultivar. Additionally, the effect of

different active ingredient combinations on SR development, grain yield, and kernel and test weight were evaluated on a susceptible cultivar.

2.3. MATERIAL AND METHODS

2.3.1. Field experimental settings

Field trials were conducted in 2015 and 2016 at La Estanzuela (LE) Research Station, Instituto Nacional de Investigación Agropecuaria (INIA), Colonia, Uruguay (34° 20' S, 57° 41' W) on a clay-loam soil (organic matter 2.1 to 4%, pH 6.2 to 7.0, Al<0.1 meq/100g, P>5 ppm). Conventional tillage was used and experiments were maintained under rainfed conditions.

Experimental units were 5 m long six-row plots , with an inter-row space of 0.17 m. Three-row early grow triticale was planted on either side of each plot to avoid fungicides drift. Trials were planted on July 21st each year, using an experimental seeder (Plotman, Wintersteiger®, Austria) at a 110 kg/ha seeding rate. Plots were fertilized with nitrogen at sowing, ZGS 22 and ZGS 30 (Zadoks *et al.* 1974) following a Nitrogen Fertilization Model (Baethgen 1992) and maintained free of weeds and insects. To ensure high and uniform SR infection, plots were inoculated twice to three times. Inoculations were made either with a suspension of uredinospores in Soltrol 170 mineral oil (Phillips Petroleum Co., Borger, TX) late in the afternoon with an ultralow volume sprayer (Ulva+®, Micron) or injecting tillers from tillering stage (ZGS 22) with a suspension of spores in water with few drops of Tween 20, until disease onset was observed. Inoculum consisted of a mixture of the three most representative *P. graminis* local races (RHKTF, RRKTF and SPLKC) maintained at the Rust Laboratory at INIA, increased on the susceptible cultivar Little Club under greenhouse conditions. After crop maturity, plots were harvested using an experimental harvester (Classic, Wintersteiger®, Austria).

2.3.2. Spraying strategies experiment

During 2015 and 2016, two experiments per year with one SR susceptible (S) cultivar and one SR moderately susceptible (MS) cultivar (Castro *et al.* 2016) were planted in a split-plot design with four replicates. In each experiment, spraying time was the main plot (factor A) and nozzle type was the subplot (factor B). Factor A included fungicide applications at: i) disease onset (DO) and ii) at watery – milky grain stage (WMG, ZGS 75). Factor B included: i) standard hollow cone nozzles (TXR ConeJet-model, TeeJet®, 95 L/ha and 0.5 m spacing) and ii) Turbo TwinJet 60° nozzles (TTJ60, TeeJet®, 284 L/ha and 0.5 m spacing). Swing Plus® (epoxiconazole 27.5 g/L + metconazole 37.5 g/L) was applied at 1.5 L/ha with a CO₂ propellant backpack sprayer. Non-treated plots served as control.

2.3.3. Fungicides efficiency experiment

A randomized complete block design (RCBD) with four replicates was used in 2015 and 2016 with susceptible SR cultivar Baguette 601 (Castro *et al.* 2016). Active ingredients included triazoles (DMIs), strobilurins (QoIs) and carboxamides (SDHIs) regarding their wide use to control stem rust and other relevant foliar diseases in Uruguay. Fungicide treatments were: i) tebuconazole (Tebucale 430®) at 0.58 L/ha, ii) metconazole + epoxiconazole (Swing Plus®) at 1.50 L/ha, iii) pyraclostrobin + epoxiconazole (Abacus®) at 0.50 L/ha, iv) azoxystrobin + cyproconazole (Amistar Gold®) at 0.40 L/ha, v) fluxapyroxad + prothioconazole (Vigo® + Proway®) at 0.30 and 0.50 L/ha, respectively, and vi) pyraclostrobin + epoxiconazole + fluxapyroxad (Xantho®) at 1.20 L/ha. The non-treated plots served as control, while plots including applications of 0.40 L/ha azoxystrobin + cyproconazole twice during the disease development served as a “disease-free” control treatment. Fungicides were applied at first visible symptoms with a CO₂-propellant backpack sprayer equipped with four standard hollow cone nozzles (HCN, TXR ConeJet-model, TeeJet® Spraying Systems Co., Wheaton, IL 60188) 0.5 m apart, in the central four rows of each plot, at a water volume of 95 L/ha and 3.06 kg/cm² of pressure. During 2015, due to

unfavorable weather conditions, fungicides application was delayed and stems had 50% SR severity by that time.

2.3.4. Disease assessment

SR was assessed four times: 1) at disease onset (first visible symptoms), 2) watery-milky grain stage (ZGS 75), 3) hard dough (ZGS 87) and 4) physiological maturity (ZGS 99). The visual modified Cobb's scale (Peterson *et al.* 1948) was used to score SR severity (SRS, percentage of tissue infected). Stem rust infection response (IR) was also scored, according to Roelfs *et al.* (1992), where susceptible, moderately susceptible, moderately resistant, and resistant reaction, corresponded to S, MS, MR and R, respectively. For each IR category, a coefficient was assigned as follows: 1, 0.8, 0.4, and 0.2, respectively (Stubbs *et al.*, 1986) to calculate the SR coefficient of infection ($CI = SRS \times IR \text{ coefficient}$). The area under disease progress curve (AUDPC) was calculated (Campbell and Madden, 1991) based on CI data.

2.3.5. Grain yield and quality

Whole plots were harvested for grain yield estimations (GY, kg/ha) using a standard scale. Thousand kernels weight (TKW, g) and test weight (TW, kg/hl) were estimated from random samples taken from the total harvested grains from each experimental plot.

2.3.6. Grain fungicide residue

Additionally, grain samples from all treatments in the fungicide strategies experiments from 2016 were analyzed for epoxiconazole and metconazole residues in grain at the *Departamento de Química del Litoral, Estación Experimental Mario A. Cassinoni*, Paysandú, Uruguay.

Samples were ground (IKA, mill, Campinas, Brazil) to obtain flour fractions. Then, the samples were extracted using a modification of the original QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe). The extraction step was

performed on 5 g -sample, extracted with 10 mL of ultrapure water and 10 mL of acetonitrile in a Stuart SSL1 orbital shaker (Staffordshire, UK) at room temperature for one hour at 250 rpm. Afterwards, 4 g MgSO₄ and 1 g NaCl were added, manually shaken for one minute, and centrifuged for three minutes at 5000 rpm. Extract purification consisted of an overnight freeze-out step of the extract at -20 °C. Then, 4 mL aliquot of the extract was transferred to a 15 mL polytetrafluorethylene (PTFE) tube containing 0.6 g MgSO₄, 0.1 g C-18, and 0.6 g of primary and secondary amine (PSA). The extract were shaken in a Vortex mixer for 30 s and centrifuged at 5000 rpm for three minutes. Finally, 1 mL of the extract was filtered through a 0.45 µm PTFE filter for HPLC-QqQ/MS determination in the multiple reaction monitoring mode (MRM).

Values were compared to the Maximum Limit of Residue in grain (MLR) settled by Codex Alimentarius (Joint FAO/WHO Codex Alimentarius Commission, 2007).

2.3.7. Data analysis

InfoStat® statistical package, version 2016 (Di Rienzo *et al.* 2016) was used for the analysis of variance (ANOVA) of CI, AUDPC, GY, TW and TKW. Mean values were compared by Tukey HSD test at $\alpha=0.05$, whenever the treatment effect was deemed significant. Lineal regressions were used to estimate GY, TKW and TW losses associated to SR severity.

2.4. RESULTS

2.4.1. Stem rust development

SR was firstly observed at flowering stage (ZGS 65) in the S cultivar and MS cultivar both, in 2015 and 2016. Final SRS levels in the non-fungicide treatment (NF) were 90% on the S cultivar and 50% on the MS cultivar in 2015, whereas these levels were 90% and 20%, respectively in 2016. Although final SRS was similar both years on the S cultivar, SR during disease development was significantly higher in 2015.

2.4.2. Grain yield, thousand kernel weight and test weight losses associated to stem rust infection

Based on a simple linear regression with SRS data collected in 2015 and 2016, % losses per 1% increase of SRS between ZGS 71 and ZGS 87 were estimated for GY, TKW and TW. GY losses of the potential GY for every 1% increase of SRS were 0.62% with a high coefficient of determination of 0.75 for the S cultivar and 0.29% with a lower coefficient of determination of 0.26 for the MS cultivar (Figure 1).

TKW losses increased 0.19% with a coefficient of determination of 0.57 and 0.16% with a coefficient of determination of 0.41 of the potential TKW for every 1% increase of SRS in the S cultivar and the MS cultivar, respectively.

TW losses increased 0.43% with a coefficient of determination of 0.64 and 0.71% with a coefficient of determination of 0.55 of the potential TW for every 1% increase of SRS in the S cultivar and the MS cultivar, respectively.

Based on all information collected, preliminary stem rust damage estimation equations on a percentage basis were obtained (Table 1). Although simple, these might guide fungicide application to manage SR between GS71 and GS87 on wheat.

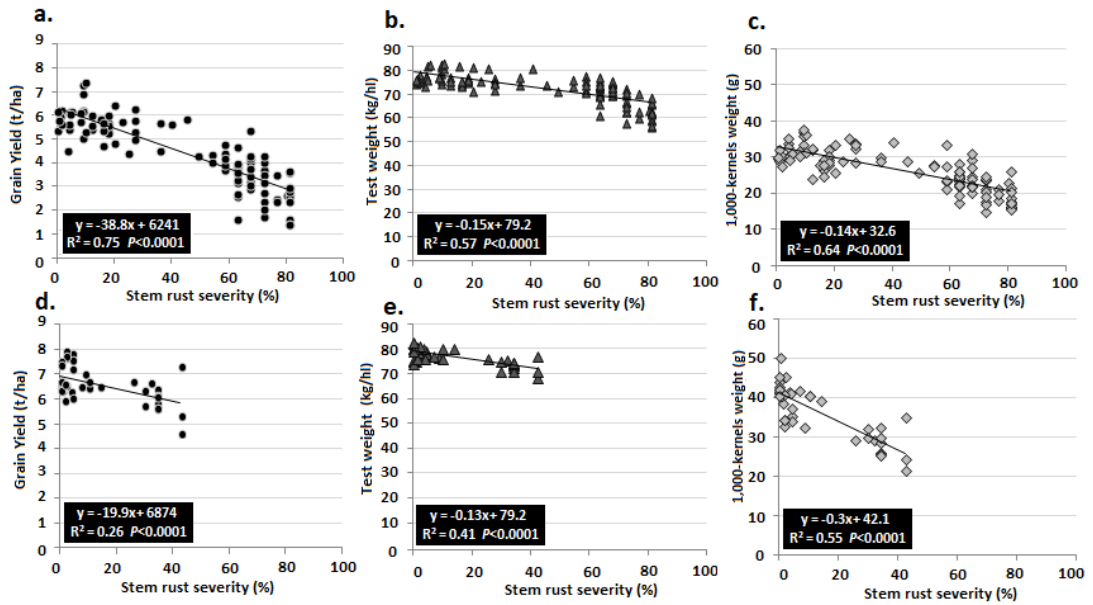


Figure 1: Linear regression between a) grain yield and stem rust severity, b) test weight and stem rust severity and c) thousand-kernels weight and stem rust severity in a susceptible cultivar (cv. Baguette 601), and d) grain yield and stem rust severity, e) test weight and stem rust severity and f) thousand-kernels weight and stem rust severity in a moderately susceptible cultivar (cv. Baguette 501), during 2015 and 2016.

Table 1. Estimated percent grain yield (GY), thousand-kernel weight (TKW) and test weight (TW) losses caused by 1% increase of SRS from ZGS 71 to ZGS 87.

Susceptible cultivar	GY = 100 - 0.63 SRS
	TW = 100 - 0.19 SRS
	TKW = 100 - 0.43 SRS
Moderately susceptible cultivar	GY = 100 - 0.29 SRS
	TW = 100 - 0.16 SRS
	TKW = 100 - 0.71 SRS

GY: Grain yield, TW: Test weight, TKW: thousand-kernels weight, SRS: stem rust severity

2.4.3. Spraying strategies: timing of fungicide spraying and nozzle type

2.4.3.1. Susceptible cultivar

The timing of fungicide spraying had a significant effect ($P>0.05$) on SR CI in the disease assessment at watery-milky stage (DA_2), disease assessment at hard dough (DA_3), disease assessment at physiological maturity (DA_4), AUDPC, GY, TKW and both, in 2015 and 2016 (Table 2). The use of different nozzle types only caused significant differences in the final disease assessment (DA_4) in 2015 and GY in 2016.

Table 2. Effect of fungicide spraying time and nozzle types on stem rust infection coefficient, area under the disease progress curve, grain yield, test weight and thousand-kernel weight on a susceptible cultivar in 2015 and 2016.

	2015								2016							
	CI				AUDPC	GY	TW	TKW	CI				AUDPC	GY	TW	TKW
	DA_1	DA_2	DA_3	DA_4	(t/ha)	(kg/ha)	(g)	DA_1	DA_2	DA_3	DA_4	(t/ha)	(kg/ha)	(g)		
NF	2.2 a	60.0 b	73.1 c	81.0 d	1639 b	1.7 b	59.5 c	17.0 b	1.6 a	25.0 b	69.8 c	78.8 b	1213 c	3.3 c	65.4 c	20.3 b
WMG-CH	2.3 a	58.8 b	65.9 b	68.6 cd	1468 b	3.2 b	72.3 b	21.6 b	0.8 a	22.0 b	63.4 b	73.1 b	1103 b	3.6 c	69.1 b	20.4 b
WMG-TT	0.9 a	53.8 b	66.4 b	67.5 c	1434 b	3.3 b	72.2 b	25.3 b	1.2 a	22.6 b	59.6 b	61.8 b	1029 b	4.4 b	72.3 b	25.6 b
DO-CH	1.2 a	3.9 a	11.3 a	25 b	368 a	5.6 a	81.5 a	32.8 a	1.9 a	2.3 a	2.2 a	9.2 a	99 a	5.9 a	75.9 a	28.6 a
DO-TT	1.2 a	1.9 a	5.0 a	7.1 a	240 a	6.0 a	82.5 a	32.6 a	2.3 a	2.6 a	1.5 a	5.6 a	83 a	5.8 a	75.1 a	27.9 a
p-value A	NS	***	***	***	***	***	***	**	NS	***	***	***	***	***	***	**
p-value B	NS	NS	*	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS
CV(%)	33.5	18.2	5.6	9.4	6.6	10.8	2.6	10.0	34.7	24.3	8.42	16.8	7.8	5.0	2.9	11.0

CI: stem rust coefficient of infection, AUDPC: area under the disease progress curve, GY: grain yield, TW: test weight, TKW: thousand-kernels weight, NF: non-fungicide treatment, WMGR-CH: watery-milky grain stage fungicide application with cone hallow nozzle treatment, WMG-TT: watery-milky grain stage fungicide application with Turbo TwinJet 60 nozzle treatment, DO-CH: disease onset fungicide application with cone hallow nozzle treatment, DO-TT: disease onset fungicide application with Turbo TwinJet 60 nozzle treatment, CV: coefficient of variation. Values followed by the same letter are not significantly different at $P<0.05$, according to Tukey HSD test.

Fungicide application at disease onset (DO) at flowering, had a 93.6% of SR control efficiency at DA_2, 85.4% at DA_4 and 86.1% lower AUDPC, compared with the NF treatment. This spraying at DO avoided losing 57% of the GY (5.8 vs. 2.5 t/ha), 21% of TW (78.8 vs. 62.4 kg/hl) and 39% of TKW (30.5 vs. 18.7 g) in comparison with NF application (Figure 2). Fungicide spraying at WMG, when SRS reached up to 60%, significantly reduced CI compared with the NF treatment. However, the lower SR infection did not always result in a significantly lower AUDPC and a higher GY than the NF treatment. In 2015, AUDPC, GY and TKW means in plots with late application at the watery-milky stage were not significantly different from the NF treatment, whereas in 2016 significant differences were found between fungicide and NF treatments on the three variables. Overall, the late spraying determined a 10.5% efficiency of SR control at the disease assessment made after the fungicide application (DA_3), 15.1% at the final disease assessment made at physiological maturity (DA_4) and 9.9% lower AUDPC than the NF treatment. This SR management avoided 31% losses of the GY (3.6 vs. 2.5 t/ha), 13% of TW (71.5 vs. 62.4 kg/hl) and 19% of TKW (23.2 vs. 18.7 g) in comparison with NF application. Fungicide spraying at DO as compared to WMG (average 11 days between fungicide applications) prevented 38% of GY (5.8 t/ha vs. 3.6 t/ha), 9% of TW (78.8 vs. 71.5 kg/hl) and 24% of TKW (30.5 vs. 23.2 g) losses in 2015 and 2016.

In 2015, nozzle types had a significant effect in SR CI ($P < 0.05$) when fungicide application was at DO. Particularly, Turbo TwinJet 60° nozzle (TT) had lower CI at the final SR assessment (DA_4) as compared with the hollow cone nozzle (CH). Yet, there were not significant differences among nozzle types for AUDPC, GY, TW or TKW. In 2016, no differences in any of the variables were observed. In the late fungicide application, SR at DA_4 differed from the NF treatment only when TT nozzle was used (no differences were obtained between late fungicide application with CH nozzle and NF treatment). Even though in general terms no statistical differences were obtained comparing the two nozzle types, SR control and GY using TT were consistently higher in both years.

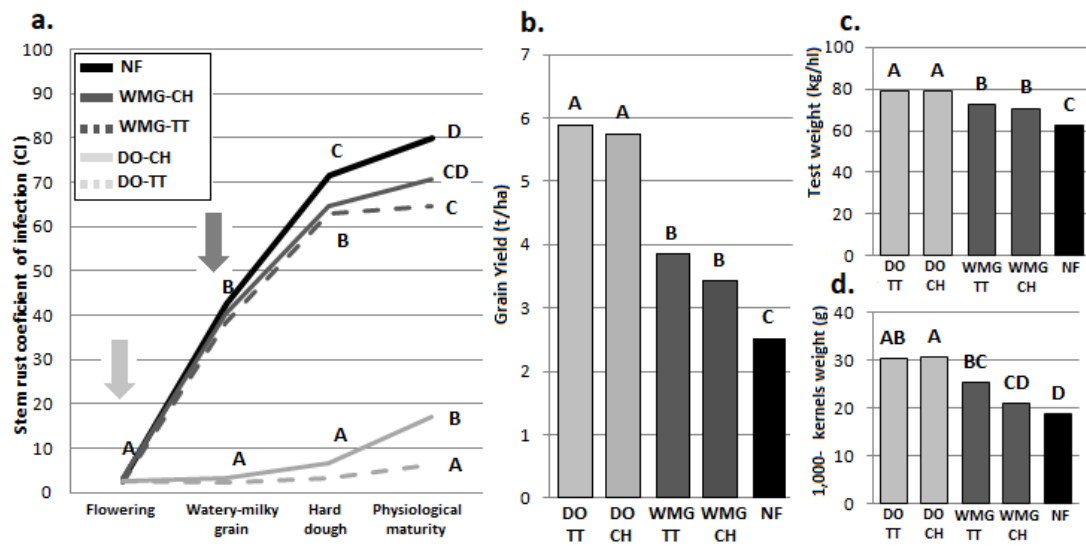


Figure 2. Mean comparison from joint analysis of 2015 and 2016 of (a) Stem rust development in two fungicides spraying times and nozzle types in a susceptible cultivar and its impact in (b) grain yield , (c) test weight and (d) thousand kernels weight. No significant differences are indicated by same letters at $P < 0.05$, according to Tukey HSD test. NF: non-fungicide treatment, WMG: watery-milky grain fungicide spraying, DO: disease onset fungicide spraying, TT: Turbo TwinJet 60° nozzle, CH: cone hallow nozzle. Gray arrow: disease onset fungicide spraying time. Gray dark arrow: watery-milky grain fungicide spraying time.

2.4.3.2. Moderately susceptible cultivar

In 2015, fungicides sprayed at DO at flowering on the MS cultivar determined an 80% SR control efficiency in the subsequent disease assessment (DA_2) and 78% lower AUDPC, compared with the NF treatment (Table 3 and Figure 3). This resulted in avoiding 29% (7.5 vs. 5.4 t/ha), 9% (78.1 vs. 70.8 kg/hl) and 29% (34.4 vs. 24.4 g) of GY, TW and TKW losses when compared with NF treatment. No significant differences in the SR CI were observed between late sprayed plots compared to the NF treatment. Disease assessments after the late fungicide spraying (DA_3) and AUDPC were 17 and 11% lower than the NF treatment. Although there were not significant differences in SR control, fungicide application

at watery-milky stage significantly avoided 14% (6.2 vs. 5.4 t/ha), 5% (74.3 vs. 70.8 kg/hl) and 18% (29.6 vs. 24.4 g) of GY, TW and TKW losses in comparison with the NF treatment. Fungicide spraying at DO in a MS cultivar prevented 17% of GY, 5% of TW and 14% of TKW losses in 2015, as compared to late application (13 days later). No differences were observed between nozzle types in CI, GY, TWK and TW in the MS cultivar.

In 2016, SR development on the MS cultivar was lower than in 2015 (Table 3). Although plots sprayed at DO had significantly ($P < 0.05$) lower CI than those sprayed at watery-milky stage and NF treatment, the SR reduction did not cause significant differences in GY, TW or TWK. No significant differences were observed between nozzle types tested in the MS cultivar.

Table 3: Effect of the fungicide spraying time and nozzle type on stem rust coefficient of infection, area under the disease progress curve, grain yield, test weight and thousand-kernels on a moderately susceptible cultivar (cv. Baguette 501) in 2015 and 2016.

	2015								2016							
	CI				AUDPC	GY	TW	TKW	CI				AUDPC	GY	TW	TKW
	DA_1	DA_2	DA_3	DA_4	(t/ha)	(kg/hl)	(g)	DA_1	DA_2	DA_3	DA_4	(t/ha)	(kg/hl)	(g)		
NF	0.4 a	14.5 b	38.3 b	---	282 b	5.4 c	70.8 c	24.4 c	1.8 a	1.8 b	4.1 b	9.5 b	98 c	6434 a	78.2 a	40.3 a
WMG-CH	0.4 a	14.0 b	31.9 b	---	255 b	6.2 b	73.5 b	31.2 b	1.5 a	1.8 b	2.0 a	3.2 a	53 b	6512 a	78.4 a	42.6 a
WMG-TT	0.4 a	13.8 b	31.4 b	---	250 b	6.3 b	75.1 b	28.1 b	1.8 a	1.2 b	1.2 a	1.1 a	32 b	6385 a	78.0 a	42.6 a
DO-CH	0.4 a	2.4 a	14.9 a	---	79 a	7.4 a	77.7 a	34.7 a	1.0 a	0.1 a	0.2 a	0.2 a	8.0 a	6705 a	79.0 a	41.8 a
DO-TT	0.4 a	3.3 a	2.3 a	---	44 a	7.6 a	78.6 a	34.2 a	2.0 a	0.1 a	0.1 a	0.2 a	7.0 a	6810 a	79.8 a	43.2 a
p-value A	NS	**	**	---	**	***	***	**	NS	**	**	**	***	NS	NS	NS
p-value B	NS	NS	NS	---	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	4.1	20.0	40.4	---	25.8	1.7	1.1	4.9	10.0	38.9	44.1	43.1	35.9	2.8	3.2	7.5

CI: stem rust coefficient of infection, AUDPC: area under the disease progress curve, GY: grain yield, TW: test weight, TKW: thousand-kernels weight, NF: non-fungicide treatment, WMGR-CH: watery-milky grain stage fungicide spraying with cone hallow nozzle treatment, WMG-TT: watery-milky grain stage fungicide spraying with Turbo TwinJet 60 nozzle treatment, DO-CH: disease onset fungicide spraying with cone hallow nozzle treatment, DO-TT: disease onset fungicide spraying with Turbo

TwinJet 60 nozzle treatment, CV: coefficient of variation. Values followed by the same letter are not significantly different at $P < 0.05$, according to Tukey HSD test.

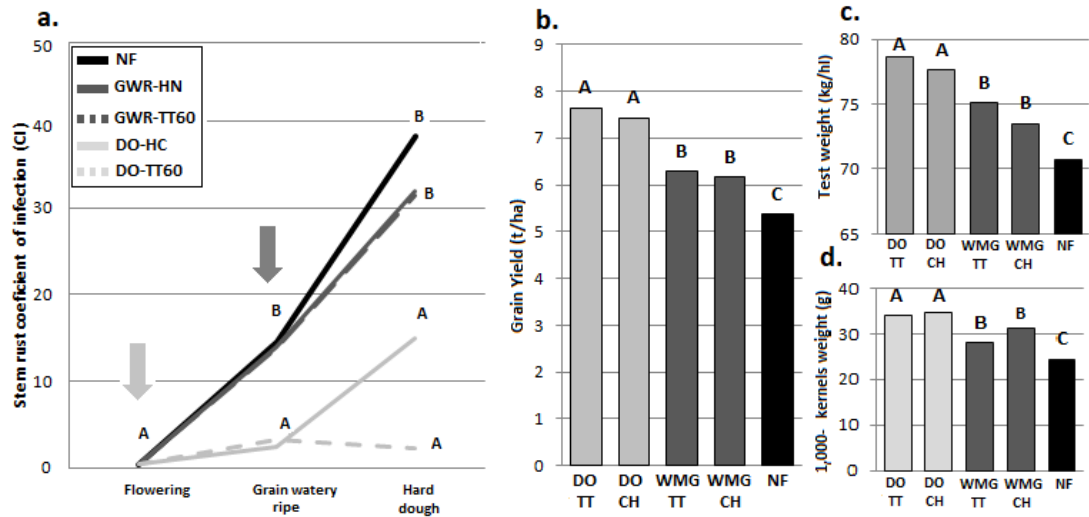


Figure 3. Mean comparison of (a) stem rust development in two fungicides spraying times and nozzle types in a moderately susceptible cultivar in 2015 and its impact in (b) grain yield, (c) test weight and (d) thousand kernels weight. No significant differences are indicated by same letters at $P < 0.05$, according to Tukey HSD test. NF: non-fungicide treatment, WMG: watery-milky grain fungicide spraying, DO: disease onset fungicide spraying, TT: Turbo TwinJet 60° nozzle, CH: cone hollow nozzle. Gray arrow: disease onset fungicide spraying time. Gray dark arrow: watery-milky grain fungicide spraying time.

2.4.3.3. Fungicide residues in harvested grain

Residues of epoxiconazole and metconazole were measured in 2016 (Figure 4). Results indicated that 94% of the samples with fungicides applied at watery-milky stage had detectable levels of epoxiconazole and metconazole in grain after harvesting. None of the samples exceeded the maximum residue limit (MRL) established by the codex alimentarius proposed by the European Union (150 and 200 $\mu\text{g}/\text{kg}$ for metconazole and epoxiconazole, respectively). Only 6% of the samples, with fungicides sprayed at DO (flowering stage), had detectable grain

residue of metconazole after harvest and no epoxiconazole was detected on these samples (Figure 4).

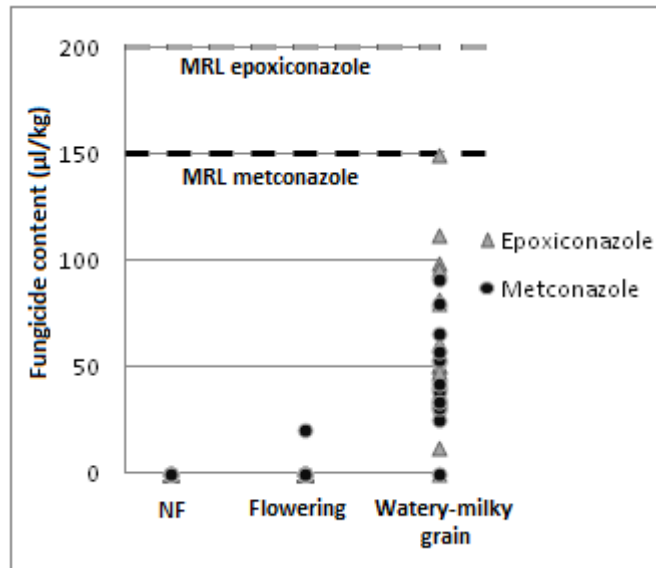


Figure 4: Residues of metconazole and epoxiconazole recovered from grains after harvesting according to fungicide spraying time (NF: non-treated with fungicide, flowering- ZGS 65, watery-milky grain-ZGS 75) in 2016. MRL: maximum residue limit.

2.4.4. Fungicides efficiency experiment

In 2015, fungicide was sprayed at DO in the complete control treatment (CCT). Due to greatly conducive conditions for SR infection and highly SR susceptibility of the cultivar, SRS reached almost 50% when fungicides were applied in the rest of the treatments. Significant differences ($P < 0.05$) were observed in SR CI, GY, TW and TKW between the CCT and the rest of the fungicide treatments (Figure 5a). Although fungicides were sprayed late, there were significant differences ($P < 0.05$) in the CI assessed at physiological maturity (DA₄) between all fungicide treatments and the NF treatment, except for pyraclostrobin + epoxiconazol and fluxapiroxad + prothioconazole treatments which did not differ from the NF treatment. Yet, differences near the end of the disease development

did not translate into higher GY in all cases. Only the late application with pyraclostrobin + epoxiconazole + fluxapyroxad, fluxapyroxad + prothioconazole or azoxystrobin + cyproconazole provided a significantly ($P < 0.05$) higher GY than the NF treatment, although lower than the CCT (Figure 5c, d and e).

In 2016, fungicides were sprayed at DO. Significant differences ($P < 0.05$) were observed in SR CI, GY, TW and TKW between the NF treatment and the rest of the fungicides treatments (Figure 5b, c, d and e). The azoxystrobin + cyproconazole treatment provided the poorest SR control, as the final disease assessment at physiological maturity (DA₄) was significantly higher than the rest of the fungicide treatments and the CCT, but lower than the NF treatment. Yet, this poorest SR control achieved GY, TW and TKW as high as the CCT and the rest of the fungicide treatments. GY and TW attained in plots applied with tebuconazole was significantly lower ($P < 0.05$) than those obtained with the pyraclostrobin + epoxiconazole + fluxapyroxad treatment.

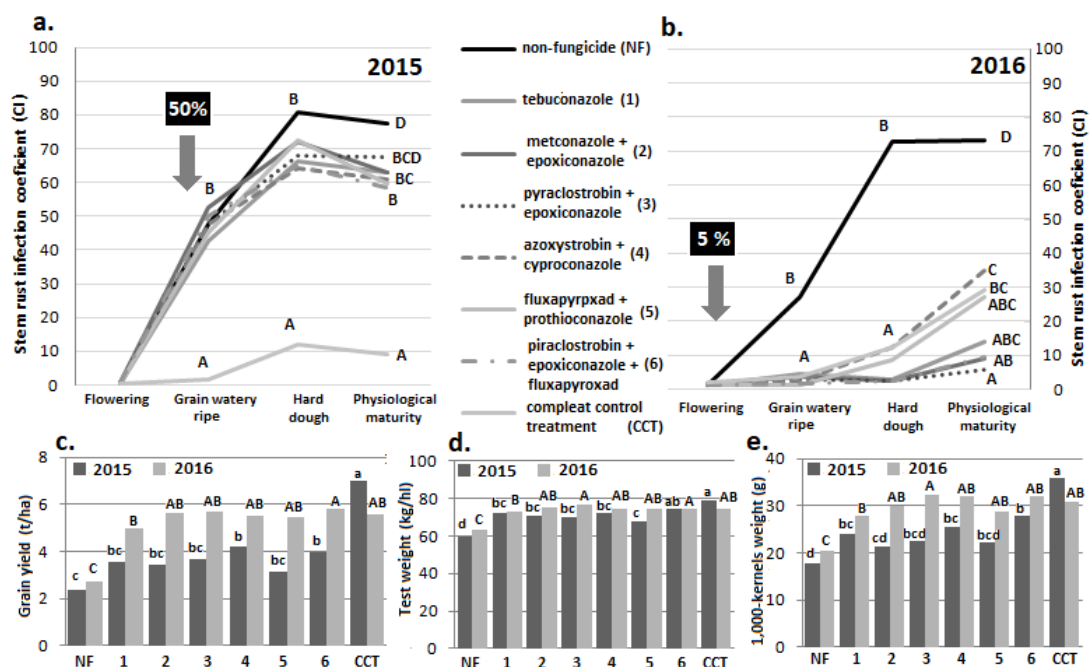


Figure 5. Stem rust coefficient of infection for different fungicide treatments in (a) 2015 and (b) 2016, and its impact on (c) grain yield, (d) test weight and (e)

thousand-kernels weight. Same letters are not significantly different at $P < 0.05$, according to Tukey HSD test. Arrows indicate when fungicides were sprayed and the respective stem rust severity at that moment.

2.5. DISCUSSION

Linear regression adjustments (R^2) between GY, TW or TKW and SRS to estimate losses due to SR were higher in all cases for the S cultivar than for the MS cultivar, demonstrating that other yield limiting factors as the interference of other diseases also had an important role affecting productivity in the MS cultivar. GY loss equations were estimated and might support the fungicide applications decision to manage SR between GS71 and GS87 in Uruguay, as it was done for other relevant diseases (Germán *et al.* 2011). In this study, GY losses were estimated in 3.8% of the potential yield every 10% of SRS, lower than yield losses reported by Greaney (1936) in spring wheat of 5.4% every 10% SRS. Probably the earlier disease onset in the wheat cycle in the previous study explains the higher yield losses as compared with this study. Also, since GY potential in present cultivars is higher than those in the 1930's, losses might be higher in the present study than in Greaney's one.

Results from this study indicated that the most important aspect to efficiently manage SR with foliar fungicides is to spray at disease onset, both in S cultivar and MS cultivar. Spraying at DO (flowering) in a S cultivar rather than applying 9 to 13 days after at WMG, determined 60%, 10% and 31% higher GY, TW and TKW, respectively. When compared with no fungicide application, 131%, 26%, and 63% higher GY, TW and TKW were achieved, respectively. These results support previous studies from Ethiopia (Tadesse *et al.*, 2010) and Australia (Loughman *et al.*, 2005), where the most efficient fungicide sprayings were those at low SR level (first symptoms), while no application was recommended when SR was already advanced or appeared late in the crop cycle (ZGS 77).

When using a S cultivar, under highly conducive conditions to SR epidemics, the use of TT nozzle for the fungicide application might lead to better SR control and lower SR infection at physiological maturity. There are no previous reports comparing different nozzle to control SR.

When using a MS cultivar, SR control efficiency and its effect in GY under favorable conditions were similar to those obtained with a S cultivar. In years where environmental conditions might not be favorable for SR development, no GY differences were obtained in a MS cultivar when foliar fungicides were applied at different timings.

Under Uruguayan conditions SR has a late disease onset, and therefore it may require later fungicide application than other diseases that affect leaves and stems. In this situation fungicide residues can be found in the harvested grain, which may be a restriction for its commercialization and consumption. Although 6% and 94% of the samples from plots with early (flowering stage) and late fungicide application (WMG) had detectable residue content of the active ingredients used in these trials (epoxiconazole and metconazole) respectively, none had higher fungicide concentration than the LMR (joint FAO/WHO Codex Alimentarius Commission, 2007). These results require further confirmation.

Further research is necessary to determine if fungicide application with some of the active ingredients combinations available in Uruguay ensure a better SR control. Preliminary results of two years indicated that, if fungicide application is done at DO (the optimum timing for controlling efficiently SR), the addition of a strobilurin in the mix did not improve SR control as compared to a triazole mix. This aspect is particularly relevant, since SR onset was at flowering stage in both years, which is the critical moment for Fusarium head blight (FHB) infection as well. The use of strobilurin increases the deoxynivalenol toxin (DON) produced by the pathogen, which is the main toxin present in Uruguay (Díaz and Pereyra 2011). Therefore, it might be relevant to confirm that it is not worth to include a strobilurin

in the mix for managing SR. Also, the addition of an SDHI in the fungicide mix did not improve SR control and GY if fungicide was sprayed at DO in comparison with a triazole mix or triazole + strobilurin mix. As a fungicide management strategy, it is highly recommended to apply SDHIs no more than once in the cropping season in order to avoid pathogens resistance to this group. Therefore, it might be relevant to consider this type of fungicide as a tool for managing other diseases as foliar blotches.

In the current study, the least SR control efficiency was obtained with the mix azoxistrobin + cyproconazole. Yet, this lowest SR control achieved GY, TW and TKW similar to the CCT and the rest of the fungicide treatments. However, only GY and TW achieved with the tebuconazole fungicide treatment was significant lower than those obtained with the pyraclostrobin + epoxiconazole + fluxapiroxad fungicide treatments. Our results with azoxistrobin + cyproconazole or tebuconazole differed from those obtained by Macharia *et al.* (2013) and Loughman *et al.* (2005), respectively, where better results were obtained with this active ingredients in comparison with other tested.

Advances towards an adjusted SR management based on foliar fungicides were attained in this study. The foliar fungicide controlled the SR development in a S cultivar and MS cultivar, directly impacting the GY, TW and TKW achieved. SR management with foliar fungicides could be used as a short term strategy until resistant cultivars are developed, as well as a complement when cultivars with inadequate resistance are used. From all these results, most outstanding outcome is the clear need of frequent crop monitoring to precisely determine the disease onset when SR requires chemical control. Furthermore, the climatic conditions during spring time frequently difficult the possibility of applying fungicides at the precise timing, which confirms that the use of resistant cultivars is the best strategy to control SR in wheat.

2.6. ACKNOWLEDGMENTS

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3. MAPPING STEM RUST RESISTANCE LOCI FROM TWO SPRING WHEAT LINESEFFECTIVE IN URUGUAY AND KENYA

3.1. SUMMARY

More than 80% of the wheat area sowed worldwide is susceptible to Ug99 race group of stem rust fungus caused by *Puccinia graminis* f.sp *tritici*. South America is not an exception, where *Sr31* and *Sr24* are the main genes conferring stem rust resistance in wheat varieties, and are not effective against Ug99 lineage. In order to be ready for the potential Ug99 spread threat, highly resistant wheat genotypes in Africa and Uruguay were identified. Stem rust resistance mapping was performed in populations derived from the cross of a susceptible parent with each of the two resistant parents 'INIA Tero ' and BR23//CEP19/PF85490, using seedling and field data from Uruguay and Kenya. Four resistance loci were detected in INIA Tero on chromosomes 2B, 3D, 6A and 7B. The four QTLs were effective to Uruguayan races, whereas the gene on chromosome 2B was the only one explaining the stem rust resistance in Kenya. Only the QTL in 7B was not present since seedling stage. In BR23//CEP19/PF85490, two stem rust loci were detected on chromosome 1B and 2B. Both explained the seedling and field resistance in Uruguay and only the one on 2B was effective in Kenya. Further studies are needed to determine the relation between resistance genes on chromosome 2B from these two lines, as well as identifying the other genes effective in Uruguay to define strategies to breed for stem rust resistance, avoiding low genetic diversity and chasing durable resistance.

Keywords: Triticum aestivum, Ug99, QTL mapping, genetic resistance.

3.2. INTRODUCTION

Bread wheat (*Triticum aestivum*) is the second most important cereal crop in the world in terms of total production (FAOSTAT 2016) and it plays an important role in feeding the world's population. In South America, wheat is sowed annually in 8.6 million ha approximately in Uruguay, Argentina, Brazil, Chile and Paraguay (FAOSTAT 2016). In this region, cereal diseases are one of the principal limitations for achieving high grain yields (Germán *et al.* 2007). Wheat stem rust (SR) caused by the pathogen *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. E. Henn. (Pgt), is considered as one of the most destructive diseases of the crop worldwide (Roelfs *et al.* 1992). Historically, yield losses due to SR epidemics were reported all around the world (Leonard and Sazbo 2005; Roelfs *et al.* 1992), becoming the most feared disease in wheat-growing countries (Singh *et al.* 2011) including South America (Germán *et al.* 2007). Due to great efforts made to breed for SR resistance, the disease was successfully controlled by genetic resistance (Singh *et al.* 2008) until the appearance of race Ug99 (TTKSK) of the SR fungus in Uganda in 1998 (Pretorius *et al.* 2000). Evolution and spread of Ug99 out of Eastern Africa is documented as one of the foremost threats to wheat production and food security (Singh *et al.* 2011). Ug99 is the first known race virulent to SR resistance gene *Sr31*, located on the 1B/1R translocation transferred from rye to wheat. *Sr31* was extensively used by the International Maize and Wheat Improvement Center (CIMMYT) as a source of SR resistance (Singh *et al.* 2008; Jin and Singh 2006) and is present in the vast majority of wheat varieties derived from CIMMYT germplasm (Singh *et al.* 2008). Until the appearance of Ug99, *Sr31* provided protection against all prevalent races of Pgt (Jin *et al.* 2008). Ug99 is also virulent to most of the *Sr* genes of wheat origin and also to extensively used *Sr38* gene of alien origin (transferred into wheat from *T. ventricosum*) (Singh *et al.* 2011). In 2006 and 2007 variants of Ug99 with additional virulence on *Sr24* and *Sr36* were detected (Jin *et al.* 2009, 2008). At that point, it was considered that about 80 to 90% of wheat varieties worldwide were susceptible to Ug99 race group (Ug99+) (Singh *et al.* 2015). In Uruguay and other

countries of South America, commercial cultivars carrying *Sr31* and *Sr24*, which are the most important genes conferring resistance to SR in this region, exceeded half of the total wheat area by 2009 (Germán *et al.* 2009). Ug99+ spread to South Eastern Africa (Pretorius *et al.* 2012) and Iran (Nazari *et al.* 2009), causing concern as Ug99+ spread can lead to major epidemics (Singh *et al.* 2013).

The use of rust resistant wheat varieties is considered the most economical, efficient and environmentally safe method to control wheat SR. To date more than 60 *Sr* genes have been identified, characterized and located on the wheat genome (McIntosh *et al.* 2014; Yu *et al.* 2014; McIntosh *et al.* 1995). Most are genes that confer resistance from the seedling stage, are expressed qualitatively and have generally been race-specific, while five are adult plant resistance (APR) genes, conferring quantitative resistance and considered race non-specific. In recent researches, several QTLs for SR resistance have also been identified, considering chromosomes 3BS, 6BS, 5BL and 2BL as hotspots since many of these QTLs were located on them (Yu *et al.* 2014). However, the Pgt population is dynamic and new races of the pathogen are likely to emerge, causing high infections on cultivars that were initially resistant. Therefore, it is essential to undertake several strategies to breed cultivars with effective and durable SR resistance, e.g.: to know which genes confer resistance in the germplasm used in breeding to avoid low genetic diversity, to identify new resistance sources in wheat germplasm, to increase diversity and develop wheat cultivars with combinations of effective resistance genes to decrease the probability of selection of virulent races, and/or to use quantitative resistance (Singh *et al.* 2011, 2006).

Marker-based approaches are valuable methodologies to identify the genetic base of disease resistance. The use of bi-parental mapping populations is the standard approach to determine the number and chromosomal location of resistance loci by relating phenotypic information to genotypic data obtained from molecular markers. Alternatively to this methodology, genome wide association

studies (GWAS) can be used to explore germplasm collections or natural populations. These tools can identify both, major and minor resistance genes and estimate their effect across environments (Gupta *et al.* 2005).

Hard efforts have been undertaken around the world to identify SR resistance to genetically control Ug99+ (Gao *et al.* 2017; Babiker *et al.* 2016; Li *et al.* 2016; Lopez-Vera *et al.* 2014; Singh *et al.* 2013; Haile *et al.* 2012; Hiebert *et al.* 2010; Kaur *et al.* 2009). In the context of the threat caused by the possible spread of Ug99+ to South America and the need of breeding for Ug99+ resistance in our region, some materials highly resistant to SR in Uruguay and Kenya were identified. The objective of this study was to identify the genetic base of the resistance to Ug99+ and local Pgt races of two lines, the Uruguayan wheat cultivar 'INIA Tero' and the Brazilian advanced line BR23//CEP19/PF85490, using QTL mapping tools.

3.3. MATERIALS AND METHODS

3.3.1. Plant materials

To study the genetic basis of the resistance to SR races present in Uruguay and Kenya, two stem rust resistant hexaploid wheats, the Uruguayan cultivar INIA Tero (I.Tero, LI107/C-CH-91-1642) and the Brazilian line BR23//CEP19/PF85490 (BR23//C/P, EMBRAPA-CNPTrigo) were crossed with the susceptible cultivar Baguette 13 (B.13) to develop two RIL populations (F_6) of 174 and 107 lines, respectively.

3.3.2. Field resistance

Four field trials were carried out to evaluate field response of the two populations to local races in Uruguay and to Ug99+ races in Kenya. Uruguay trials were located at: i) Instituto Nacional de Investigación Agropecuarias (INIA) La Estanzuela (LE), Colonia (34°20'18"S, 57°41'26"W) during main season 2015 (LE-15); ii) LE during off-season 2016 (LEv-16); and iii) Universidad de la República, Facultad de Agronomía, M. Cassinoni Experimental Station, Paysandú, (EEMAC) (32°22'49"S,

58°03'09"W) during main season 2016 (EEMAC-16). In Kenya trials were carried out at Kenya Agricultural Research Institute (KARI) at Njoro, during off-season 2017/2018 (K-18).

In Uruguay, an incomplete block design with two replications at each environment was used with the three parents as repeated checks. Only one repetition was used in Kenya. Single 1.0 m length rows with 0.40 m spacing between rows were used in Uruguay and two 0.7 m rows at 0.20 m spacing at Kenya. To facilitate uniform disease build-up within the nursery, spreader rows with a mixture of susceptible lines were planted perpendicular to all entries on one side of the plots, in the middle of alleys, and around the field. In addition to the natural infection, trials were also artificially inoculated by spraying the spreaders rows two to three times with a lightweight mineral oil Soltrol 170 (Phillips Petroleum Co., Borger, TX) spore suspension. In Uruguay, two additional inoculations were made, injecting a suspension of uredinospores on distilled water into two tillers per plot. Freshly collected uredinospores of races RHKTF, RRTTF and SPLKC were used in Uruguay, and races TTKSK, TTKST, TTTSK, TTKTK and TTKTT (Jin *et al.* 2008) in Kenya.

Days to heading was recorded in LE-15 and LEv-16. SR severity (DS) was scored using the modified Cobb's scale (Peterson *et al.* 1948). Infection response (IR) was scored following Roelfs *et al.* (1992), as susceptible (S), moderately susceptible (MS), moderately resistant (MR) and resistant (R). Coefficient of infection ($CI = DS \times IR$) assigning coefficients to each IR (R=0.2, MR=0.4, MRMS=M=0.6, MS=0.8 and S=1, Stubbs *et al.*, 1986) was used for QTL analysis. DS, IR and growing stage (GS) were assessed at the peak of disease development.

3.3.3. Seedling resistance

Greenhouse seedling tests were carried out during 2017 at La Estanzuela, Uruguay. RIL populations were sown in trays of 28 lines (incomplete blocks, two replications), where the first material was always the susceptible parent B.13. Pgt

race RHKTF (US nomenclature, Jin *et al.* 2008) with avirulence/virulence *Sr8a*, *Sr9e*, *Sr11*, *Sr24*, *Sr31*, *Sr36/Sr5*, *Sr6*, *Sr7b*, *Sr9a*, *Sr9d*, *Sr9g*, *Sr10*, *Sr17*, *Sr21*, *Sr30*, *Sr38*, *SrTmp*, *SrMcN* and race RRTTF *Sr8a*, *Sr9e*, *Sr24*, *Sr31/Sr5*, *Sr6*, *Sr7b*, *Sr9a*, *Sr9d*, *Sr9g*, *Sr10*, *Sr11*, *Sr17*, *Sr21*, *Sr30*, *Sr36*, *Sr38*, *SrTmp*, *SrMcN* were used individually to inoculate seedlings with fully expanded leaves (8-10 days after planting). For spraying, uredinospores were suspended in light mineral oil Soltrol 170. After inoculation, seedlings were incubated in a dew chamber overnight and then kept in the greenhouse at average 20°C. Infection types (IT) were assessed 14 days after inoculation using the 0-4 scale developed by Stakman *et al.* (1962), were IT 0 to 2+ were considered resistant (R) and 3 or higher were considered susceptible (S). Chi-square (χ^2) test was performed to test for goodness-of-fit to models for one, two or more resistance genes. For mapping seedling resistance, IT data was converted into a quantitative scale of seedling reaction (avirulent phenotypes: 0=0, ;=0, 1=1, 2=2, virulent phenotypes: 3=3, 4=4, +=+0.25, -=-0.25), according to Maccaferri *et al.* (2010).

3.3.4. Statistical analysis of phenotypic data

Analysis of variance of field CI at the peak of disease development and seedling reaction were carried out using the General Linear Model Procedure in R statistical software (R Development Core Team 2014). Mean value for each genotyped RIL across replications within each location (field data) or Pgt race (seedling data) was calculated in R, considering genotype as fixed effect and incomplete blocks nested within complete blocks as random effects. Adjusted means were used in QTL analysis. Analysis with and without days to flowering as co-variable included into the model was performed.

3.3.5. Genotyping

DNA was isolated from pooled leaves of six seedlings of each RIL and parents, using CTAB method following CIMMYT protocols (Dreisigacker *et al.* 2016). The presence of seedling resistant genes *Sr24* and *Sr31* in I.Tero and BR23//C/P

were postulated using the flanking markers *Sr24#12* (Mago *et al.* 2005), *iag95* (Mago *et al.* 2002), respectively. Markers for *Sr24* and *Sr31* were also used in the two RIL populations. CIMMYT protocols were followed for gels electrophoresis (Dreisigacker *et al.* 2016).

Single nucleotide polymorphisms (SNPs) markers were obtained using the genotyping by sequencing (GBS, Feed the Future Innovation Lab for Applied Wheat Genomics, Kansas State University, USA) approach following the library construction protocol with two-enzymes, *PstI* and *MspI*, as described by Poland *et al.* (2012), and sequenced with Illumina HiSeq2000. The SNPs markers were called using TASSEL 5 GBS v2 pipeline (Glaubitz *et al.* 2014) and the unique sequence tags were aligned to the reference Chinese Spring Wheat Assembly v1.0 of the International Wheat Genome Sequencing Consortium, 2014 (IWGSC). The SNPs filtering parameters used were minor allele frequency (MAF) < 0.25, missing data > 20% and all heterozygous marker were excluded. Principal coordinate analysis (PCA) was performed with SNP information.

3.3.6. Map construction

Two linkage maps were constructed using QTL IciMapping software version 4.1 (<http://www.isbreeding.net/>). Redundant markers which were completely correlated within each population were deleted. Linkage maps were constructed by grouping, ordering and rippling. Grouping was done considering the chromosome in which each tag was aligned with a minimum LOD of 5. Ordering was done by the two-opt heuristic algorithm (nnTwoOpt). Rippling was done by permutation of a window of 5 markers ($m=5$) with the Sum of Adjacent Recombination Frequencies (SARF) criteria. Recombination frequencies were converted to centiMorgans (cM) using the Kosambi mapping function (Kosambi 1944) to calculate genetic distances between markers. The information of the presence/absence of markers for *Sr24* and *Sr31* was included as SNPs markers in the map construction.

3.3.7. QTL mapping and BLAST

QTL analysis was made using the R/qtl package of R (Broman *et al.* 2003). The composite interval mapping function (CIM) was used with a 50 cM window and considering a QTL as significant if its LOD was greater than the threshold value determined by 1,000 permutation tests, at type I error $\alpha=0.01$. For each detected QTL, the percent of phenotypic variance explained (R^2) and allelic effects were estimated. QTL mapping of flowering time was done to verify that QTL determining this variable were different from QTLs involved in SR resistance.

Flanking marker sequences of detected QTL were blasted to the reference Chinese Spring Wheat Assembly of the IWGSC (2018) and the most probable physical interval position was obtained for each QTL.

3.4. RESULTS

3.4.1. Field resistance

In the four environments where SR field resistance was recorded, the susceptible parent B.13 had uniformly high final CI scores. The highest CI were obtained in K-18, where the susceptible parent had CI 80 to 90. The lowest SR infection was obtained in LEv-16, where the susceptible parent had CI 40 to 50. Both resistant parental lines (I.Tero and BE23//C/P) had high levels of resistance in all environments (CI 1 or lower).

In Uruguay none RIL of either population had a higher CI than the susceptible parent B.13. In Kenya, few RILs had CI 100 higher than B.13 (Figure 1). The ANOVA of the phenotypic data indicated highly significant genotype effects on SR CI (data not shown). Days to heading was not significant as co-variable and was removed from the model.

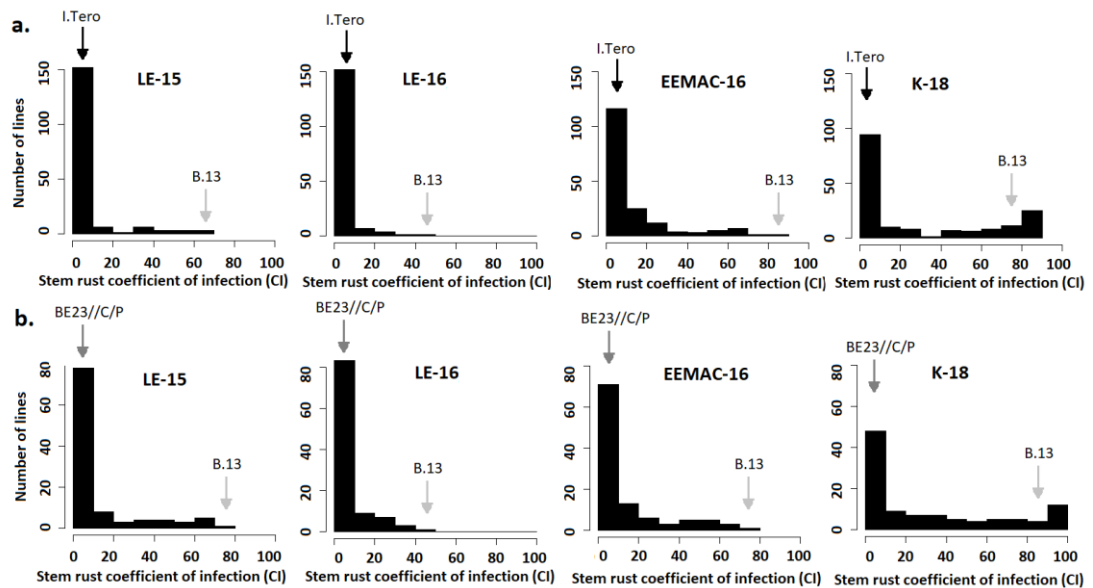


Figure 1: Frequency distribution of SR CI at La Estanzuela main season 2015 (LE-15), La Estanzuela off-season 2016 (LE-16), Experimental Estation M. A. Cassinoni 2016 (EEMAC-16) and Kenya off-season 2018 (K-18) of a) INIA Tero/Baguette 13 and b) BR23//C/P19/PF85490/3/Baguette 13 RIL populations.

3.4.2. Seedling resistance

Cultivar I.Tero had a low IT that ranged from fleck (;) to low IT (;1=) to race RHKTF and 0 to 1= to race RRTTF, whereas the line BR23//C/P had IT that ranged from 1- to 2- to race RHKTF and 1= to 2= to race RRTTF. The susceptible parent B.13 had high IT 4 to both races. The distribution of seedling reactions in both populations is shown in Figure 2.

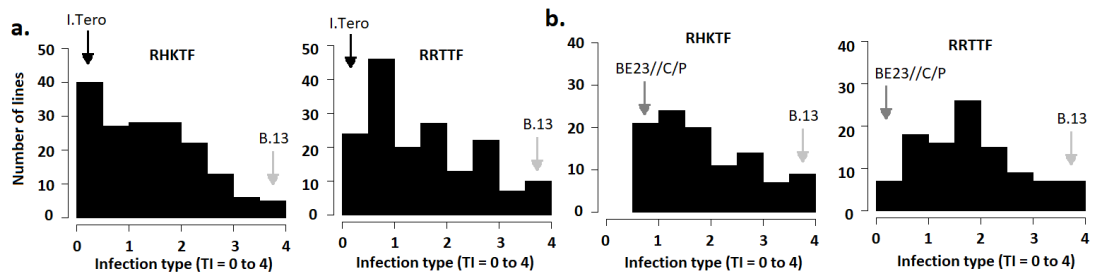


Figure 2: Frequency distribution of the seedling reaction in the RIL populations a) INIA Tero/Baguette 13 and b) BR23//CEP19/PF85490/3/Baguette 13, to *Puccinia graminis* races RHKTF and RRTTF.

3.4.3. Genotypes and genetic linkage maps

Principal coordinate analysis (PCoA) with SNP information showed that RILs from both populations were distributed between the corresponding parents (Figure 3).

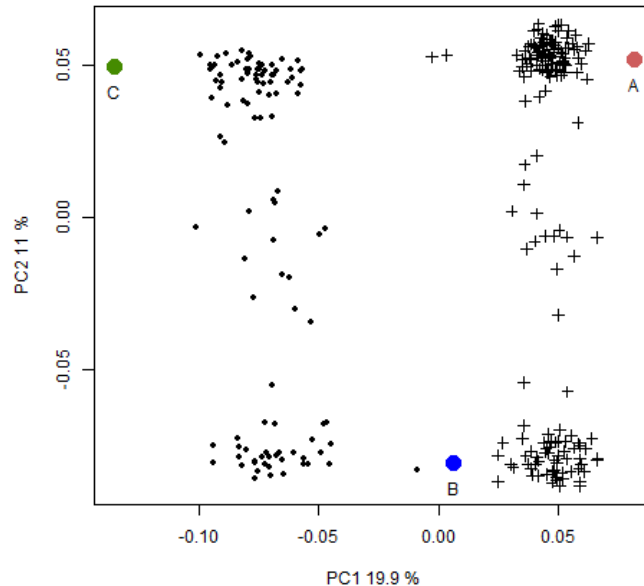


Figure 3. Principal coordinate analysis (PCoA) of INIA Tero/Baguette 13 and BR23//CEP19/PF85490/3/Baguette 13 RIL populations, including the three parental lines. Each data point represents a genotype, “A” is I.Tero, “B” is Baguette 13 and

“C” is Br23//C/P. Crosses corresponds to INIA Tero/Baguette 13 individuals and dots to BR23//CEP19/PF85490/3/Baguette 13 individuals.

Linkage maps with 21 linkage groups were obtained from each population, corresponding to each of the wheat chromosomes. The I.Tero/B.13 linkage map had 1,262 SNP markers, with a total genetic distance of 2,816 cM and average distance between markers of 2.27 cM (Figure 4a). The BR23//C/P/3/B.13 linkage map had 1,209 SNP markers, with a total genetic distance of 3,236 cM and average distance between markers of 2.72 cM (Figure 4b). In average, genome A was covered with 82 and 79 markers, genome B with 78 and 73, and genome D with 20 and 21 markers per chromosome in the I.Tero/B.13 and BR23//C/P/3/B.13 RIL populations, respectively.

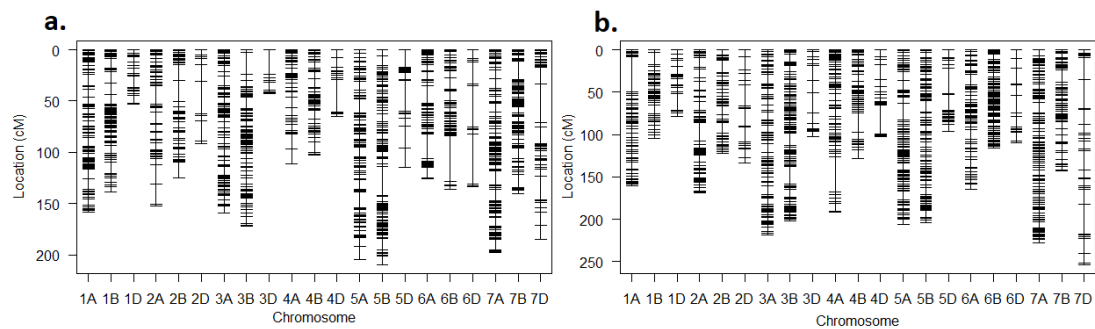


Figure 4: Linkage maps with 21 linkage groups a) INIA Tero/Baguette 13 and b) BR23//CEP19/PF85490/3/Baguette 13 RIL populations.

3.4.4. QTL mapping of days to heading

Three QTLs located on chromosomes 2D, 5A and 5D (position 37.5, 78.6 and 112.5 cM, respectively) associated to days to heading were detected in the I.Tero/B.13 population, and two QTLs on chromosomes 2D and 7A (position 95 and 157 cM, respectively) in the BR23//C/P/3/B.13 population.

3.4.5. Inheritance of seedling resistance

Markers *Sr24#12* (Mago *et al.* 2005) and *iag95* (Mago *et al.* 2002) indicated the presence of *Sr24* in I.Tero and *Sr31* in BR23//C/P. The resistant:susceptible segregation ratio of I.Tero/B.13 population to race RHKTF fitted the 3:1 expectation for two resistance genes ($\chi^2= 0.071$, $P= 0.79$), while the segregation ratio of the 73 lines without *Sr24* fitted the 1:1 expectation for one resistance gene ($\chi^2= 2.06$, $P = 0.15$). The segregation to race RRTTF fitted the 7:1 expectation for three resistance genes ($\chi^2= 0.78$, $P = 0.38$) while the segregation of the lines without *Sr24* fitted the 3:1 expectation for two genes ($\chi^2= 0.057$, $P = 0.81$). The resistant:susceptible segregation ratio of BR23//C/P/3/B.13 population to both races fitted the 3:1 expectation for two resistance genes ($\chi^2= 0.32$, $P = 0.57$, and $\chi^2= 0.029$, $P = 0.87$, respectively), while the resistant:susceptible segregation ratio of the 65 lines without *Sr31* fitted the 1:1 expectation for one resistance ($\chi^2= 0.55$, $P = 0.46$ and $\chi^2= 1.27$, $P = 0.26$, respectively).

3.4.6. Mapping stem rust field and seedling resistance

Four QTLs were detected in I.Tero/B.13 population on chromosomes 2B, 3D, 6A and 7B (Figure 5a), whereas three QTLs were detected in the BR23//C/P/3/B.13 population on chromosomes 1B, 2B and 6A (Figure 5b). All QTLs were inherited from the resistant parents. In general, a high proportion of the phenotypic variance in each test was explained by these QTLs (Table 1).

In the I.Tero/B.13 population, the three QTLs detected in EEMAC-16, the QTLs detected in K-18 and the four QTLs detected in LE-15 explained 61.3%, 55.6% and 47.9% of the phenotypic variance, respectively. The three QTLs detected in LEv-16 explained a lower proportion of the phenotypic variance (31.6%). The two and three QTLs associated to seedling resistance to races RHKTF and RRTTF explained 59.6% and 51.1% of the phenotypic variance, respectively.

In the BR23//C/P/3/B.13 population, the QTL detected in K-18 explained 53.9% and the two QTLs detected in both LE-15 and LEv-16, explained 43.9% and 31.9% of the phenotypic variance, respectively. No QTLs were detected in EEMAC-16 in this RIL population. The two QTLs associated to seedling resistance to races RHKTF and RRTTF explained 67.4% and 54.0% of the phenotypic variance, respectively.

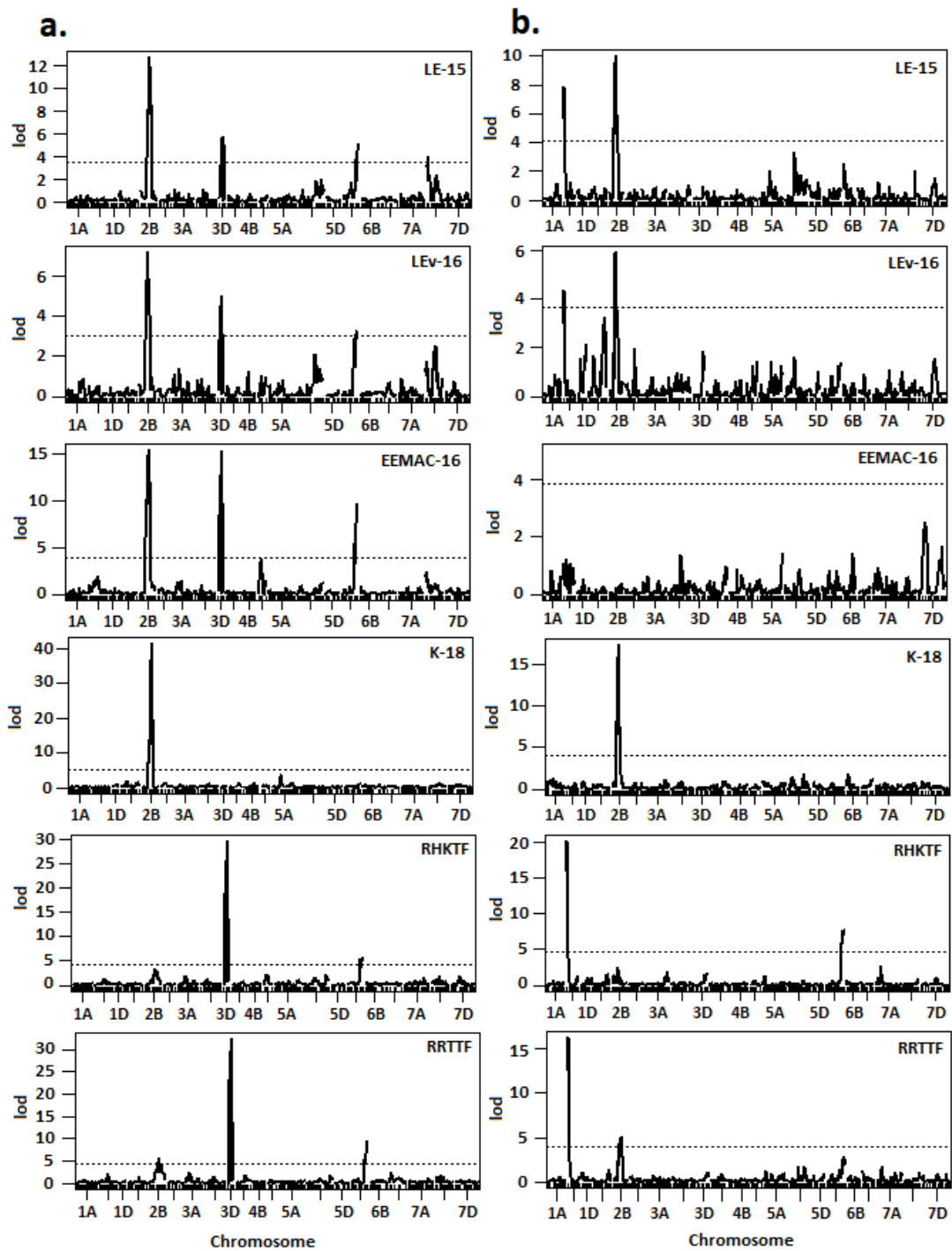


Figure 5: QTL mapping of field resistance in four environments (LE-15, LEv-16, EEMAC-16, K-18) and seedling resistance to races RHKTF and RTTF for a) INIA Tero/Baguette 13 and b) BR23//CEP19/PF85490/3/Baguette 13 RIL populations.

Table 1: QTLs for seedling resistance to Pgt races RHKTF and RRTTF and field resistance to stem rust in four environments detected by composite interval mapping in I.Tero/Baguette 13 and BR23//CEP19/PF85490/3/Baguette 13 RIL populations.

Population/ Race/ Environment/	Chr	Pos. (cM)	Marker interval	LOD	AE ^a	R ² ^{cb} (%)	Total R ² (%)
I.Tero/B.13, field resistance							
LE-15	2B	67.8	S2B_PART1_58321611 - S2B_PART2_316804988	12.49	-6.97	23.5	47.9
	3D	23.9	S3D_PART2_93294483 - S3D_PART1_298356803	5.64	-4.14	9.2	
	6A	122.5	S6A_PART1_19599107 - S6A_PART1_2869043	4.92	-4.49	9.0	
	7B	3.7	S7B_PART2_295419700 - S7B_PART1_113572123	3.65	-3.42	6.2	
LEv-16	2B	67.6	S2B_PART1_31382110 - S2B_PART2_316804988	6.38	-2.64	14.9	31.6
	3D	25.0	S3D_PART2_93294483 - S3D_PART1_298356803	4.61	-2.28	10.7	
	6A	117.5	S6A_PART1_48519635 - S6A_PART1_2869043	3.15	-1.76	6.0	
EEMAC-16	2B	72.5	S2B_PART2_272907378 - S2B_PART2_316804988	15.77	-9.86	23.0	61.3
	3D	27.7	S3D_Sr24 - S3D_PART2_130761093	14.53	-9.22	24.4	
	6A	125.7	S6A_PART1_9321817 - S6A_PART1_2869043	8.07	-6.89	13.9	
K-18	2B	72.5	S2B_PART1_142867158 - S2B_PART2_316804988	42.70	-29.76	55.6	55.6
I.Tero/B.13, seedling resistance							
RHKTF	3D	27.5	S3D_Sr24 - S3D_PART2_130761093	29.37	-0.70	51.2	59.6
	6A	122.5	S6A_PART1_48519635 - S6A_PART1_2869043	5.91	-0.31	8.4	
RRTTF	2B	75.0	S2B_PART1_63135770 - S2B_PART2_335467964	4.90	-0.24	4.6	51.1
	3D	25.0	S3D_Sr24 - S3D_PART2_25814641	32.33	-0.79	36.5	
	6A	126.0	S6A_PART1_9321817 - S6A_PART1_2869043	9.67	-0.33	10.0	
Br23//C/P///B.13, field resistance							
LE-15	1B	5.0	S1B_Sr31 - S1B_PART2_87711697	6.42	-8.72	19.1	43.9
	2B	52.5	S2B_PART1_216469337 - S2B_PART1_71304355	8.23	-10.59	24.8	
LEv-16	1B	3.16	S1B_Sr31 - S1B_PART2_87711697	3.57	-3.34	11.0	31.9
	2B	55.0	S2B_PART1_216469337 - S2B_PART1_71304355	5.91	-4.93	21.9	
K-18	2B	57.5	S2B_PART1_216469337 - S2B_PART1_71304355	17.40	-27.51	53.9	53.9
Br23//C/P///B.13, seedling resistance							
RHKTF	1B	3.2	S1B_Sr31 - S1B_PART2_87711697	19.37	-0.76	50.0	67.4
	6A	162.5	S6A_PART1_12313749 - S6A_PART1_2409547	8.31	-0.71	17.4	
RRTTF	1B	3.2	S1B_Sr31 - S1B_PART2_87711697	16.74	-0.65	44.5	54.0
	2B	70.0	S2B_PART2_343379354 - S2B_PART1_41105268	5.05	-0.34	9.5	

^a AE= additive effect of the QTL, ^b R²= phenotypic variance explained by the QTL, ^c Total R² = total phenotypic variance explained with QTLs detected at each environment

In the I.Tero/B.13 population, the QTL on chromosome 2B (QTL-2B-I.T.) was the most consistent and had the largest effect in Uruguay, and is the only one explaining the resistance in Kenya. QTL-2B-I.T. had a mean additive effect (AE) of 12.3 and explained in average 29.3% of the field phenotypic variances (R^2), reaching a R^2 as high as 55.6% in K-18 (with a LOD = 42.7). In seedlings QTL-2B-I.T. was detected only to race RHKTF with a low LOD score, low R^2 of 4.6%, AE 0.24 and lines carrying only this QTL expressed mean seedling reaction of 2.2 (IT 2+ approximately). The QTL on chromosome 3D (QTL-3D-I.T.) had the second largest effect. It was detected in all Uruguayan environments, with mean AE of 5.21 and R^2 of 14.8%. In seedlings, QTL-3D-I.T. was significant to both races, had a high average R^2 of 43.9%, mean AE of 0.75, and lines carrying only this QTL expressed a mean seedling reaction of 0.70 and 0.75 (IT 1-) to races RHKTF and RRTTK, respectively. The QTL on chromosome 6A (QTL-6A-I.T.) was detected in all Uruguayan field tests (mean AE of 4.38 and R^2 of 9.7%) and in seedlings tests to both races, where it had a R^2 of 10.0%, mean AE of 0.32 and lines carrying only this QTL expressed mean seedling reaction of 1.5 and 1.7 (IT 2= and 2- approximately) to RHKTF and RRTTF, respectively. QTL on chromosome 7B (QTL-7B-I.T.) was only detected in LE-15 and was not significant in the seedling stage.

In the BR23//C/P/3/B.13 population, QTL on chromosome 1B (QTL-1B-BR.) was detected in field tests in LE, Uruguay, expressing a mean AE of 6.03 and average R^2 of 15.1%. QTL-1B-BR. had the highest effect on seedling resistance to both races with average R^2 of 47.3%, mean AE of 0.71 and lines carrying only this QTL expressed mean seedling reaction of 1.25 and 1.20 (IT 1+ approximately) to RHKTF and RRTTF, respectively. The QTL on chromosome 2B (QTL-2B-BR.) was the most important conferring field SR resistance in LE (Uruguay) and the only QTL detected in Kenya, expressing a mean AE of 14.24 and explaining an average of 33.5% of the field phenotypic variance, reaching R^2 53.9% in K-18. QTL-2B-BR. was also detected in the seedling stage to race RRTTF, with a relatively low LOD, R^2 and AE, and lines carrying only this QTL expressed a mean seedling reaction of 2.75 (IT 2+3

approximately). The QTL on chromosome 6A (QTL-6A-BR.) was only significant at the seedling stage to race RHKTF, had an intermediate LOD, R² and AE, and lines carrying only this QTL expressed a mean seedling reaction of 2.00 (IT 2).

Figure 6 graphically shows the effect of single QTLs and the increase of resistance resulting from their accumulation in both RIL populations.

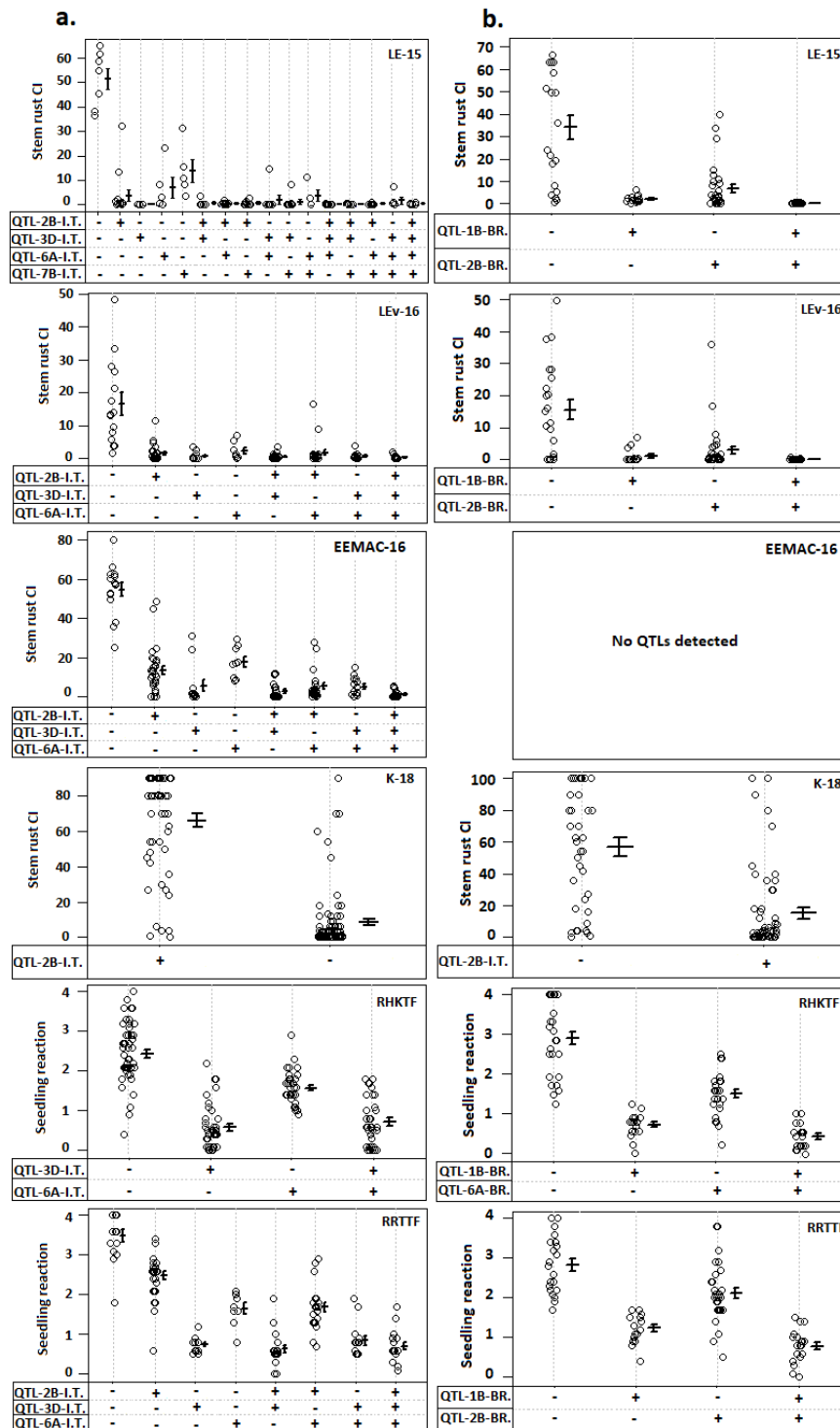


Figure 6: Effect of single QTL and the increase of field and seedling resistance resulting from their accumulation in a) INIA Tero/Baguette 13 and b) BR23//CEP19/PF85490/3/Baguette 13 RIL populations. “+” indicates lines carrying

the resistant allele of the QTL, “-“ indicates lines carrying the susceptible allele of the QTL.

3.4.7. BLAST of loci detected in QTL mapping for SR resistance

Intervals where the possible physical location of the SR resistance loci detected on chromosome 2B in each population were large (QTL-2B-I.T.: 627.2 and QTL-2B-BR.:145 MB) (Table 2). These two intervals overlapped from 142.9 to 216.5 MB. QTL-6A-I.T. was located on the distal region of the chromosome while QTL-6A-BR. was located in the proximal region of the chromosome, without physical overlapping. QTL-7B-I.T. was located in a relatively short interval of the chromosome.

Table 2: BLAST to the reference genome of the International Wheat Genome Sequencing Consortium of markers flanking each QTL detected.

QTL	Markers	Linkage position (cM)	Interval (cM)	Physic Position (MB)	Interval (MB)
2B-I.T.	S2B_PART1_142867158 - S2B_PART2_316804988	69.9 to 76.1	6.2	142.9 to 770.0	627.2
6A-I.T.	S6A_PART1_9321817 - S6A_PART1_2869043	114.8 to 125.7	11.0	286.9 to 932.2	645.3
7B-I.T.	S7B_PART2_295419700 - S7B_PART2_266995962	0 to 10.6	10.6	720.8 to 749.2	28.4
2B-BR.	S2B_PART1_216469337 - S2B_PART1_71304355	51.0 to 59.3	8.3	71.3 to 216.5	145.2
6A-BR.	S6A_PART1_12313749 - S6A_PART1_2409547	152.9 to 165.1	12.2	2.4 to 12.3	9.9

3.5. DISCUSSION

3.5.1. Phenotypic analysis

Precise phenotypic assessment is necessary for QTL mapping. According to SR infection on the susceptible parent B.13, used as susceptible check, SR epidemics were homogeneous and high in all four environments used for field testing, which allowed detecting statistical phenotypic differences and associations with genotypes. Frequency distributions of the adjusted mean SR CI in both populations at the four environments was skewed to lower infections scorings. The high proportion of lines with low infections in all environments probably indicates the involvement of major SR resistant genes. B.13 seedling SR infection was also consistently high for the two races tested indicating that reliable information was obtained.

Maturity is usually associated to the level of disease infection. In this study, days to heading was not associated with SR resistance as it was not significant when used as co-variate in the ANOVA model. QTLs detected for days to heading were different to QTLs for SR resistance since these were located on different chromosomes.

3.5.2. QTL mapping

According to the PCoA analysis (Figure 3), the distribution of genotypic information was as expected for the two crosses, where all RILs were distributed between the corresponding parental lines.

Molecular mapping of SR response in four environments and two seedling tests indicated the presence of at least four SR resistance QTLs in the I.Tero/B.13 population and three in the Br23//C/P/3/B.13 population. The I.Tero/B.13 population of 174 RILs allowed more precise detection of QTLs, since no lines without detected QTL were resistant (Figures 6a). The smaller population size of 107 lines of the Br23//C/P///B.13 population resulted in less variation of SR

resistance explained by the detected QTLs. In the latter population, additional resistance undetected by the QTL analysis is probably present, since the phenotypic values of some RILs without any of the detected QTL still showed some degree of resistance (Figures 6b).

3.5.2.1. Comparing QTL mapping and inheritance of seedling resistance

In the I.Tero/B.13 population, the QTL mapping and the chi-squared test indicated the presence of the same number of QTL/genes: two loci to race RHKTF and RRTTF (QTL-3D-I.T. and QTL-6A-I.T.) and an additional locus to race RRTTF (QTL-2B-I.T.), which was not significant to race RHKTF. Lines carrying only QTL-2B-I.T. had an intermediate IT since the mean seedling reaction was 2.2 (IT 2+ approximately) to race RRTTF and 2.5 (IT 2+3) to race RHKTF. QTL-2B-I.T. LOD score for race RRTTF was slightly higher, and the estimated threshold LOD was slightly lower than that for race RHKTF, a difference probably leading to its significant detection only to race RRTTF. Environmental variations within and between tests carried out three weeks apart might explain why this gene with low phenotypic effect was detected for one race and not for the other.

In the BR23//C/P/3/B.13 population, the inheritance of seedling resistance and QTL mapping determined the presence of two genes to races RHKTF and RRTTF. QTL-1B-BR. was detected to both races whereas QTL-6A-BR. was detected to race RHKTF and QTL-2B-BR to RRTTF. Seedling reactions of lines carrying only QTL-2B-BR. were similar to both races. The difference in the detection of this QTL to both races could be due to its intermediate resistant reaction. Seedling reactions of lines carrying only QTL-6A-BR. were also similar to both races (2.0 or IT 2 to race RHKTF and 2.1 or IT 2+ to race RRTTF). The difference in the detection of this QTL between races could be due to environmental conditions or experimental error.

3.5.2.2. QTL mapping in INIA Tero/Baguette 13

QTL-2B-I.T. had the largest effect in the adult plant stage, was consistently detected across all Uruguayan environments and was the only one explaining the resistance to Ug99+. Chromosome 2B has been reported as a hotspot of SR resistance loci since 11 designated *Sr* genes and 15 QTLs have been located on this chromosome (Yu *et al.* 2014; Jin *et al.* 2007). *Sr9h*, *Sr28*, *Sr32*, *Sr36*, *Sr39*, *Sr40* and *Sr47* are effective to the original Ug99 race TTKSK, although *Sr39* and *Sr36* became ineffective to the variant TTTSK (Yu *et al.*, 2014; Jin *et al.* 2009). Races TTKSK, TTKST, TTTSK, TTKTK and TTKTT were artificially inoculated in K-18 environment so QTL-2B-I.T. cannot be *Sr36* or *Sr39*. *Sr40* is unsuitable for utilization in breeding for resistance, due to linkage with undesirable traits in the translocation (Singh *et al.* 2015). *Sr47* is a very important resistance gene in tetraploid wheat (Klindworth *et al.* 2012; Faris *et al.* 1999), so it is unlikely to be the gene detected in this study. *Sr32* is located on 2BS (Dundas *et al.* 2007) while QTL-2B-I.T. was most probably located on 2BL, since flanking markers blasted to the interval between 172.9 and 770 MB of the IWGSC reference genome, which corresponds to the long arm of the chromosome, where also markers *wmc332*, *wmc175* and *wmc344* are located. Marker *wmc332* is linked to *Sr28* (Rouse *et al.* 2012), marker *wmc344* to *Sr40* (Wu *et al.* 2009) and markers *wmc332* and *wmc175* to *Sr9h* (Hiebert *et al.* 2010). QTL-2B-I.T. could be either *Sr28*, *Sr9h*, QTLs previously reported in the same region (Yu *et al.* 2011, Hiebert *et al.* 2010, Kaur *et al.* 2009), a new allele of *Sr9* or a new *Sr* gene. Specific markers for *Sr9h*, *Sr28*, *Sr40* should be used to confirm if QTL-2B-I.T. is any of these previously reported genes. If QTL-2B-I.T. is *Sr9h*, precautions have to be taken in breeding for resistance since TTKSF+, the latest race detected from the Ug99 lineage, reported in South Africa and Zimbabwe (Pretorius *et al.* 2012), is virulent to this allele (Singh *et al.* 2015).

QTL-3D-I.T. with the second largest effect was effectively detected in all Uruguayan environments and in seedling tests to the two Pgt races. This resistant

locus must be *Sr24* because the marker linked to this gene is present in I.Tero and was the marker linked to the detected QTL. *Sr24* is also effective in Uruguay and ineffective in Kenya. The pedigree information of I.Tero is limited so it was not possible to identify the source of *Sr24*.

QTL-6A-I.T. also explained a high proportion of the field and seedling SR resistance in Uruguay. This locus was not detected in Kenya so this resistance is ineffective to Ug99+. Four *Sr* genes, eight QTL (reviewed by Yu *et al.* 2014) and one QTL reported by Bariana *et al.* (2001) have been located on chromosome 6A. *Sr13*, *Sr26*, and *Sr52* are probably absent in I.Tero because these genes are effective to Ug99+. Additionally, until recently *Sr26* was not used in commercial varieties due to its associated yield penalty (Dundas *et al.* 2007) and *Sr52* was introgressed from *Dasypyrum villosum* (L.) lately (Qi *et al.* 2011). *Sr8a* is located on the short arm of chromosome 6A (McIntosh 1972) and it is effective to local RHKTF and RRTTF races. Blast with QTL-6A-I.T. flanking markers indicated it is physically located within the interval 286.9 to 932.2 MB, which is different from the *Sr8* reported location. Therefore, this QTL probably corresponds to a new *Sr* gene effective to Uruguayan races used in this study but ineffective to Ug99+. QTL-6A-I.T. could correspond to QTLs on 6A effective to Ug99 summarized by Yu *et al.* (2014) which are ineffective to other races of the Ug99 lineage present in field experiment K-18.

QTL-7B-I.T. was only detected at the adult plant stage in LE-15. Unlike *Sr17* and *Sr25* reported on chromosome 7B, this QTL was not detected in the seedling stage. In addition, *Sr25* is effective to Ug99+, therefore it is not likely that I.Tero has either *Sr17* or *Sr25*. Blast analysis indicated that QTL-7B-I.T. is located between 720.8 and 749.2 MB of the reference genome of the IWGSC and is likely an adult plant SR QTL. As discussed for QTL-6A-I.T., QTL-7B-I.T. could also correspond to some of the QTLs summarized by Yu *et al.* (2014) on chromosome 7B if these were ineffective to races present in K-18 or a new QTL.

3.5.2.3. QTL mapping in BR23//C/P/3/Baguette 13

The QTL on chromosome 1B explaining high proportion of the SR resistance in seedling and field tests in Uruguay (QTL-1B-BR.), corresponds to *Sr31*, as the peak of the QTL was located at the *Sr31* specific marker *iag95* (Mago *et al.* 2002). As expected this QTL was not detected in Kenya. *Sr31* probably was inherited from Alondra (Singh and Rajaram 1991) which is in the pedigree of BR23//C/P.

The QTL explaining the SR resistance in Kenya and in all Uruguayan environments in the BR23//C/P/3/B.13 population was detected on chromosome 2B (QTL-2B-BR.), physically located in the interval between 71.3 and 216.5 MB. This interval overlapped from 142.9 to 216.5 MB with QTL-2B-I.T.. In the interval where QTL-2B-BR. was blasted, only the marker *wmc344* linked to *Sr40* was located in the reference genome. As discussed for I.Tero, it is unlikely that *Sr40* could be present in BR23//C/P because of the reported yield penalty associated to this gene. It is possible that QTL-2B-BR. is *Sr28* gene since Kota, which carries *Sr28* (McIntosh 1978), is in the BR23//C/P pedigree. However, *Sr28* marker *wmc332* is close but not within the interval where this QTL blasted. It is also possible that *Sr9h* or an allele corresponds to this resistance. For these reasons, QTL-2B-BR. is most possibly QTL-2B-I.T.. Allelism test with a large population might be carried out to determine if QTL-2B-I.T. and QTL-2B-BR. correspond to the same gene.

QTL-6A-BR. was significant only to race RHKTF in the seedling stage. Among *Sr* genes and QTLs reported on chromosome 6A (Yu *et al.* 2014), *Sr8a* is probably the gene present in BR23//C/P as it is located in the same region as QTL-6A-BR, its resistant IT is 2- to 3, similar to BR23//C/P seedling reaction, and has limited field response (McIntosh *et al.* 1995). *Sr8a* is present in the Brazilian cultivar Frontana (McIntosh *et al.* 1995), which is an ancestor of BR23//C/P.

3.6. CONCLUSIONS

This study confirms the presence of at least one QTL for SR resistance effective to Ug99+ race complex, that could correspond to a previously reported gene or a new *Sr* gene. I.Tero carries four SR resistance loci: QTL-2B-I.T. which could correspond to Ug99+ effective genes *Sr9h*, *Sr28* or a new resistant gene; QTL-3D-I.T. which is *Sr24*; QTL-6A-I.T. which is possibly a new *Sr* gene effective in Uruguay and QTL-7B-I.T. which could correspond to one of the QTLs reported on chromosome 7B or a new QTL. BR23//C/P carries three SR resistance loci: QTL-1B-BR. which is *Sr31*, QTL-2B-BR. that could correspond to the same QTL detected in I.Tero and QTL-6A-BR. which could correspond to *Sr8a*. Most of these QTLs appear to be major genes expressing resistance from the seedling stage. Most seedling major genes are race-specific and have not conferred durable resistance. Therefore it is necessary to use them in combination with other effective resistance genes to increase their durability. As I.Tero and BR23//C/P are being extensively used in Uruguay in breeding for SR resistance, it is relevant to confirm if the genes on chromosome 2B from both resistant parents are the same or different genes to avoid redundancy in the genes used in breeding which leads to low genetic diversity and more probable pathogen adaptation. To provide longer SR resistance effective to local and Ug99+ it will be necessary to use the QTLs located on chromosome 2B in combination with other effective major resistance genes and/or adult plant minor resistance gene, to obtain a durable SR resistance to Ug99+ and Uruguayan races (Singh *et al.* 2005).

3.7. ACKNOWLEDGMENTS

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4. DISCUSIÓN Y CONCLUSIONES GENERALES

La roya del tallo (RT) fue la enfermedad más temida del cultivo de trigo a nivel mundial durante el siglo XX (Singh *et al.* 2011) por causar pérdidas totales en cultivares susceptibles (Germán y Verges, 2007). A través del uso generalizado de cultivares con resistencia genética, esta enfermedad no causó daños en las últimas tres décadas (Singh *et al.* 2008).

El área sembrada con cultivares susceptibles a RT en Uruguay y Argentina se incrementó significativamente con la introducción de materiales europeos de alto potencial de rendimiento susceptibles a esta enfermedad (Germán *et al.* 2009) a partir de comienzos de la década del 2000, lo que explica la re-emergencia a nivel epidémico de la roya de tallo en Uruguay a partir del año 2014. La aparición de la raza Ug99 (Pretorius *et al.* 2000) y el grupo de razas derivadas (Ug99+) generó una amenaza mundial ya que más del 80% del área mundial de trigo es susceptible a Ug99+ (Singh *et al.* 2015). En Uruguay y en la región, los genes *Sr24* y *Sr31* son los que confieren resistencia efectiva a roya de tallo en una alta proporción de los cultivares utilizados, lo que significa un gran riesgo en el caso de que alguna de las razas del linaje Ug99+ virulenta sobre ambos genes (Pretorius *et al.* 2000, Jin *et al.* 2008) migre a nuestra región. El aumento de temperatura global genera condiciones más propicias para el desarrollo de esta enfermedad. Estos tres factores indican que la importancia económica de la enfermedad probablemente incrementará en forma significativa.

Con el objetivo de controlar esta enfermedad, el objetivo del presente trabajo fue ajustar el manejo de RT con fungicidas que puede estar disponible en el corto plazo, y estudiar la base genética de la resistencia a razas locales y Ug99+ de dos fuentes de resistencia, para generar información que permita su uso más eficiente en mejoramiento genético para el desarrollo de cultivares resistentes.

La aplicación de fungicidas foliares en el momento de detección de los primeros síntomas es la clave para el manejo eficiente de RT tanto en un cultivar susceptible como en uno moderadamente susceptible. La aplicación a primeros síntomas en un cultivar susceptible logró reducir significativamente el desarrollo de la enfermedad, permitiendo concretar un 60.3% más de rendimiento en comparación con aplicaciones tardías en grano acuoso-lechoso (Z70 – Z75) (enfermedad ya avanzada), y un 130.9% más en comparación al testigo sin aplicar. Se midió el contenido de residuo de fungicidas en grano y, si bien los niveles no sobrepasaron el límite máximo de residuo (LMR) establecido por la Unión Europea, el 94% de las muestras de parcelas aplicadas tardíamente tuvieron residuo de los dos principios activos utilizados. Únicamente una muestra de una parcela donde se aplicó fungicida a primeros síntomas (floración) se detectó residuo en grano y la cantidad detectada fue mínima.

La utilización de una boquilla Turbo TwinJet 60 para realizar la aplicación de fungicidas en un cultivar susceptible determinó un desarrollo más lento de la enfermedad y niveles finales de infección de RT inferiores en comparación a la aplicación con una boquilla de cono hueco. El mejor control de RT no se tradujo en un mayor rendimiento ni calidad física de grano. Estas diferencias entre tipos de boquillas no se observaron en un cultivar moderadamente susceptible.

Deben llevarse a cabo más estudios para identificar ventajas específicas de algunos principios activos frente a otros. Los resultados preliminares mostraron que no se logró un mejor control con la adición de una estrobilurina ni de una carboxamida, en comparación con la mezcla de triazoles. Estos resultados son promisorios ya que la aparición de los primeros síntomas de RT ocurrió en floración en los dos años evaluados (2015 y 2016), que es también el momento crítico para la infección de fusariosis de la espiga. La aplicación de estrobilurinas provoca un aumento de la producción de la toxina deoxynivalenol (DON) de *Fusarium graminearum sensu stricto*, la especie más importante del complejo de especies

causantes de fusariosis de espiga (Díaz y Pereyra, 2011), por lo que contar con un manejo compatible para las dos enfermedades es muy valioso en las condiciones de producción de Uruguay.

A pesar de ser el manejo de la RT con fungicidas foliares fundamental para contar con herramientas de control en el corto plazo, el mejor método para el control de las royas es el uso de resistencia genética. La siembra de cultivares resistentes no sólo reduce los daños causados por la enfermedad, sino también frena su diseminación (Johnson, 1981). Para realizar eficazmente mejoramiento genético por resistencia a enfermedades es necesario conocer la base genética de la resistencia de las fuentes utilizadas de manera de evitar baja diversidad genética y poder desarrollar materiales con combinaciones de genes para obtener una resistencia duradera (Singh *et al.* 2015). Se identificaron dos materiales de trigo altamente resistentes a razas locales de Pgt y Ug99+, el cultivar uruguayo INIA Tero y la línea brasilera BR23//CEP19/PF85490. Los dos materiales resistentes se cruzaron con un cultivar susceptible y se estudió la base genética de la resistencia a través de mapeo de QTL en las dos poblaciones RILs desarrolladas.

Cuatro QTLs en los cromosomas 2B, 3D, 6A y 7B (QTL-2B-I.T., QTL-3D-I.T., QTL-6A-I.T. y QTL-7B-I.T., respectivamente) explicaron la resistencia en el cultivar INIA Tero. Todos los QTLs encontrados explicaron la resistencia a las razas locales, mientras que únicamente QTL-2B-I.T. fue efectivo a Ug99+ en Kenia. Los QTLs QTL-2B-I.T., QTL-3D-I.T. y QTL-6-I.T. se expresaron en el estado de plántula y confirieron resistencia a campo, mientras que el QTL-7B-I.T. confirió únicamente resistencia a campo. Se confirmó que el QTL-3D-I.T. es el gen de resistencia *Sr24*. Se estima que el QTL-2B-I.T. puede ser el *Sr28*, *Sr9h*, un nuevo alelo del *Sr9* o un nuevo gen *Sr* efectivo en África, mientras que el QTL-6A-I.T. puede ser un nuevo gen *Sr* efectivo a razas locales e inefectivo a Ug99+ y que el QTL-7B-I.T. puede ser uno de los 10 QTL reportados previamente en el cromosoma 7B (Yu *et al.* 2014).

Tres QTLs explicaron la resistencia de la línea experimental BR23//CEP19/PF85490. Se confirmó que el QTL detectado en el cromosoma 1B (QTL-1B-BR. corresponde al gen *Sr31*, el cual explicó en gran proporción la resistencia en plántula y a campo a las razas locales. El QTL-2B-BR. fue efectivo frente a las razas locales en plántula y a campo y el que explicó la resistencia a Ug99+. La posición física más probable de este gen obtenida al blastear los marcadores flanqueantes contra el genoma de referencia del International Wheat Genome Sequencing Consortium, 2014 (IWGSC), demuestra la posibilidad de que este gen sea el mismo que el detectado en INIA Tero. Deben llevarse a cabo estudios de alelismo y genotipado con marcadores moleculares específicos para los genes *Sr28* y *Sr9h* para dilucidar la identidad de estos genes. Por último, el QTL-6A-BR. fue detectado sólo en el estado de plántula frente a la raza local RHKTF, y se estima que puede ser *Sr8a*, previamente reportado en el brazo corto de este cromosoma (McIntosh, 1972).

Ambas líneas parentales resistentes están siendo utilizadas como fuentes de resistencia para el desarrollo de variedades resistentes a RT por lo que conocer la identidad de el o los genes del cromosoma 2B es fundamental para evitar redundancias y diseñar estrategias para asegurar diversidad genética de la resistencia. Este o estos valiosos genes efectivos a Ug99+ y razas locales, deben ser utilizados en combinación con otros genes efectivos para obtener resistencia más duradera.

5. BIBLIOGRAFÍA GENERAL

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