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MAPEO ASOCIATIVO DE LA RESISTENCIA A ENFERMEDADES DEL TALLO Y LA VAINA EN GERMOPLASMA AVANZADO DE ARROZ

por

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Dedico este trabajo a mi familia.

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RESUMEN

En Uruguay se aplican fungicidas en casi el 100% del área arrocera, debido principalmente a la susceptibilidad de los cultivares locales a las enfermedades del tallo causadas por *Nakataea oryzae* (NO) y *Rhizoctonia oryzae sativae* (ROS). Para obtener nuevos cultivares resistentes se requieren metodologías de selección más eficientes. Se compararon cinco métodos para evaluación de la resistencia a NO y ROS en invernáculo, identificándose el más adecuado. Una población de 641 líneas avanzadas de tipo *indica* y *japonica* tropical fue fenotipada para resistencia a NO y ROS en invernáculo y en ensayos de campo. Se realizó un estudio de asociación (GWAS) entre polimorfismos de un nucleótido (*single nucleotide polymorphisms*, SNPs) genómicos y las medias fenotípicas de resistencia corregidas por altura de planta y tiempo de floración. El análisis de GWAS detectó 29 QTL asociados con resistencia a las enfermedades estudiadas, independientes de altura de planta y largo de ciclo. Los QTL encontrados explicaron hasta el 43% y 21% de la varianza fenotípica en ensayos de campo e invernáculo, respectivamente. Se identificó una región en el cromosoma 9 que explicó más del 15% de la varianza fenotípica de las enfermedades estudiadas. Los SNPs identificados pueden ser utilizados para selección asistida de la resistencia a NO y ROS en el programa de mejoramiento genético de arroz de INIA, sin afectar la altura de planta y largo del ciclo, y con una eficiencia comparable a la de los actuales ensayos de campo.

Palabras clave: fenotipado, GWAS, mancha agregada de las vainas, pudrición del tallo, tizón de las vainas.

GENOME-WIDE ASSOCIATION MAPPING OF RESISTANCE TO STEM AND SHEATH DISEASES IN ADVANCED RICE GERMPLASM

SUMMARY

Fungicides are sprayed over almost 100% of the Uruguayan rice crop area, mostly due to susceptibility of local cultivars to stem and sheath diseases caused by *Nakataea oryzae* (NO) and *Rhizoctonia oryzae sativae* (ROS). To breed new resistant cultivars, more efficient selection methods are required. Five greenhouse methods for greenhouse evaluation of resistance to NO and ROS were compared. A population of 641 *indica* and tropical *japonica* type advanced breeding lines were phenotyped for resistance to NO and ROS in greenhouse and in field trials. A genome-wide association study (GWAS) was performed to analyze the association between single nucleotide polymorphisms (SNPs) and the field and greenhouse phenotypic means, adjusted by plant height and flowering time. GWAS analysis detected 29 QTL for resistance to the studied diseases, independent of plant height and flowering time, explaining up to 43% and 21% of the phenotypic variance observed in field and greenhouse trials, respectively. A region on chromosome 9 explained more than 15% of the phenotypic variance of studied diseases. The identified QTL are useful for assisted selection of resistance to NO and ROS in INIA's rice breeding program, without effects in plant height and flowering time, and with an efficiency comparable to that of the currently used field trials.

Keywords: phenotyping, GWAS, aggregate sheath spot, stem rot, sheath blight.

1. INTRODUCCIÓN

El arroz (*Oryza sativa* L.) es la base alimenticia de más de la mitad de la humanidad, y juega un rol clave en la seguridad alimentaria mundial. En Uruguay el arroz es actualmente el cuarto principal rubro de exportación, detrás de la carne, soja y productos forestales. Con más de 500 millones de dólares anuales, representa aproximadamente el 7% del valor exportado por el país (Uruguay XXI 2013). Las exportaciones uruguayas de arroz representan el 95% de la producción nacional de arroz (Zorrilla 2015), colocando a Uruguay entre los siete mayores exportadores del mundo y primero de Latinoamérica (Pérez del Castillo y Alfaro 2010). Este perfil exportador, en un mercado internacional caracterizado por fuertes subsidios de los principales países exportadores (John 2014) ha moldeado la cadena agroindustrial del arroz en Uruguay. Es así que el sector arrocero nacional se caracteriza por la amplia adopción de tecnologías desarrolladas localmente, incluyendo prácticas de manejo del cultivo y los cultivares más sembrados (Pittelkow et al. 2016). La necesidad de mantener altos niveles de competitividad hace que sea un sector que demanda constantemente a la investigación nacional el desarrollo de tecnologías y productos que aumenten la productividad disminuyendo los costos (Zorrilla 2015). Uno de los factores que atentan contra la productividad son las enfermedades que afectan al cultivo, lo cual además requiere un aumento de los costos de producción, llevando a que se aplique fungicidas al 94% del área sembrada (Martínez 2016). Si bien la enfermedad más importante en nuestro país es el *brusone* o quemado del arroz, el principal motivo de la alta dependencia en fungicidas son las enfermedades del tallo y la vaina (Martínez 2016).

Las principales enfermedades que afectan tallos y vainas del arroz en nuestro país son la podredumbre del tallo, causada por el hongo *Nakataea oryzae* (Catt.) (NO) (previamente, *Sclerotium oryzae* Catt.), y la mancha confluente de las vainas, causada por el hongo *Rhizoctonia oryzae-sativae* (Sawada) Mordue (ROS) (= *Ceratorhiza oryzae-sativae* (Sawada) R. T. Moore). NO causa la segunda enfermedad en importancia en el país, y está presente en todas las zonas arroceras del mundo

(Webster y Gunnell 1992). Las pérdidas por NO reportadas en Uruguay alcanzan hasta un 24% en cultivares susceptibles y años favorables a la enfermedad (Avila 2000). Si bien ROS es considerado un patógeno de importancia secundaria a nivel mundial, es un problema creciente en regiones templadas y subtropicales como California, el sudeste de Australia y Uruguay (Lanoiselet et al. 2007). La actual intensificación del cultivo aumenta los niveles de inóculo y consecuentemente la prevalencia y severidad de ambas enfermedades (Cintas y Webster 2001; Lanoiselet et al. 2005; Blanco et al. 2010). Este escenario de vulnerabilidad frente a enfermedades del tallo y la vaina requiere un manejo integrado que permita disminuir la dependencia de fungicidas, incluyendo el uso de cultivares con resistencia genética aumentada (McKenzie et al. 1994; Lanoiselet et al. 2007).

Los niveles de resistencia a NO y ROS en el germoplasma uruguayo de arroz son variables, predominando la susceptibilidad a ROS en los materiales de tipo *japonica* tropical, y niveles de susceptibilidad intermedia a NO en materiales de tipo *indica* (Blanco et al. 2016). La selección fenotípica por resistencia a NO y ROS que se realiza actualmente en el Programa de Mejoramiento consiste en ensayos de campo en parcelas con infección natural. Esta estrategia presenta algunas dificultades que limitan el avance genético en estos rasgos: 1) se requiere un número de individuos por genotipo que sólo es alcanzable en etapas avanzadas de evaluación (Jia et al. 2007); 2) la infección natural hace que la cantidad de inóculo dentro y entre las parcelas experimentales y entre distintos años sea heterogénea, imponiendo un fuerte efecto ambiental (McKenzie et al. 1994; Ni et al. 2001), lo que genera bajas correlaciones entre años y en algunos casos bajas heredabilidades en los ensayos (Fernando Pérez de Vida, comunicación personal, febrero de 2013; y 3) la evaluación de los síntomas se realiza sobre el fin del ciclo del cultivo, momento en que la senescencia de hojas y vainas de algunos genotipos puede ser un factor de confusión en dicha evaluación (Sebastián Martínez, comunicación personal, febrero de 2013). Esto hace deseable el desarrollo e implementación de métodos alternativos para seleccionar por resistencia a NO y ROS, como pueden ser el fenotipado en

condiciones ambientales controladas y la selección asistida por marcadores moleculares asociados a resistencia.

Debido a la importancia secundaria de NO y ROS entre los principales patógenos que afectan al arroz a nivel mundial, estos patógenos han sido poco estudiados y hay diversas interrogantes no resueltas. Entre ellas, no hay reportes concluyentes sobre el mejor método de inoculación para evaluar la resistencia a NO en condiciones controladas (Carreres et al. 1994; Cother y Nicol 1999; Kumar et al. 2003), y no hay métodos reportados específicos para ROS. Por el contrario, el tizón o añublo de las vainas, causado por el hongo *Thanatephorus cucumeris* (Frank) Donk (TC) (previamente, *Rhizoctonia solani* Kühn) es una enfermedad con ciclo de vida similar al de NO y ROS que afecta principalmente zonas tropicales y ha sido profusamente estudiada (Zheng et al. 2013). Diversos métodos de fenotipado de la resistencia a TC en condiciones ambientales controladas han sido desarrollados y comparados en varios estudios (Jia et al. 2013). Debido a las similitudes de las afecciones causadas por TC con las causadas por NO y ROS, es esperable que algunos de los métodos utilizados para TC puedan adaptarse a NO y ROS.

La utilidad de la selección asistida por marcadores moleculares para un determinado rasgo depende, entre otros factores, de la arquitectura genética de este rasgo (Bernardo 2008). Para evaluar la viabilidad de la selección de resistencia a enfermedades del tallo y la vaina es necesario por lo tanto conocer la forma en que estos caracteres están genéticamente regulados en la población de interés. Las resistencias a TC y a NO han sido reportadas como caracteres cuantitativos, regulados por múltiples loci (QTL) (Ni et al. 2001; Lanoiselet et al. 2007; Srinivasachary et al. 2013). Mientras que hay sólo un estudio publicado sobre identificación de QTL de resistencia a NO (Ni et al. 2001), no existen a la fecha reportes a sobre el control genético de la resistencia a ROS. El mapeo asociativo genómico o *genome-wide association study* (GWAS, estudio de asociación genómico) es una herramienta para identificación de QTL en poblaciones con distintos niveles de parentesco entre los individuos que las integran (Jannink et al. 2001). El análisis de GWAS habitualmente

empleado en plantas consiste en estimar la probabilidad de asociación entre cada marcador molecular y el fenotipo de interés. Los modelos estadísticos empleados más comúnmente incluyen, además del efecto del marcador, uno o más términos para modelar el *background* genético y/o las relaciones de parentesco entre los genotipos (Yu et al. 2006). Estos términos son necesarios a fin de corregir las asociaciones entre marcadores sin relación causal con el fenotipo pero cuyos estados alélicos se correlacionan con subpoblaciones o familias (Marchini et al. 2004). El uso de modelos mixtos permite incluir a los genotipos como efectos aleatorios, con una matriz de covarianzas entre los genotipos determinada por los marcadores moleculares (Kennedy et al. 1992; Habier et al. 2007). Es posible incorporar al modelo de asociación un término para uno o más marcadores moleculares adicionales al estudiado, para encontrar así QTL independientes entre sí (von Zitzewitz et al. 2011). Los estudios de resistencia a TC enfatizan la necesidad de controlar factores de confusión de tipo morfológico o fenológico, como la atura de planta o el largo del ciclo (Willocquet et al. 2011; Lore et al. 2013; Zeng et al. 2015). Estudios de QTL para resistencia a enfermedades de cultivos en poblaciones biparentales han utilizado marcadores asociados a factores de confusión como la altura de la planta para identificar QTL de resistencia a enfermedades, independientes de la altura de la planta (Nelson et al. 2012). Análogamente, es posible incorporar al modelo de GWAS el efecto de marcadores asociados a factores de confusión en la evaluación fenotípica como altura de planta o largo del ciclo, permitiendo de esta forma la identificación de QTL de resistencia a enfermedades del tallo y la vaina que sean independientes de éstos.

Las hipótesis planteadas en la presente tesis son:

1. Los métodos reportados para la inoculación y evaluación en invernáculo de resistencia a TC y NO difieren en su repetibilidad (heredabilidad) cuando son aplicados en la evaluación de la resistencia a NO y ROS del germoplasma de mejoramiento de arroz de INIA.

2. En el germoplasma de mejoramiento de arroz de INIA al menos un grupo de marcadores moleculares ubicados a menos de 1 Mb de distancia entre sí se asocian con la resistencia a NO, ROS, y/o TC, en forma independiente de la estructura de la población y de factores de confusión como altura de planta y largo del ciclo.

En forma concordante con las hipótesis planteadas, los objetivos de la tesis son:

1. Identificar un método de inoculación y evaluación en invernáculo de la resistencia a NO y ROS con mayor heredabilidad que los ensayos de campo.
2. Evaluar la resistencia a TC y NO en una población representativa del programa de mejoramiento de arroz de INIA, con el método identificado en el objetivo 1.
3. Estudiar la asociación de marcadores moleculares distribuidos en el genoma con la resistencia a NO y ROS evaluada en ensayos de campo e invernáculo, y con la resistencia a TC evaluada en invernáculo, independiente de factores de confusión.
4. Evaluar la conveniencia desde el punto de vista genético de los nuevos métodos de selección por resistencia a NO y ROS en el programa de mejoramiento genético de INIA.

2. COMPARISON OF PHENOTYPING METHODS FOR RESISTANCE TO STEM ROT AND AGGREGATED SHEATH SPOT IN RICE

2.1. RESUMEN DE CONTENIDO Y APORTE

Las principales enfermedades que atacan vainas y tallos del arroz en Uruguay son las causadas por *Nakataea oryzae* (NO) y *Rhizoctonia oryzae-sativae* (ROS). El avance genético de la resistencia a estos patógenos está limitado por la heterogeneidad ambiental difícil de controlar en ensayos en parcelas a campo, con heredabilidades muchas veces inferiores a 0,2. La evaluación bajo condiciones ambientales controladas en invernáculo es una alternativa posible, pero no existen reportes sobre métodos de inoculación y evaluación en invernáculo para ROS, y los que hay para NO no son concluyentes. *Thanatephorus cucumeris* (TC) es un patógeno de arroz muy estudiado y cercano filogenética y fitopatológicamente a NO y ROS. En este artículo, cinco métodos reportados para TC y/o NO, fueron adaptados y evaluados para la determinación de la resistencia a NO y ROS en ensayos de invernáculo. Este fue el primer reporte en comparar sistemáticamente los principales métodos de inoculación para enfermedades del tallo y la vaina en arroz en base a su repetibilidad y capacidad de discriminar entre genotipos. El método de mejor desempeño fue utilizado para estudiar la resistencia a NO y ROS en una población de 641 líneas representativas del programa de mejoramiento genético de arroz de INIA, obteniéndose heredabilidades de 0,65 a 0,89 en los distintos ensayos. El método de evaluación reportado permite una evaluación de la resistencia a NO y ROS de mayor precisión que los ensayos de campo, y es aplicable para el fenotipado masivo de poblaciones de mejoramiento o de mapeo asociativo. Este artículo fue publicado en 2016 en la revista *Crop Science* (Rosas et al. 2017).

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RESEARCH

Comparison of Phenotyping Methods for Resistance to Stem Rot and Aggregated Sheath Spot in Rice

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ABSTRACT

Stem and sheath diseases caused by *Sclerotium oryzae* Cattaneo (SCL) and *Rhizoctonia oryzae-sativae* Sawada Mordue (ROS) can severely reduce rice (*Oryza sativa* L.) yield and grain quality. Genetic resistance is the best strategy to control them. Phenotypic selection for resistance is hampered due to a heterogeneous distribution of the inoculum in the soil that generates high environmental variability and decreases genetic gain. To have higher selection accuracy it is necessary to develop phenotyping methods with high repeatability and discriminative power. Comparison of greenhouse methods have been reported for *Rhizoctonia solani* Kühn, a more invasive pathogen than SCL and ROS, and for SCL, but no such comparisons are reported for ROS. Our study compares five inoculation methods for SCL and ROS to identify the more discriminant and repeatable method and to apply it for high-throughput phenotyping of hundreds of rice lines. A method that uses an agar disc with growing mycelium attached to the base of stems was found to have the best balance between discrimination among genotypes and variability among replicates of the same genotype for both pathogens. This method was used in five greenhouse experiments for phenotyping resistance to SCL and ROS in a population of 641 rice advanced breeding lines. Heritabilities of resistance ranged from 0.36 to 0.71 in these experiments. These findings have a direct application in screening for resistance of rice to SCL and ROS, and in high-throughput phenotyping for mapping loci associated to disease resistance.

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Abbreviations: INIA, Instituto Nacional de Investigación Agropecuaria; ROS, *Rhizoctonia oryzae-sativae*; SCL, *Sclerotium oryzae*.

Stem rot and aggregated sheath spot are among the major biotic constraints in temperate rice production worldwide (Ferreira and Webster, 1976; Lanoiselet et al., 2007). Growing cultivars with genetic resistance is the most efficient strategy to cope with these diseases (Savary et al., 2012). However, efforts for breeding resistant cultivars must overcome the inconsistencies in disease evaluation due to inoculum and environmental variabilities often found in field experiments (Madhav et al., 2013; Srinivasachary et al., 2013).

Stem rot and sheath spot of rice and their causal agents have received little attention compared to other rice diseases and pathogens. Stem rot is caused by *Nakataea oryzae* (Cattaneo) J. Luo and N. Zhang, best known in the sclerotial state as *Sclerotium oryzae* Cattaneo (SCL). Stem rot has been documented in almost every rice-growing country, but the more relevant research has

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been focused to the conditions, isolates, and cultivars of California and Australia. Yield losses due to SCL have been roughly estimated in various countries and crop conditions, ranging from 5 to 75% (Chauhan et al., 1968; Ou, 1985). In experimental plots with high inoculum density and a susceptible cultivar, a maximum loss of 22% was found (Cintas and Webster, 2001).

Aggregated sheath spot of rice is caused by *Rhizoctonia oryzae-sativae* (≡ *Ceratorhiza oryzae-sativae*) Sawada Moore (ROS). As for stem rot, aggregate sheath spot is a widespread rice disease, but is considered a major rice production constraint in temperate rice growing regions like California, the Mediterranean, Australia and the Southern Cone of South America (Gunnell and Webster, 1984; Lanoiselet et al., 2007; Chaijuckam and Davis, 2010; Martínez et al., 2014). It was not until 2007 that the first comprehensive review about ROS was undertaken (Lanoiselet et al., 2007). Regarding losses caused by ROS, up to 20% yield reductions were found in field experiments in Australia and 9% in commercial field conditions in Uruguay (Lanoiselet et al., 2005).

Phenotyping for resistance to stem rot and aggregated sheath spot of rice is challenging (McKenzie et al., 1994; Ni et al., 2001). Three main procedures for evaluating the response of rice to SCL were reported (Krause and Webster, 1973; Cother and Nicol, 1999; Kumar et al., 2003), mainly differing in the level of environmental control and in the inoculation method used. Briefly, one method involves spreading sclerotia or infected grain husk over the irrigation water surface or over soil surface immediately prior to flooding (Krause and Webster, 1973; Cother and Nicol, 1999). The rough amount of viable sclerotia per plant ranged from 200 to 7500 spreading at 21 d after seeding. Stems are expected to be infected by floating sclerotia on contact mimicking field conditions, or infection can be favored by artificially injuring the stems at the water level. Another method involves the attachment of an agar disc with SCL mycelium to tillers (Cother and Nicol, 1999). An agar disc is secured against each stem at water level with grafting tape and a paper clip at 50 to 60 d after seeding. Finally, another method involving attachment of infected 5-mm long pieces of *Typha angusifolia* L. or susceptible rice cultivars at the base of tillers was described by Kumar et al. (2003). Comparative studies among these methods found a similar variability of symptoms displayed with spreading of sclerotia and agar discs (Cother and Nicol, 1999), while higher mean disease incidence was produced with spreading of sclerotia and infected plant pieces than with agar discs (Kumar et al., 2003). However, it is not clear if methods with higher variability and mean incidence enabled a better discrimination among resistance levels of rice genotypes. For rating susceptibility of cultivars to SCL, Krause and Webster (1973) scored with a 1–5 scale, where 1 = healthy tillers and 5 = dead tillers, and then calculated a disease index as the ratio between the summation of the infection score of each tiller divided by the total number of tillers. Alternatively, Cother and Nicol (1999) used canonical variable analysis to identify a linear function of the presence of sclerotia in the sheath, outer and inner culms. Finally, Kumar et al. (2003) applied the Standard Evaluation System for Rice (IRRI, 2002) which consists of a 0–9 scale with 0 = no lesions observed and 9 = lesions observed in more than 65% of the plant height.

Few inoculation and rating procedures specific for ROS are reported. Chaijuckam et al. (2010) used a cut-tiller method to evaluate pathogenicity of ROS isolates. Briefly, an agar plug of 6 mm in diameter was placed on the surface of the stem and incubated at room temperature in transparent closed trays with high relative humidity. Evaluation was performed by measuring the length of the lesions at 4 d after inoculation.

Inoculation methods for other stem and sheath diseases were reported. Most of the literature about evaluation of resistance to rice sheath diseases was focused on *Rhizoctonia solani* Kühn, the causal agent of sheath blight. Methods for studying *R. solani* Kühn resistance in rice were reviewed elsewhere (Jia et al., 2013), showing a number of diverse inoculation procedures including colonized toothpicks and agar plugs (Zou et al., 2000; Rodrigues Peters et al., 2001; Eizenga et al., 2002), mixtures of infected rice grain and hull (Pan et al., 1999), and sclerotia (Wasano et al., 1983; Singh et al., 2002). While most of these methods are suspected to introduce variability into the infection process, Park et al. (2008) report a 100% of infection rate for their method, done by placing a liquid cultured mycelia ball beneath each leaf sheath.

In summary, three inoculation methods were reported for SCL, and one for ROS. Several methods were reported for other rice sheath diseases like *R. solani* Kühn but these have not been tested in SCL or ROS. It is not clear which inoculation method enables the most discriminant and accurate disease phenotyping. Therefore, the information about methods for controlled evaluation of rice resistance to stem rot and aggregated sheath spot remains critically and important. The objective of this work was to evaluate five inoculation methods both for SCL and ROS, and to compare them based on their precision and ability to discriminate among different levels of genetic resistance. The best performing method was verified in a high throughput greenhouse phenotyping of 641 advanced rice breeding lines for both diseases.

MATERIALS AND METHODS

Inoculation Methods

Five inoculation methods were compared using six rice cultivars for two diseases, ROS and SCL, at the Instituto Nacional de Investigación Agropecuaria (INIA), Treinta y Tres, Uruguay.

Plant Materials

Six rice genotypes ('El Paso 144', 'INIA Olimar', 'Tetep', 'INIA Tacuarí', 'Parao' and 'Lemont') were chosen to have a wide range of susceptibility to rice stem and sheath diseases. El Paso 144 (Yan et al., 2007) and INIA Olimar (Blanco et al., 2004) are the two most widely grown *indica*-type cultivars in Uruguay and have an intermediate response to SCL and ROS (Martínez and Escalante, 2012). Tetep (Yan et al., 2007) is a Vietnamese *indica* traditional cultivar with resistance to SCL (Chien, 1977) and to *R. solani* Kühn (Bhuiyan and Arai, 1994), and has unreported response to ROS. INIA Tacuarí (Blanco et al., 1993) and Parao (Molina et al., 2011) are the two most widely grown tropical *japonica*-type cultivars in Uruguay, with intermediate response to SCL and ROS (Martínez and Escalante, 2012). Lemont (Bollich et al., 1985) is tropical *japonica* cultivar in the southern United States that is highly susceptible to SCL (Mazzanti de Castañón et al., 1994), *R. solani* Kühn, and other rice diseases (Li et al., 1995), and unknown response to ROS.

Inoculum Production

Sclerotia of both pathogen species were produced with a Krause and Webster (1972) modified method. Substrate consisted of 2:1 (v/v) of rice seeds to rice hulls in 1 L of distilled water amended with 1 g $Ca(NO_3)_2$, 4 g $CaCO_3$, 0.25 g $MgSO_4$, and 2 g dextrose. Polypropylene bags were half filled with substrate, the open side closed with a cotton plug and autoclaved twice for 45 min with a 24-h interval. After cooling the substrate, each bag was inoculated with 7-d old mycelia obtained from cultures grown in 90-mm Petri dishes containing potato-dextrose agar solid medium (Oxoid Limited, Hampshire, UK). These bags were incubated for 25 d at 23°C. After inoculation, the content was removed of the bag, dried at 40°C in an electric oven, and the sclerotia separated in a 1-mm mesh. Sieved sclerotia were conserved at 4°C until utilization.

Fungal Isolates

Two fungal isolates were used in this study. Ten SCL isolates were obtained from different rice cultivars in naturally infected experimental plots from the Experimental Unit of Paso de la Laguna (UEPL, 33°16′ S, 54°10′ W), Treinta y Tres, Uruguay, in 2011. The ten isolates were tested on eight common Uruguayan rice cultivars, and the isolate with the highest discriminative power was chosen (Martínez and Escalante, 2012). Similarly, ten ROS isolates were obtained from soil after rice cultivation with the tropical *japonica* rice cultivar INIA Tacuari from the Experimental Unit of Paso de la Laguna in 2003, and the isolate with the highest discriminative power was chosen (Martínez and Escalante, 2012). Both isolates are stored in potato-dextrose agar slants at 4°C and maintained at the Laboratorio de Patología Vegetal, INIA, Treinta y Tres, Uruguay.

Experimental Design

Five simultaneous inoculation experiments were conducted in greenhouse conditions (28:18°C day/night, 80– 90% relative humidity, and 12 h light time) during September 2013 at INIA Treinta y Tres Experimental Station (33°15′ S, 54°25′ W). For each method, a completely randomized design with six replicates was used. For *Methods I to IV*, experimental units consisted of 180 cm^3 pots with four single stem plants each uniformly distributed in a cross pattern in the pot. Each pot was fertilized with 160 kg N ha^{-1} before sowing. All pots were placed in trays of 30-cm depth. At 3-leaves stage, five inoculation methods were tested. *Method I* is a modification of the agar disc inoculation procedure described by Cother and Nicol (1999). Each one of the four single stem plants was inoculated with a 5-mm agar disc with growing mycelium attached to the base of the stems at 3 cm above the soil surface. Isolates were grown for 7 d at 25°C in 90-mm Petri dishes containing potato-dextrose agar. Agar discs taken from the border of an actively growing colony were fastened to the base of the stem with Parafilm 'M' (Pechiney Plastic Packaging, Chicago, IL) and oriented with their bottom side contacting the stem. *Method II* is a modification of the sclerotia spreading inoculation procedure described by Cother and Nicol (1999). The flooded trays containing pots with four single stem plants each were inoculated manually by spreading the sclerotia over water surface at 2.5 cm above soil surface at 1.5 mg cm^{-2} (SCL) or 7.5 mg cm^{-2} (ROS). Sclerotia were sieved with a 0.6-mm sieve for SCL and a 1.4-mm sieve for ROS to remove plant remains and other debris from inoculum. *Method III* is a new method that consisted in each one of the four single stem plants being inoculated at water surface level with 1 mL of an 8% suspension of carboxymethyl cellulose (90000 g mol^{-1}, Sigma-Aldrich, St. Louis, MO) with 240 mg mL^{-1} (SCL) or 1200 mg mL^{-1} (ROS) of sieved sclerotia. This highly viscous and adhesive suspension was manually applied to the base of the stems at 3 cm from the soil surface before flooding. *Method IV* is a modification of *Method III* where the carboxymethyl cellulose-sclerotia suspension was covered with an aluminum foil to prevent it from being washed by the flooding water. Trays for *Methods I* through *IV* were flooded up to 2.5 cm above the soil surface. *Method V* is a modification of the detached stems procedure proposed by Chaijuckam et al. (2010). A single stem plant from each pot was detached at 3-leaves stage and incubated in test tubes with sclerotia. Stems were sanitized with 70% ethanol for 1 min and 5% sodium hypochlorite for 3 min, rinsed with sterile water, and each one put in a test tube with 15 mL of sterile distilled water. The lower 4 cm of stems were submerged in water. Sieved sclerotia were added to each tube (2.4 mg SCL and 12.0 mg ROS) and incubated at 25°C with 85% relative humidity.

Disease Rating

Diseases were scored at 45 d (*Methods I* to *IV*) or 15 d (*Method V*) post-inoculation. An adaptation of the scoring scale proposed by IRRI (2002) for *R. solani* Kühn was used to rate the diseases in *Methods I* to *IV*. Disease was scored with a 0 to 9 severity scale in which 0 = no infection is observed, 1 = lesions are limited to 20% of the plant height, 3 = lesions cover 21 to 30%, 5 = lesions cover 31 to 45%, 7 = lesions cover 46 to 65%, and 9 = lesions cover >65%. For *Method V*, the disease score was the average length of visible symptoms along both sides of the stem length (cm).

Statistical Analysis

The model used for evaluation of inoculation methods and their interaction is shown in Eq. [1]:

$$Y_{ijk} = \mu + g_i + m_j + (gm)_{ij} + \varepsilon_{ijk} \quad [1]$$

where Y_{ijk} is the disease score for the i^{th} genotype in its k^{th} replicate with the j^{th} inoculation method, μ is the intercept, g_i is the fixed effect of the i^{th} genotype, m_j is the fixed effect of the j^{th} inoculation method, $(gm)_{ij}$ is the interaction effect of the i^{th} genotype with the j^{th} inoculation method, and ε_{ijk} is the residual for the i^{th} genotype in its k^{th} replicate with the j^{th} inoculation method, with $i = \{1, \ldots, 6\}$, $j = \{1, \ldots, 5\}$ and $k = \{1, \ldots, 6\}$. Genotype and inoculation main effects as well as genotype by inoculation method interaction effect were tested using g_i, m_j, and $(gm)_{ij}$ as fixed effects. If the interaction term from an ANOVA was significant ($\alpha = 0.05$), then a Tukey's HSD test ($\alpha = 0.05$) was used. Pearson correlation of genotypic means was estimated between each pair of inoculation methods. Statistical analyses were run in R software (R Core Team, 2014) using *lm* for fitting the model and *agricolae* for Tukey analysis (De Mendiburu, 2014).

The model for estimating the variance components and repeatability of inoculation methods is shown in Eq. [2]:

$$Y_{ij} = \mu + g_i + \varepsilon_{ij} \quad [2]$$

where Y_{ij} is the disease score for the i^{th} genotype in its j^{th} replicate, μ is the intercept, g_i is the random effect of the i^{th} genotype with $g_i \sim N(0, \sigma^2_G)$, and ε_{ij} is the residual for the i^{th} genotype in its j^{th} replicate, with $i = \{1, \ldots, 6\}$ and $j = \{1, \ldots, 6\}$. Statistical analyses of this model were run in R software with *lme4* package (Bates et al., 2005).

Methods were also compared based on their repeatability (H^2) calculated on a genotype mean basis (Eq. [3]).

$$H^2 = \frac{\sigma^2_G}{\sigma^2_G + (\sigma^2_\varepsilon / r)} \quad [3]$$

where σ^2_G is the genetic variance, σ^2_ε is the residual variance, and r is the number of replicates. Standard errors and confidence intervals for variances and repeatability were estimated with a bootstrap data resampling technique as recommended by Holland et al. (2003) and implemented in the *boot* R package (Canty and Ripley, 2015).

High-Throughput Phenotyping

The disease performance of a rice population of advanced inbred lines for ROS and SCL resistance was evaluated using the best inoculation method chosen from the inoculation experiments.

Plant Materials

A total of 641 advanced inbred lines, 316 *indica* and 325 tropical *japonica*, from the National Rice Breeding Program of INIA Uruguay were evaluated in five greenhouse phenotyping experiments. Inoculation was performed with *Method I*, the most discriminating inoculation method, as described above. Two experiments were performed for SCL, from July to November 2012 and from September 2013 to January 2014. Three experiments for ROS were run from May to November 2013, from January to May 2014, and from March to August 2014. A Federer's unreplicated experiment in an augmented randomized complete blocks design (Federer, 1961) with twelve blocks was used in each experiment. Cultivars El Paso 144, INIA Olimar, INIA Tacuarí, Parao, and Lemont were used as replicated checks. Ten seeds were sown in 12-cm diameter pots. After emergence, thinning was conducted to leave four plants per pot. Plants were inoculated with *Method I* as described above. At about 90 d after inoculation, diseases were scored using the 0 to 9 scale as described above, and the average of the four plants per pot was used as the response variable.

Statistical Analysis

Two models (Eq. [4] and [5]) were compared for high throughput greenhouse experiments based on genetic variances, generalized heritability, and model fitness using the Akaike information criterion (Akaike, 1974). Model BAS is the baseline model where disease score is a function of genotype and block effects (Eq. [4]). Model SPA is the baseline model with spatial correction (Eq. [5]):

$$\text{Model BAS, } Y_{ij} = \mu + \gamma_i + G_j + \varepsilon_{ij} \quad [4]$$

$$\text{Model SPA, } Y_{ijmn} = \mu + \gamma_i + G_j + R_{m(i)} + C_{n(i)} + \varepsilon_{ijmn} \quad [5]$$

where Y_{ijmn} is the disease score; μ is the intercept; γ_i is the random block effect with $\gamma_i \sim N(0, \sigma^2_B)$ and $i = \{1, \ldots, 12\}$; G_j is the genotypic effect, $j = \{1, \ldots, 646\}$; and ε_{ij} is the residual. G_j is modeled as $G_j = g_k + C_l$, where g_k is the random effect of the k^{th} genotype line, with $k = \{1, \ldots, 641\}$ and $g_k \sim N(0, \sigma^2_G)$ for estimation of genetic variances and as fixed effect for estimating adjusted genotypic means for Pearson correlation analysis; and C_l is the fixed effect of the l^{th} check, with $l = \{1, \ldots, 5\}$. In Eq. [5], $R_{m(i)}$ is the random row effect nested within blocks, with $R_{m(i)} \sim N(0, \sigma^2_R)$ and $C_{n(i)}$ is the column effect nested within blocks, with $C_{n(i)} \sim N(0, \sigma^2_C)$ with $m = \{1, \ldots, 35\}$ and $n = \{1, \ldots, 26\}$. Analyses of these mixed linear models were performed with the R packages *lme4* (Bates et al., 2005) and *lsmeans* (Lenth, 2016).

The generalized heritability (H^2_g) was estimated following Cullis et al. (2006) with Eq. [6]:

$$H^2_g = 1 - \frac{\overline{v}_{BLUP}}{2\sigma^2_G} \quad [6]$$

where $\overline{v}_{BLUP}$ is the average pairwise variance error of BLUPs estimated with the *arm* R package (Gelman and Su, 2015). Standard errors for σ^2_G, $\overline{v}_{BLUP}$, and H^2_g were estimated with a bootstrap data resampling technique as recommended by Holland et al. (2003) and implemented in the *boot* R package (Canty and Ripley, 2015). Pearson correlation of phenotypic means was estimated between each pair of greenhouse experiments.

RESULTS

Inoculation Methods Comparison

A significant genotype by inoculation method interaction was found ($P < 0.0001$ for SCL and $P = 0.0012$ for ROS) when considering all five inoculation methods. When *Method V* was removed, no interaction was found in SCL experiments ($P = 0.79$) and genotypic effect was significant ($P < 10^{-15}$) with genotypic means highly correlated across methods (Fig. 1a). For *Methods I* to *IV* in ROS experiments, a genotype by inoculation method interaction was still found ($P = 0.037$). However, there were no

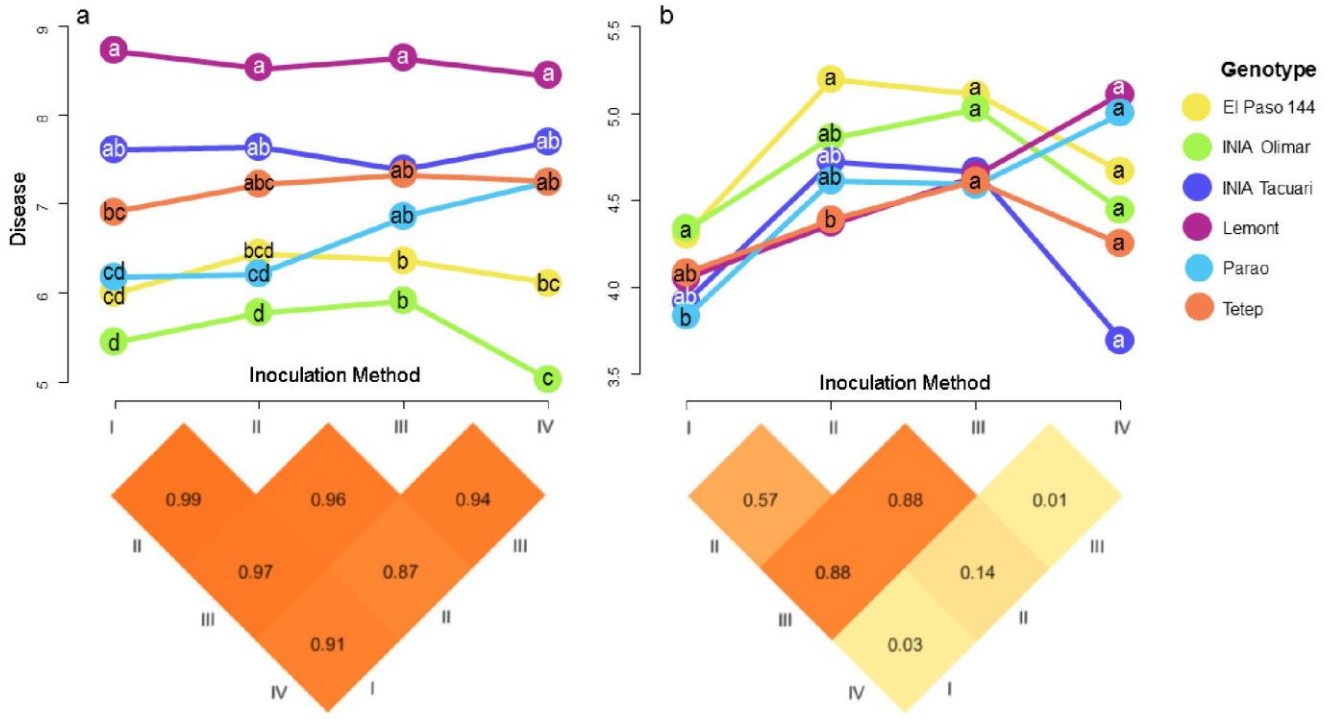

Fig. 1. Average disease score for *Sclerotium oryzae* (a) and *Rhizoctonia oryzae-sativae* (b) in five inoculation methods evaluated for six genotypes, and Pearson correlations among methods. Pairs of genotypic means with different letters were statistically different with a Tukey's HSD test ($\alpha = 0.05$) for each inoculation method.

differences in genotype ranking between *Methods I* and *II* (Fig. 1b).

The range of disease score means was higher for SCL (5.4 to 8.8) than for ROS (3.7 to 5.2). Therefore, genotypic responses were better discriminated in SCL experiments (Fig. 1a), ranging from moderately resistant (INIA Olimar), susceptible (El Paso 144, Parao, Tetep, INIA Tacuarí), to highly susceptible (Lemont). In ROS experiments (Fig. 1b) all genotypes had a moderately resistant response. Parao was somewhat closer to resistance, INIA Tacuarí, Lemont, and Tetep were intermediate, and the least resistant were El Paso 144 and INIA Olimar.

Experiments for resistance to SCL enabled a better comparison between methods than experiments for ROS. Inoculation *Methods I* and *II* had the highest discriminative power for both pathogens. *Method I* statistically differentiated eight pairs of genotypic means in SCL experiments (El Paso 144 vs. INIA Tacuarí and Lemont; INIA Olimar vs. INIA Tacuarí, Lemont and Tetep; and Lemont vs. Parao and Tetep), whereas *Method II* differentiated seven pairs, failing to discriminate between Lemont and Tetep (Fig. 1a). *Methods III*, *IV*, and *V* differentiated two, six and one pairs of genotypic means, respectively. In ROS experiments (Fig. 1b), *Method I* differentiated two pairs of genotypic means (Parao from El Paso 144 and from INIA Olimar) and *Method II* differentiated two pairs as well (El Paso 144 from Lemont and from Tetep). *Methods III* and *IV* failed to differentiate any pair of genotypes, and *Method V* only discriminated Parao vs. Lemont and Parao vs. Tetep.

Repeatability was high and similar across all inoculation methods (Table 1), with statistical differences only between *Methods I* (i.e., the highest) and *III* (the lowest) for ROS (Table 1). Additionally, *Methods III* and *V* had the widest standard errors and confidence intervals of repeatability estimates for both pathogens (Table 1).

Table 1. Performances of five inoculation methods for rice diseases *Sclerotium oryzae* and *Rhizoctonia oryzae-sativae*. Genetic variance (σ^2_G), residual variance (σ^2_ε) and repeatability (H^2) with their standard error (in parentheses) and 95% confidence intervals of repeatability, CI(H^2), are reported.

	σ^2_G	σ^2_ε	H^2	CI(H^2)
		Sclerotium oryzae		
Method I	1.35 (0.32)	0.56 (0.13)	0.94 (0.02)	0.88–0.96
Method II	0.94 (0.25)	0.61 (0.16)	0.90 (0.04)	0.81–0.96
Method III	0.73 (0.29)	1.05 (0.30)	0.81 (0.10)	0.58–0.97
Method IV	1.31 (0.34)	1.00 (0.28)	0.89 (0.05)	0.77–0.97
Method V	0.92 (0.66)	2.04 (0.34)	0.73 (0.13)	0.43–0.93
		Rhizoctonia oryzae-sativae		
Method I	0.03 (0.02)	0.06 (0.01)	0.75 (0.16)	0.46–0.96
Method II	0.07 (0.06)	0.20 (0.06)	0.67 (0.26)	0.22–1.00
Method III	0.00 (0.05)	0.31 (0.08)	0.05 (0.66)	0.00–0.17
Method IV	0.16 (0.21)	0.69 (0.25)	0.58 (0.24)	0.12–0.83
Method V	1.25 (1.45)	5.24 (1.19)	0.59 (0.38)	0.04–0.99

High-Throughput Phenotyping

Method I was chosen for high-throughput greenhouse phenotyping of SCL and ROS resistance in a large collection of rice breeding germplasm. In all phenotyping experiments for both pathogens models without (BAS) and with spatial correction (SPA) had similar variances and high and similar heritability (i.e., both models gave heritability of 0.81 in experiment ROS1, and 0.76 in experiment SCL2; Table 2). Spatial correction did not improve significantly the model fitness nor heritability (Table 2). Thus, model BAS was selected.

Correlation between the two SCL experiments was low (Fig. 2a), suggesting strong genotype by environment interaction between greenhouse experiments. This interaction was also observed among the three ROS trials (Fig. 2b).

DISCUSSION

All five inoculation methods performed similarly and had good repeatabilities, with the exception of *Method III* in ROS experiments. *Method IV*, an improvement of *Method III* that prevents carboxymethyl cellulose from being dissolved, outperformed *Method III* in both diseases. Although there were no statistical differences in repeatability (i.e., 0.81 and 0.89 for *Methods III* and *IV* in SCL, and 0.05 and 0.58 for *Methods III* and *IV* in ROS), *Method IV* discriminated genotypes better in SCL based on the Tukey analysis. *Method V* is easy to implement and requires few resources, but provided inconsistent results and ranked genotypes incongruently with respect to the other methods (i.e., large confidence intervals for repeatability and had no ability to differentiate genotypes, data not shown). *Method II* had good performance, and the production and application of inoculum was easy. However, its throughput in larger trays or tanks with uneven spread of sclerotia may create heterogeneity in the amount of the inoculum and add noise to the infection process. Furthermore, although both *Methods I* and *II* had high heritability, *Method I* had a larger power to differentiate cultivars based on the Tukey analysis. *Method I*, our modification of the inoculation method described by Cother and Nicol (1999) consisting of attaching mycelium-growing agar discs to

Table 2. Comparison of baseline (BAS) model and the baseline with spatial correction (SPA) model used to fit disease scores from greenhouse phenotyping experiments, two for *Sclerotium oryzae* (SCL) and three for *Rhizoctonia oryzae-sativae* (ROS). Genetic variance (σ^2_G), average pairwise BLUP variance ($\overline{v}_{BLUP}$), generalized heritability (H^2_g) with standard error (in parentheses), and Akaike information criterion (AIC) for each model are reported. The best fitting model for each experiment is underlined.

Phenotyping experiment	σ^2_G		$\overline{v}_{BLUP}$		H^2_g		AIC	
	BAS	SPA	BAS	SPA	BAS	SPA	BAS	SPA
SCL 1	1.18 (0.10)	1.18 (0.12)	0.42 (0.05)	0.42 (0.05)	0.64 (0.02)	0.65 (0.02)	<u>11100</u>	11149
SCL 2	1.51 (0.08)	1.54 (0.12)	0.36 (0.04)	0.36 (0.04)	0.76 (0.01)	0.76 (0.01)	<u>11621</u>	11645
ROS 1	1.70 (0.06)	1.64 (0.12)	0.20 (0.07)	0.18 (0.07)	0.88 (0.01)	0.89 (0.01)	<u>9105</u>	9137
ROS 2	1.12 (0.05)	1.05 (0.08)	0.21 (0.08)	0.20 (0.08)	0.81 (0.01)	0.81 (0.01)	7671	<u>7669</u>
ROS 3	1.77 (0.14)	1.63 (0.17)	0.56 (0.13)	0.54 (0.12)	0.68 (0.02)	0.67 (0.02)	<u>11248</u>	11263

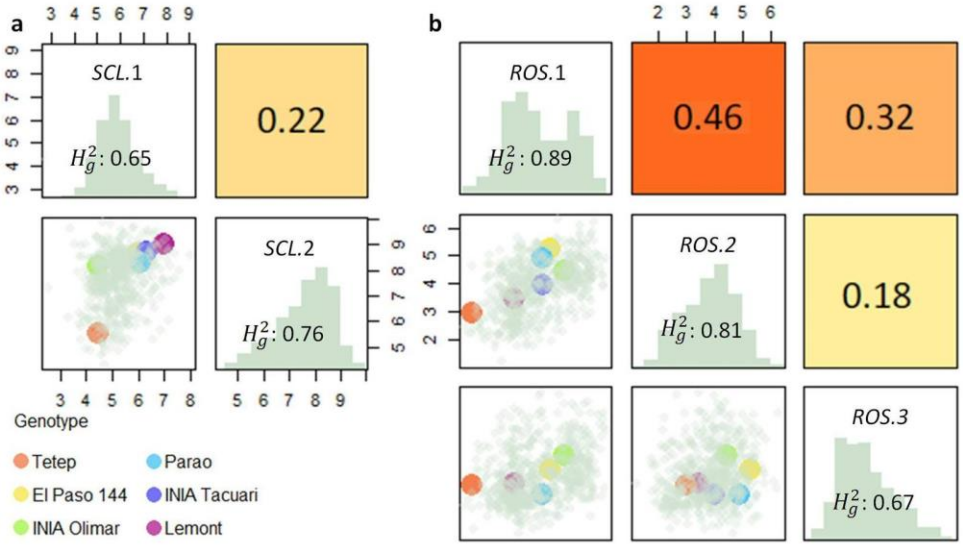

Fig. 2. Disease performance of 641 advanced inbred lines of rice evaluated in two greenhouse experiments for *Sclerotium oryzae* (SCL, a) and three for *Rhizoctonia oryzae-sativae* (ROS, b). Diagonal plots are histograms with distribution of each phenotypic variable and their generalized heritability (H^2_g). Lower-diagonal are scatter plots with common checks color-coded. Above-diagonal are Pearson correlations between genotypic means from each pair of experiments.

the base of rice stems was found to have high repeatability and discriminative power. An advantage of *Method I* over the others is that is uses fresh mycelium instead of sclerotia (Park et al., 2008). Fresh mycelium facilitates more homogeneous infection due to much closer contact between growing mycelium and rice outer sheath. Since every single tested stem is faced with a mycelium growing agar disc, its scalability appeared more reliable. Thus, *Method I* was chosen for high-throughput phenotyping of resistance to SCL and ROS, and was successfully applied in five greenhouse experiments achieving high heritabilities for both traits. However, we found *Methods I* to *IV* to perform similarly without a clear superiority of any of them. From our results using any of *Methods I* to *IV* may yield a similar and consistent phenotyping and enable a good rating of the resistance to both pathogens. Furthermore, *Methods I* to *IV* can equally identify resistant genotypes but *Method I* can further discriminate these selections.

Genotypic response to the diseases in inoculation *Methods I* to *IV* generally agreed among each other and with previous reports. Susceptible and intermediate genotypes were ranked according to reported responses, but there were some discrepancies between our inoculation methods results and reported resistance response to SCL. INIA Olimar usually has an intermediate response to SCL similar to that of El Paso 144 in experimental fields and productive conditions (Blanco et al., 2004), whereas in our inoculation methods experiments it had the most resistant response. This was probably due to the isolate used in our experiments being slightly less virulent than the average Uruguayan SCL isolates (Martínez and Escalante, 2012). Cultivar Tetep is widely reported as resistant to SCL, but behaved as intermediate in our inoculation methods experiments. Conversely, Tetep response in the high throughput experiments was clearly resistant in agreement with previous reports. We hypothesize that Tetep's progress response may have an early intermediate response stage followed by a recovery that leads to a final resistant response. Since diseases were rated 45 d earlier in inoculation experiments than in high throughput phenotyping, a different stage in Tetep's response curve may have been rated in each set of experiments. Resistance levels of cultivars used for evaluation of inoculation methods and as checks in high throughput phenotyping experiments were therefore broadly consistent, exception made of the above mentioned Tetep.

Cother and Nicol (1999) found low correlation and noticeable genotype by method interaction between variations of *Method I* vs. *II* applied to SCL, and this was attributed to phenological differences in inoculation time. We found broadly consistent genotypic ranking for both methods since we eliminate this source of variation by inoculating the plants at the same phenological stage.

The same two inoculation methods were previously compared by Kumar et al. (2003). They used the mean incidence of the disease to select methods. We found no differences in mean incidence across methods. However, we believe that the ability of the method to consistently discriminate genotypes is the most relevant feature of a good phenotyping method. In our study, the methods varied in their abilities to discriminate genotypes. Since these differences were subtle between some methods, logistics and scalability were also considered when choosing between similarly performing inoculation methods. Local resources will also be important in selecting methods.

The high throughput phenotyping experiments with the chosen inoculation method had similar heritability using a statistical model with or without spatial correction. Thus, we did not find evidence of spatial patterns affecting the response to the diseases in our greenhouse experiments that were not corrected by blocking. The experimental design and the inoculation methods used were enough to discriminate the genotypes in a large population and the spatial correction was not needed.

The estimated generalized heritability in our high throughput phenotyping experiments was medium to high according to Boopathi (2013). Phenotypic data within these ranges of heritability is acceptable for disease resistant traits and is suitable for association mapping studies (Zila et al., 2014). However, the somewhat low correlations and ranking differences for genotypes used as checks found between some pairs of phenotyping experiments indicate genotype by environment interaction. Since experiments were run at different seasons through a two year lapse, season and year effects (mostly due to variations in daylight time and solar radiation intensity) may be underlying these interactions. Therefore, further study of genotype by environment interaction should be attempted (Allard and Bradshaw, 1964; van Eeuwijk et al., 2005).

The phenotypic values of cultivars used as checks in the phenotyping experiments methods were widely distributed in the scatter plots showing a good representation of the whole breeding population levels of resistance to SCL and ROS, and their resistance levels were as expected. Furthermore, the presence of lines with somewhat lower disease scores in experiments with successful susceptible checks infection suggests a wider and useful genetic variability for these traits.

We compared inoculation methods for ROS and SCL and selected a successful inoculation method that could be applied to massive phenotyping experiments. We showed how this method performed in high-throughput phenotyping of breeding germplasm. Our results will enable high-throughput phenotyping of lines for breeding purposes in either traditional selection, QTL mapping (Bernardo, 2008), GWAS (Breseghello and Sorrells, 2006) or Genomic Selection (Heffner et al., 2009) contexts.

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3. RESISTANCE TO MULTIPLE TEMPERATE AND TROPICAL STEM AND SHEATH DISEASES OF RICE

3.1. RESUMEN DE CONTENIDO Y APORTE

El segundo artículo de la tesis consiste en el análisis de asociación de marcadores genómicos con las tres enfermedades del tallo y la vaina estudiadas: pudrición del tallo (*Nakataea oryzae*, NO), mancha agregada de las vainas (*Rhizoctonia oryzae-sativae*, ROS), y tizón de las vainas (*Thanatephorus cucumeris*, TC). El artículo constituye el primer reporte sobre el control genético de la resistencia a ROS, así como el primer estudio de mapeo asociativo para ROS y para NO. También es el primer reporte de una región en el cromosoma 9 asociada a la resistencia a las tres enfermedades del tallo y la vaina mencionadas. La altura de planta y el largo de ciclo son factores de confusión en la evaluación de resistencia a estas enfermedades. Para remover su efecto se emplearon dos estrategias complementarias: 1) en los ensayos de campo se utilizaron la altura de planta y el largo de ciclo como covariables para estimar las medias fenotípicas de resistencia a enfermedades, y 2) en el análisis de mapeo asociativo con los datos de fenotipado de campo e invernáculo, se utilizaron los SNPs asociados a altura de planta y largo de ciclo como cofactores en el GWAS, para identificar asociaciones independientes. De esta forma se logró identificar QTL asociados a resistencia a las enfermedades del tallo y la vaina, que no afectan la altura ni el ciclo de la planta. Este artículo fue aceptado para publicación en la revista *The Plant Genome* el 16 de mayo de 2017, y publicado en la página "*First look*" de dicha publicación el 22 de setiembre de 2017 (Anexo en página 84 de esta tesis).

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RESISTANCE TO MULTIPLE TEMPERATE AND TROPICAL STEM AND SHEATH DISEASES OF RICE

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Running head: Resistance to Rice Stem and Sheath Diseases

Key words: Rice, aggregated sheath spot, sheath blight, stem rot, GWAS, QTL.

Abbreviations: FT, flowering time; GBS, genotyping-by-sequencing; GWAS, genome wide association study; Ind, *indica* ssp.; INIA, Instituto Nacional de Investigación Agropecuaria; NO, *Nakataea oryzae*; PH, plant height; QTL, quantitative trait loci; ROS, *Rhizoctonia oryzae-sativae*; SNP, single nucleotide polymorphism; SW, stem width; TC, *Thanatephorus cucumeris*; Trj, tropical *japonica* ssp.; UEPL, Paso de la Laguna Experimental Unit.

Core Ideas:

- Reaction to sheath blight, stem rot, and aggregated sheath spot were tested in 641 tropical *japonica* and *indica* rice lines.
- Disease resistance was mapped independently of flowering time and plant height.
- QTL of major effect for resistance to the three diseases were found.
- A multiple disease resistance QTL was found on chromosome 9 across tropical *japonica* and *indica* populations.

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ABSTRACT

Stem rot and aggregated sheath spot of rice are the two major stem and sheath diseases affecting rice (*Oryza sativa* L.) in temperate areas. A third fungal disease, sheath blight, is a major disease in tropical areas. Resistance to these diseases is a key objective in rice breeding programs, but phenotyping is challenged by the confounding effects of phenological and morphological traits such as flowering time (FT) and plant height (PH). This study sought to identify quantitative trait loci (QTL) for resistance to these three diseases after removing the confounding effects of FT and PH. Two populations of advanced breeding germplasm, one with 316 tropical *japonica,* and the other with 325 *indica* genotypes, were evaluated in field and greenhouse trials for resistance to the diseases. Phenotypic means for field and greenhouse disease resistance, adjusted by FT and PH were analyzed for association with 29,000 SNPs in tropical *japonica* and 50,000 SNPs in *indica*. A total of 29 QTL were found for resistance that were not associated with FT or PH. Multilocus models with selected resistance-associated SNPs were fitted for each disease to estimate their effects on the other diseases. A QTL on chromosome 9 accounted for more than 15% of the phenotypic variance for the three diseases. When resistance-associated SNPs at this locus from both the tropical *japonica* and *indica* populations were incorporated into the model, resistance was improved for all three diseases with little impact on FT and PH.

There is a large gap between yield potential and yields obtained by farmers in most rice-growing countries (Laborte et al., 2012). Rice diseases explain a substantial proportion of this yield gap (Savary et al., 2000; Van Nguyen and Ferrero, 2006). In temperate and subtropical areas like the Southern Cone of South America, the yield gap has been reduced significantly over the last decades thanks to the widespread adoption of modern cultivars and technology (Martínez et al., 2014; Pittelkow et al., 2016). However, high yields in these areas rely on the intensive use of fungicides, as most of the top cultivars still lack adequate genetic resistance to one or more pathogens affecting the crop (Jia et al., 2011; Lanoiselet et al., 2007; Martínez et al., 2014). Sheath blight is a major global rice disease caused by the fungus *Thanatephorus cucumeris* (A. B. Frank) Donk (TC) (previously, *Rhizoctonia solani* J. G. Kühn). Yield loss due to TC can reach up to 40% in susceptible cultivars (Groth, 2008), and this percentage of yield loss tends to increase with higher yields (Willocquet et al., 2011). Among the most prevalent pathogens affecting rice in temperate areas worldwide are *Nakataea oryzae* (Catt.) J. Luo & N. Zhang (NO) (previously, *Sclerotium oryzae* Catt.), causal agent of stem rot, and *Rhizoctonia oryzae-sativae* (Sawada) Mordue (ROS) (previously, *Ceratorhiza oryzae-sativae* (Sawada) R. T. Moore), causal agent of aggregated sheath spot (Clément and Roumen, 1992; Krause and Webster, 1973; Lanoiselet et al., 2007; McKenzie et al., 1994). Yield losses have been quantified as up to 22% due to NO (Krause and Webster, 1973), and 20% for ROS (V. L. Lanoiselet et al., 2005). Hence, susceptibility to stem and sheath diseases is a major concern for breeding programs for large and small yield gap conditions, and for tropical and temperate rice growing regions.

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TC, NO and ROS are sclerotial pathogens with similar life cycles and display predominantly monocyclic infections, sporulating in rice plants only late in the season under field conditions (M. A. Lanoiselet et al., 2005; Ou, 1985). Sclerotia and mycelium can overwinter in rice stubble and soil, and infect rice sheaths at the water line (Krause and Webster, 1973; Lanoiselet et al., 2007). TC and ROS progress upwards, whereas NO invades the inner stem (Ou, 1985). Plant morphology and phenology affect disease progress and are considered to be associated with disease escape mechanisms in several crops and pathosystems (Danon, 1982; Kicherer et al., 2000; Lore et al., 2013; Simón et al., 2004; Zhu et al., 1999). In particular, plant height (PH) and flowering time (FT) have been found to be correlated with resistance to sheath blight and are considered to be two of the major confounding effects on disease rating (Srinivasachary et al., 2011; Zeng et al., 2015).

Studies that do not consider the effect of phenology and morphology on disease rating lead to many QTL for resistance that overlap with those of phenological and morphological traits (Zeng et al., 2015). For example, Li et al. (1995) identified sheath blight resistance QTL that also affected FT and PH, and Sharma et al. (2009) identified one QTL for sheath blight resistance colocalizing with the semidwarf gene *sd-1* in chromosome 1. In most cases it is impossible to distinguish between linkage and pleiotropy at those QTL (Wiesner-Hanks and Nelson, 2016). Proposed strategies for minimizing the confounding effect of FT and PH for evaluation of resistance to confounded diseases include: the use of controlled phenotyping methodologies such as microchambers and mist chambers (Jia et al., 2007; Liu et al., 2009); rating systems not influenced by PH (Zeng et al., 2015); mapping populations that do not segregate for FT or PH (Liu et al., 2013); and using FT and PH as covariates for correcting

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to find independent QTL (von Zitzewitz et al., 2011, Locatelli et al., 2013; McCouch et al., 2016). Multi-QTL models may be used to jointly test the effect of all GWAS-discovered QTL (Locatelli et al., 2013; Gutierrez et al., 2015; Malosetti et al., 2007b).

The objective of the present study is to map the genetic resistance to the three fungal pathogens, TC, NO, and ROS, independent of FT and PH, in tropical *japonica* and *indica* rice breeding germplasm. This is the first study to focus on the genetics of resistance to ROS, and the first report on GWAS for the identification of QTL for resistance to NO and ROS. The QTL identified in this work will contribute to improving resistance to the three studied diseases in both tropical *japonica* and *indica* genetic backgrounds.

MATERIALS AND METHODS

Plant material and phenotyping

Resistance to the three fungal pathogens, TC, NO, and ROS, was individually evaluated in 641 advanced inbred lines (325 tropical *japonica* and 316 *indica*) from the National Agricultural Research Institute of Uruguay (INIA) rice breeding program. Resistance to NO and ROS was evaluated in both greenhouse and field trials in Uruguay, whereas resistance to TC was evaluated in greenhouse trials in Colombia. Greenhouse experiments for NO and ROS have been described elsewhere (Rosas et al., 2016). Briefly, two trials for NO and three trials for ROS were conducted from 2012 to 2014 at INIA Treinta y Tres Experimental Station, Treinta y Tres, Uruguay (33°15'S, 54°25'W). The cultivars *El Paso 144*, *INIA Olimar*, *INIA Tacuarí*, *Parao*, and

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Lemont were used as replicated checks. Resistance to TC was evaluated in 2015 in two greenhouse trials at CIAT, Valle del Cauca, Colombia (3°29'N, 76°21'W) with the mist-chamber method (Jia et al., 2013). The varieties *Oryzica 3* and *Lemont* were used as replicated resistant and susceptible checks, respectively. For all greenhouse experiments, a Federer's unreplicated design (Federer and Raghavarao, 1975) with augmented checks in randomized complete blocks was used, and the environmental conditions were 28/18°C day/night, 80/90% relative humidity and 12 h light time. Ten seeds were sown in 12-cm diameter pots. After plant emergence, four (for NO and ROS) or two (for TC) plants per pot were grown for inoculation. At the 3-leaf stage plants were inoculated with 5-mm agar discs with fresh mycelium at the base of the stem. An additional disc in the third leaf was used for TC. Diseases were scored at 45 days post inoculation using the percentage of relative lesion length based on modifications of the International Rice Research Center's Standard Evaluation System for rice (IRRI, 2002) described in Martínez (2016) and depicted in Supplementary figures 1 and 2 for NO and ROS, respetively, and in Jia et al. (2013) for TC. Stem width (SW) at inoculation time and phenological stage at scoring time were recorded. Phenological stage was used as a proxy for FT in the greenhouse experiments.

Field trials for NO and ROS were performed from 2009 to 2013 at Paso de la Laguna Experimental Unit (UEPL), Treinta y Tres, Uruguay (33°54´S 54°38´W). Trials from 2009 to 2012 used naturally infected six-row plots of 1.2 X 3.5 m laid out in a randomized complete block design with three replicates. Three points in each plot were visually assessed for NO and ROS sympthoms separately, rating disease severity based on a 0-to-9 scale (IRRI, 2002) depicted in Supplementary figure 1. The 2013 field trials were artificially inoculated hill plots of ~10 adult

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plants in an augmented alpha-lattice design (Piepho et al., 2006) with six replicates. Plots were treated with 0.6 g m^{-2} (NO trials) or 1.8 g m^{-2} (ROS trials) of inoculum sprinkled from about 50 cm over the flooding water surface at the beginning of the tillering stage. Flowering time (FT) and plant height at maturity (PH) were measured in all field experiments and used as covariates to remove confounding effects on disease resistance.

Adjusted phenotypic means were estimated using the model in Equation [1]:

$$Y_{ijmn} = \mu + \gamma_i + G_j + \beta_1 X1_{ij} + \beta_2 X2_{ij} + R_{m(i)} + C_{n(i)} + \varepsilon_{ijmn} \qquad [1]$$

where Y_{ijmn} is the response variable; μ is the intercept; γ_i is the random block effect with $\gamma_i \sim N(0, \sigma^2_B)$; G_j is the genotypic effect; $X1_{ij}$ and $X2_{ij}$ are the covariates in the j^{th} genotype and the i^{th} block, and β_1 and β_2 are the regression slopes of the covariates (FT and SW for greenhouse trials, and FT and PH for field trials, and none for estimation of phenotypic means of FT and PH); $R_{m(i)}$ and $C_{n(i)}$ are the random row and column effects nested within blocks, with $R_{m(i)} \sim N(0, \sigma^2_R)$ and $C_{n(i)} \sim N(0, \sigma^2_C)$; ε_{ijmn} is the residual. The genotypic effect was modeled as $G_j = g_k + c_l$, where g_k is the effect of the k^{th} genotype, and c_l is the fixed effect of the l^{th} check. For estimation of genetic variances, g_k was modeled as a random effect with $g_k \sim N(0, \sigma^2_G)$, and for Pearson correlation analysis g_k was modeled as a fixed effect. This notation for genotypic effects follows that of Eckermann et al. (2001) and Verbyla et al. (2003). To illustrate the consequences of not correcting for FT and PH, a naïve model without covariates was also used to estimate the disease adjusted means from field experiments. Generalized heritability was estimated as $1 - v_{BLUP}/2\sigma^2_G$, where v_{BLUP} is the mean pairwise variance error of the genotypic BLUPs (Cullis et al., 2006), and its standard error was estimated with a bootstrap data resampling technique, as recommended by Holland et al. (2003). Phenotypic means were

averaged and weighted by the generalized heritability of each individual trial. Two-sample Student's t-tests were performed to compare weighted phenotypic means of each trait in tropical *japonica* vs. *indica* populations. Analyses were performed in R using packages *lme4* (Bates et al., 2005), *lsmeans* (Lenth, 2016), *arm* (Gelman and Su, 2016), and *boot* (Canty and Ripley, 2015).

Fungal isolates and inoculum production

Monosclerotial strains were isolated from naturally infected rice plants and soil and selected to have intermediate aggressiveness on rice. Strain SO-Samba (NO) was isolated in 2001 from the *temperate japonica* rice cultivar Samba in naturally infected experimental plots at Paso de la Laguna Experimental Unit (UEPL). Strain ROS-17 (ROS) was obtained from soil after rice cultivation with the tropical *japonica* rice cultivar INIA Tacuarí in UEPL in 2003. SO-Samba and ROS-17 strains caused good discrimination among rice genotypes, and are maintained at the Culture Collection of the Plant Pathology Laboratory of INIA Treinta y Tres (Rosas et al., 2016). Strain 1953-1 (TC) was collected in 1985 in Tolima, Colombia, and is maintained at the Culture Collection of the CIAT Pathology Laboratory. For greenhouse experiments, isolates were grown for 5-7 d at 25°C in 90 mm Petri dishes containing potato dextrose agar (Oxoid) for NO and ROS, or rice bran agar for TC. Sclerotia for inoculation of field trials in 2013 were produced separately from sterilized mixtures of 2:1 rice seeds and rice hulls that were inoculated with 7 d old mycelia, incubated for 25 d at 23°C, dried at 40 °C, and conserved at 4 °C until utilization (Krause and Webster, 1973).

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Genotyping

Rice DNA was isolated in the Biotechnology Unit of INIA (Canelones, Uruguay) with the DNeasy kit (Qiagen) and genotyped using genotyping-by-sequencing (GBS) with the *ApeK*1 enzyme in the Biotechnology Resource Center at Cornell University (US). SNP calling was performed with the TASSEL version 3.0 GBS pipeline (Bradbury et al., 2007). SNPs were aligned to the *Nipponbare* reference genome MSU version 7.0 using Bowtie 2 (Langmead and Salzberg, 2012). Missing SNP data was imputed with the FILLIN algorithm (Swarts et al., 2014) and SNPs with minor allele frequency <1% and/or >50% of missing data were removed. Final genotypic matrices contained 49,589 SNPs for *indica* and 28,850 SNPs for tropical *japonica*.

Population structure, GWAS scan, multilocus and LD block analyses

Due to the deep population structure existent within the *Oryza sativa* species, *indica* and tropical *japonica* populations were analyzed separately. To assess population structure within each subspecies, principal component analysis (PCA) was performed on each genotypic matrix. GWAS and multilocus analyses were tested by fitting the mixed linear model $y=X\beta+Zu+e$, where y is a vector of adjusted phenotypic means; β is a vector of fixed effects (single SNP for the GWAS scan, and multiple selected SNPs for the multilocus analysis); u is a vector of random genotypic effects with $u\sim N(0, K\sigma^2_G)$; e is a vector of residual effects with $e\sim N(0, I\sigma^2_e)$; X and Z are incidence matrices that relate y to β and to u respectively; K is the realized genotypic

relationships matrix calculated as the variance-covariance matrix of the PCA from SNP data, following Price et al. (2006), Malosetti et al. (2007a), and Gutiérrez et al. (2011); σ^2_G is the genetic variance; I is an identity matrix, and σ^2_e is the residual variance. The GWAS was performed using the R package *lmem.gwaser* (Gutierrez et al., 2016), with a significance threshold adjusted by the effective number of independent tests (Li and Ji, 2005). The significant SNPs on each chromosome were clustered by their physical positions using the *hclust* and *cutree* basic R functions (R Core Team, 2015) with an *h* parameter of a quarter of the maximum height of the tree. Clusters with three or more SNPs were considered to be a QTL, and the SNP with the highest $-\log_{10}(P)$ at each QTL was selected. The selected SNPs from QTL for FT and PH were used as covariates for GWAS scans of the disease traits, following von Zitzewitz et al. (2011) and Locatelli et al. (2013). To show the consequences of not correcting for those confounding factors in the GWAS analysis, a naïve model without FT- and PH-associated SNPs as covariates was also fit. The selected SNPs from QTL for NO, ROS and TC were used for multilocus estimation of the proportion of phenotypic variance explained by the QTL (PVE), and QTL effects, calculated as percentage of the phenotypic mean. Multilocus models were fit with the R package *EMMREML* (Akdemir and Okeke, 2015) following Gutiérrez et al. (2015). Regions of chromosomes 9 and 12 were analyzed for LD blocks. LD was computed as pairwise R^2 between all SNPs in the region, and limits between LD blocks were graphically assessed with the R package *LDheatmap* (Shin et al., 2006). Physical positions of QTL described in the literature for sheath blight, PH, and FT were based on the review by Zeng et al. (2015)

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RESULTS

Phenotyping

Adjusted phenotypic means of tropical *japonica* were significantly different ($P < 0.01$) from those of the *indica* population for all traits. Tropical *japonica* lines were on average taller, later to flower, and more susceptible to stem and sheath diseases than *indica* lines. Genetic variance and generalized heritability did not differ significantly between populations ($P < 0.01$). Resistance to NO and ROS had higher heritability in greenhouse than in field trials (Table 1). Field trials had intermediate to high heritability for resistance to NO, FT, and PH, and low or very low for non-inoculated ROS experiments. TC had lower heritability than NO and ROS in greenhouse trials.

FT from tropical *japonica* field trials was correlated ($P < 0.05$) with the proxy for FT from tropical *japonica* greenhouse trials, while FT from *indica* field trials was not correlated with FT from *indica* greenhouse trials (Table 2). FT was not correlated with disease severity in tropical *japonica* field trials, while it was negatively correlated with disease severity in tropical *japonica* greenhouse trials (Table 2). Inversely, FT was negatively correlated with disease severity in *indica* field trials and was not correlated or had low positive correlations with disease severity in *indica* greenhouse trials (Table 2). FT was negatively correlated with PH in tropical *japonica* and positively correlated with PH in the *indica* population. Resistance to the three diseases correlated positively in tropical *japonica*, while in *indica* resistance to TC was not correlated with resistance to NO and it was negatively correlated with resistance to ROS.

GWAS scan and multilocus analysis

A major-effect QTL for FT was found in both populations on chromosome 3 (0.5 to 2.2.6 Mb in tropical *japonica* and 0.5 to 2.1 Mb in *indica*), with PVE=30 in tropical *japonica* and PVE=24 in *indica*. PH was oligogenic in tropical *japonica*, with a major-effect QTL (PVE=27) on chromosome 1 (34.6 to 39.8 Mb), while several QTL with PVE<5 were found for PH in *indica* (Suppl. Figure 1). When naïve models without covariates were used, several QTL for resistance to the studied diseases colocalized with these FT and PH QTL (Suppl. Figure 1).The SNPs with highest -$\log_{10}(P)$ for FT and PH QTL were used as cofactors in the GWAS scan for resistance to NO, ROS, and TC. A total of 33 non-overlapping QTL for resistance to stem and sheath diseases were identified (Figure 1). The genetic architecture differed for the three diseases in tropical *japonica* and *indica*, with only five QTL identified in the same genomic locations in the two populations. Five additional QTL were found exclusively in tropical *japonica*, and 23 in *indica*. Most QTL for resistance to stem and sheath diseases (two in both populations, five in tropical *japonica*, and 22 in *indica*) did not co-localize with QTL for FT or PL, while four disease QTL did colocalize with QTL for FT and/or PH and were not included in further analyses. Multilocus analysis of QTL for disease resistance revealed major effect loci with PVE ranging from 10-43, with the exception of ROS evaluated in greenhouse trials (Figure 1).

Favorable alleles at QTL discovered for resistance to NO were also favorable or neutral for resistance to ROS, and vice versa, for all trials, environments and populations (Figure 2). Favorable alleles at QTL for resistance to NO and ROS had little effect on resistance to TC. Allele

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substitution effects of QTL for resistance to TC, NO and ROS accounted for less than 2% of the phenotypic mean on FT, and less than 4% of the phenotypic mean on PH (Figure 2).

A region on chromosome 9, from 12 to 23 Mb, encompassed multiple QTL for resistance to NO, ROS, and TC in field and greenhouse trials, in tropical *japonica* (Figure 3) and in *indica* (Figure 4). For tropical *japonica*, three out of five major LD blocks across the region (Figure 3, lower section) had SNPs associated with NO, named q9-1, q9-2, and q9-3 (Figure 3, middle section). SNP S9_21382492 was associated with resistance to NO in field and greenhouse trials and to ROS in field trials. It was not in LD with surrounding significant SNPs, and was named q9-4. SNPs S9_22275709 and S9_22544543 were in LD and together were named q9-5. QTL q9-5 was associated with resistance to NO in field and greenhouse trials, with ROS resistance in field trials, and with TC resistance in greenhouse trials in tropical *japonica*. There were no associations between SNPs and FT or PH in this region of chromosome 9 in tropical *japonica*. QTL q9-3 and q9-5 did not overlap with any previously reported QTL for FT or PH in the region (Figure 3, upper section). Together, tropical *japonica* q9-3 and q9-5 had an allele substitution effect of 5.2% of the phenotypic mean (NO field), 10.2% (NO greenhouse), 8.9% (ROS field), 17.0% (ROS greenhouse), and 6.6 (TC greenhouse). The allele substitution effect of these QTL on FT and PH were 0.2% and 0.5%, respectively. In *indica*, the LD pattern in the same region of chromosome 9 (Figure 4, lower section) suggested a much higher rate of historical recombination and less well defined LD block structure than in tropical *japonica*. However, pairwise R^2 between significant SNPs in *indica* allowed their grouping in QTL that partially overlapped those found for tropical *japonica* (Figure 4, middle section). Specifically, pairwise R^2 between significant *indica* SNPs in the region from 17 to 20 Mb differentiated two QTL, named

q9-3a and q9-3b (Figure 4). *Indica* q9-3b, co-localized with tropical *japonica* q9-3 and was associated with the same traits. Similarly, *indica* SNP S9_22756593 co-localized with tropical *japonica* q9-5 and was associated with resistance to TC as well (Figure 4, middle section). The resistant allele substitution effects of *indica* q9-3b and q9-5, estimated as percentage of the phenotypic mean, were 17.2% (NO field), 20.2% (NO greenhouse), 17.0% (ROS field), 14.0% (ROS greenhouse), and 8.1% (TC greenhouse). The resistance allele substitution effects of q9-3b and q9-5 were 0.4% of the FT mean, and 1.6% of the PH mean. Of all the disease resistance QTL found in chromosome 9 for *indica*, only q9-3a had a SNP associated with PH (P = 0.0004) (Figure 4, middle section).

DISCUSSION

QTL for resistance to stem and sheath diseases independent of FT and PH

In the population used in our study, *indica* lines were shorter, with earlier maturity and still more resistant to NO, ROS and TC than tropical *japonica* lines on average. However, when correlations were studied within the *indica* population individually, FT and PH were negatively correlated with disease incidence. This is in concordance with reports on late maturing and tall cultivars found to be more resistant to ROS and NO than modern early maturing, semi-dwarf ones (McKenzie et al., 1994)..

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In our study, variation due to FT and PH in the evaluation of disease resistance was removed statistically using covariates for adjusted phenotypic mean estimation. However, when the proxy for FT from greenhouse trials was used as a covariate, disease resistance adjusted means still correlated with FT from field trials (Table 2), and QTL found for greenhouse trials adjusted means still colocalized with FT QTL when using a GWAS naïve model (Supplementary figure 2). This may correspond in part to different phenological controls of flowering time or a strong genotype by environment interaction for this trait, particularly for *indica* trials, that may make FT measured in greenhouse trials not relevant as a confounding factor for disease rating. This supported the need for additional corrections using SNPs associated with FT and PH as cofactors in the GWAS analysis. Other developmental and morphological traits not issued in this work such as tiller number and culm angle have also been reported to affect field evaluation of TC in some studies (Loan et al., 2004; Pinson et al., 2005). However, these traits tend to be fixed or have little phenotypic variation in adapted or advanced breeding populations, no longer affecting disease evaluation (Eizenga et al., 2013; Li et al., 1995; Wang et al., 2011). Thus, the remaining genetic effect estimated in our study after removing FT and PH effects presumably corresponds predominantly to physiological disease resistance mechanisms, i.e. those not driven by phenology or morphology.

The QTL of major effect for FT and PH identified in this study correspond to the *HD9* QTL (Lin et al., 2002) and the *sd-1* gene (Cho et al., 1994), respectively. *HD9* was segregating in our material and was the most significant genetic region explaining phenology in both the tropical *japonica* and *indica* populations. The *sd-1* gene segregated only in tropical *japonica;* it was fixed in the semi-dwarf, *indica* germplasm, where PH was under polygenic control. GWAS scans for

resistance to the studied stem and sheath disease with naïve models (i.e. without SNP of major QTL for FT and PH as cofactors) identified QTL that colocalized with these FT and PH major-effect QTL, in accordance with previous studies (Zeng et al., 2015). Nelson et al. (2012) reported that one putative QTL for resistance to sheath blight was eliminated when FT and PH were used as covariates. In our study, when SNPs associated with FT and PH were used as cofactors in the GWAS analysis, the corresponding putative disease resistance QTL were eliminated.

The strategies used in this study to minimize the confounding effects of FT and PH were effective, enabling the discovery of 29 QTL for resistance to stem and sheath diseases. Notably, 17 of these QTL did not overlap with previously reported QTL for FT or PH (Zeng et al., 2015). The multi-disease resistance locus, q9-3, which was associated with resistance to TC, NO and ROS, was not associated with FT or PH in our population (Figures 3 and 4). The only exception to this was QTL q9-3a in indica, which had an allele substitution effect of 1.6% of PH mean. This evidence supports the conclusion that most of the QTL reported here contribute to physiological mechanisms of disease resistance, and SNPs within these QTL will be useful for marker-assisted breeding to improve levels of resistance to the fungal pathogens, TC, NO, and ROS, the causal agents of sheath blight, stem rot, and aggregated sheath spot, without significantly affecting either FT or PH.

QTL for multiple disease resistance

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Due to its many advantages, multiple disease resistance is a highly valuable and relevant breeding objective (Wiesner-Hanks and Nelson, 2016). In our work, all QTL associated with resistance to ROS, NO and TC in tropical *japonica* breeding materials reciprocally increased resistance to the other pathogens (Figure 3, left panel). A similar result was found for NO and ROS in *indica* (Figure 3, right panel). Furthermore, nine QTL for resistance to NO and/or to ROS on chromosomes 1, 4, 5, 6, 9, 11, and 12 co-localize with reported QTL for resistance to TC (Zeng et al., 2015). In particular, the region from 12 to 23 Mb on chromosome 9 was rich in disease resistance QTL in tropical *japonica* and *indica* (Figures 3 and 4). The QTL q9-3 and q9-5, present in both populations, were associated with resistance to the stem and sheath diseases, accounted for high PVE, and co-localized with more than 12 QTL for resistance to TC in previous studies (Zeng et al., 2015). This suggests either pleiotropy or that genes for specific responses to each pathogen are physically close together in the mapped regions (linkage). Following Wiesner-Hanks and Nelson (2016), the magnitudes of the effects of resistant alleles at q9-3 and q9-5 across diseases fit with scenarios of multiple disease resistance, with pleiotropy for NO and ROS, and with slightly uneven pleiotropy for TC vs. NO/ROS.

Conclusions

The confounding effect of some phenological and morphological traits on resistance to stem and sheath diseases can be efficiently controlled using appropriate phenotyping and analytical methodologies. When the effect of confounding factors is removed, QTL identified for disease resistance have little effect on FT and PH. The effects of the disease resistance QTL found in this

work suggest the existence of common physiological mechanisms for resistance to sheath blight, stem rot and aggregated sheath spot, three of the main fungal diseases affecting rice in temperate and tropical areas worldwide. Markers associated with these QTL can be applied in marker-assisted breeding strategies to improve resistance to these three diseases in high-yielding elite germplasm without affecting FT and PH.

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Figure 1. Manhattan plots of the GWAS scan for resistance to *Nakataea oryzae* (NO, red), resistance to *Rhizoctonia oryzae-sativae* (ROS, blue), resistance to *Thanatephorus cucumeris* (TC, orange) in field (triangles) and greenhouse (circles) trials for tropical *japonica* (left panel) and *indica* (right panel) populations. All SNPs in the region of defined QTL are highlighted in light green, and the SNP with highest $-\log_{10}(P)$ in each QTL is colored in black. Percentage of phenotypic variance (PVE) explained by the identified QTL is reported.

Figure 2. Effects of QTL for resistance to *Nakataea oryzae* (NO), *Rhizoctonia oryzae-sativae* (ROS), and *Thanatephorus cucumeris* (TC) in field and greenhouse (GH) on diseases, flowering time (FT), and plant height (PH) in tropical *japonica* (Trj) and *indica* (Ind) populations, estimated with a multi-loci model with all the QTL found in this study for each disease. Allele substitution effect are reported as the percentage of the phenotypic mean. Error bars represent standard errors.

Figure 3. Zoomed-in view of positions 11.5 to 23 Mb of chromosome 9 in tropical *japonica*. The upper section shows the physical positions of previously reported QTL for flowering time (FT, purple), plant height, (PH, green) and resistance to *Thanatephorus cucumeris* (TC, brown) reviewed by Zeng et al. 2015. QTL found in our study in this region are marked with gray bars. The middle section shows the zoomed-in view of the region with GWAS scan results for *Nakataea oryzae* (NO, red), *Rhizoctonia oryzae-sativae* (ROS, blue), TC (orange), FT (purple), and PH (green), in field (triangles) and greenhouse (circles) trials. The lower section shows the

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pairwise R^2 between all SNPs in the region. Average R^2 within and between each LD block are presented.

Figure 4. Zoomed-in view of positions 11.5 to 23 Mb of chromosome 9 in *indica*. The upper section shows the physical positions of previously reported QTL for flowering time (FT, purple), plant height (PH, green) and resistance to to *Thanatephorus cucumeris* (TC, brown) reviewed by Zeng et al. 2015. QTL found in our study in this region in tropical *japonica* (Trj) are marked in dark gray bars, and QTL found in *indica* are marked with light gray bars. The middle section shows the zoomed-in view of the region with GWAS scan results for *Nakataea oryzae* (NO, red), *Rhizoctonia oryzae-sativae* (ROS, blue), TC (orange), FT (purple), and PH (green), in field (triangles) and greenhouse (circles) trials. The lower section shows the pairwise R^2 between all SNPs in the region. Average R^2 within and between each LD block are presented.

Table 1. Generalized heritability of field and greenhouse (GH) trials for *Nakataea oryzae* (NO), *Rhizoctonia oryzae-sativae* (ROS), sheath blight caused by *Thanatephorus cucumeris* (TC), plant height (PH), and flowering time (FT) in tropical *japonica* (*Trj*) and *indica* (*Ind*) populations. Standard error of generalized heritability in parentheses.

Trial	NO		ROS		TC		PH		FT	
	Trj	Ind	Trj	Ind	Trj	Ind	Trj	Ind	Trj	Ind
Field 2009	0.56 (0.06)	-	0.41 (0.06)	-	-	-	0.66 (0.03)	-	0.82 (0.01)	-
Field 2010	0.40 (0.08)	-	0.19 (0.15)	-	-	-	0.62 (0.08)	-	0.72 (0.08)	-
Field 2011	0.52 (0.09)	0.32 (0.09)	0.02 (0.17)	0.08 (0.16)	-	-	0.62 (0.07)	0.49 (0.09)	0.75 (0.04)	-
Field 2012	0.60 (0.04)	0.52 (0.06)	0.30 (0.10)	0.10 (0.13)	-	-	0.76 (0.05)	0.66 (0.08)	0.89 (0.02)	0.83 (0.06)
Field 2013	0.62 (0.02)	0.54 (0.04)	0.43 (0.05)	0.53 (0.02)	-	-	0.62 (0.02)	0.58 (0.05)	0.64 (0.01)	0.54 (0.04)
GH 1	0.69 (0.04)	0.66 (0.04)	0.79 (0.03)	0.85 (0.04)	0.65 (0.13)	0.50 (0.12)	-	-	0.62 (0.09)	0.68 (0.02)
GH 2	0.75 (0.04)	0.79 (0.06)	0.85 (0.07)	0.76 (0.05)	0.50 (0.08)	0.52 (0.10)	-	-	0.70 (0.07)	0.71 (0.03)
GH 3	-	-	0.80 (0.02)	0.67 (0.04)	-	-	-	-	0.74 (0.02)	0.69 (0.03)

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Table 2. Pearson's correlations between weighted phenotypic means of field and greenhouse (GH) trials for *Nakataea oryzae* (NO), *Rhizoctonia oryzae-sativae* (ROS), *Thanatephorus cucumeris* (TC), plant height (PH), and flowering time (FT) in tropical *japonica* (Trj, white) and *indica* (Ind, gray shadow) populations. Only significantly different from zero correlations ($P < 0.05$) are reported.

	Trj	NO		ROS		TC	FT		PH
Ind		Field	GH	Field	GH	GH	Field	GH	Field
NO	Field		0.29	0.62	0.13	0.15	*NS*	*NS*	0.23
	GH	0.28		0.12	0.26	0.12	-0.36	-0.30	*NS*
ROS	Field	0.38	0.24		*NS*	0.20	*NS*	*NS*	*NS*
	GH	0.37	0.31	0.26		*NS*	-0.3	*NS*	0.23
TC	GH	*NS*	*NS*	*NS*	-0.13		*NS*	*NS*	*NS*
FT	Field	-0.40	-0.30	-0.20	-0.40	*NS*		0.33	-0.11
	GH	*NS*	0.22	0.14	*NS*	*NS*	*NS*		*NS*
PH	Field	-0.26	-0.20	-0.40	-0.37	*NS*	0.34	-0.12	

Supplementary table 1. Summary statistics of adjusted phenotypic means for flowering time (FT) in days, and plant height (PH) in cm in field and greenhouse (GH) experiments for tropical *japonica* (Trj) and *indica* (Ind) populations.

		FT		PH
		Field	GH	Field
Ind	Min.	90.97	51.42	57.25
	1st. Q.	94.65	62.75	63.89
	Median	95.47	64.70	65.78
	Mean	95.87	64.41	66.55
	3rd. Q.	96.73	66.60	68.71
	Max.	104.11	75.30	79.22
Trj	Min.	90.16	46.65	66.49
	1st. Q.	95.65	61.38	76.53
	Median	96.64	63.70	79.38
	Mean	97.04	63.45	79.54
	3rd. Q.	98.23	66.05	82.50
	Max.	104.18	70.67	94.20

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Supplementary figure 1. Infection types of stem rot caused by *Nakataea oryzae* (NO) and depiction of the rating scale used in this study for phenotyping resistance to NO: 0 = no symptoms (not shown); 1 = dark lesion on the outer sheath and stem without lesions, with a) superficial dark lesion in the outer sheath, and b) uncovered stem with no symptoms; 3 = lesions reach the outer part of the stem, with a) growing dark lesion in the outer sheath, and b) small dark superficial lesion in the uncovered stem (red arrow); 5 = lesion inside the stem and/or nodes, with a) growing dark lesion in the outer sheat showing some white mycelia, and b) dark lesion inside the stem (red arrow) and node (white arrow); 7 = wilted stem with mycelia and/or sclerotia inside; and 9 = necrotic and lodged stem.

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Supplementary figure 2. Infection types of aggregated sheath spot caused by *Rhizoctonia oryzae-sativae* (ROS) and depiction of the rating scale used in this study for phenotyping resistance to ROS: 0 = no symptoms (not shown); 1 = lesion limited to lower 25% of the leaf sheath, with lower sheath showing a characteristic oval lesion with green center surrounded by a brown margin; 3 = lesion present on lower 50% of the leaf sheath, with a) tiller showing lesion between 25% and 50% of the leaf sheath, and b) characteristic lesions on the lower sheath; 5 lesion present on more than 50% of the leaf sheath and lower leaves, with a) tiller showing lesion on more than 50% of the leaf sheath and lower leaves, and b) characteristic lesions on higher sheaths; 7 = lesion present on more than 75% of the leaf sheath, flag leaf affected, with a) tiller showing lesions on more than 75% and affected leaf sheath, and b) characteristic lesions on wilted sheaths; and 9 = severe infection or necrotic tiller, with a) tillers showing severe infection, and b) characteristic lesions on necrotic tillers. The same scale was used for rating resistance to *Thanatephorus cucumeris* (TC).

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Supplementary figure 3. Manhattan plots of the GWAS scan for flowering time (FT, purple), plant height (PH, green), resistance to *Nakataea oryzae* (NO, red), and resistance to *Rhizoctonia oryzae-sativae* (ROS, blue) in field (triangles) and greenhouse (circles) trials for tropical *japonica* (left panel) and *indica* (right panel) populations using the naïve model without covariates for GWAS analysis. Phenotypic disease means were estimated with the naïve model, or with FT and PH covariates correction (corr.). The SNPs in the region of defined QTL are highlighted in orange for PH and in light green for the other traits.

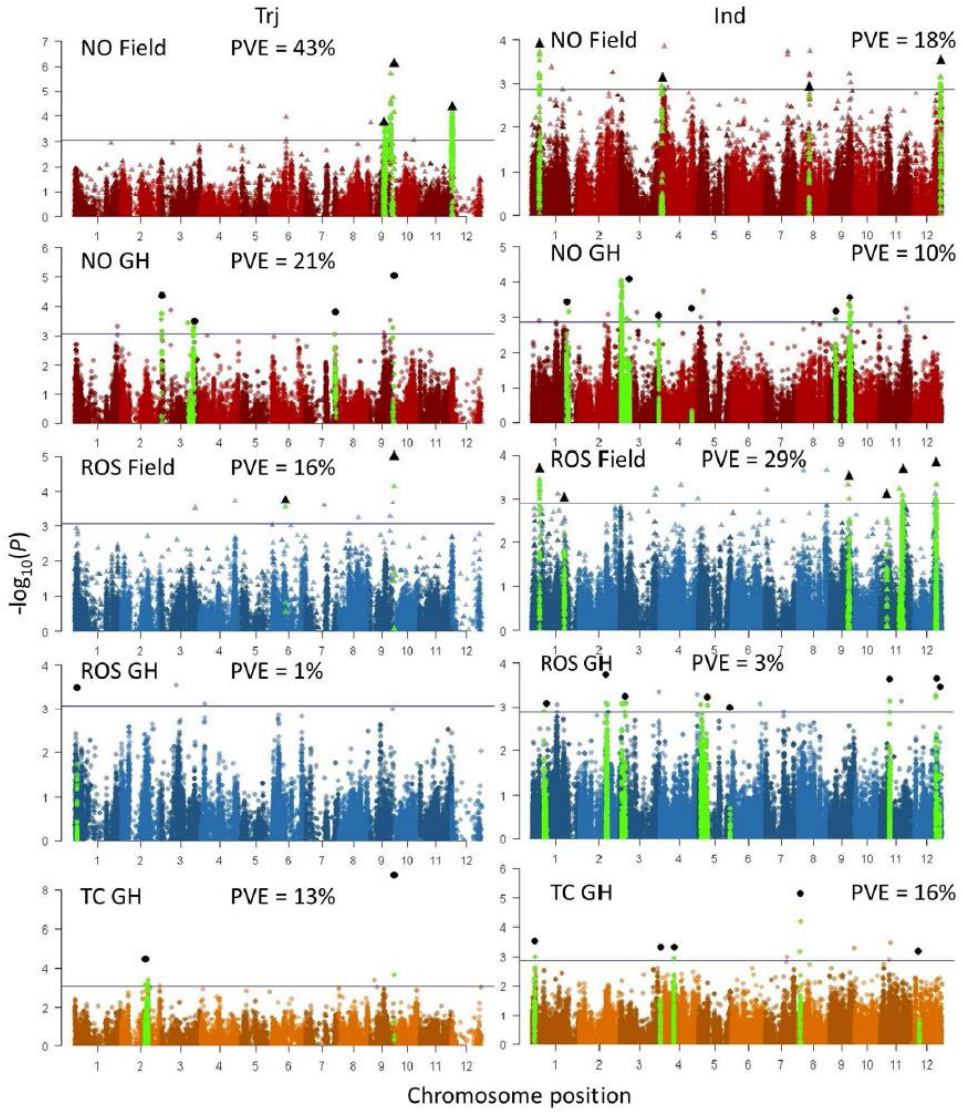

Figure 1. Manhattan plots of the GWAS scan for resistance to Nakataea oryzae (NO, red), resistance to Rhizoctonia oryzae-sativae (ROS, blue), resistance to Thanatephorus cucumeris (TC, orange) in field (triangles) and greenhouse (circles) trials for tropical japonica (left panel) and indica (right panel) populations. All SNP in the region of defined QTL are highlighted in light green, and the SNP with highest -log10(P) in each QTL is colored in black. Percentage of phenotypic variance (PVE) explained by the identified QTL is reported.

164x190mm (300 x 300 DPI)

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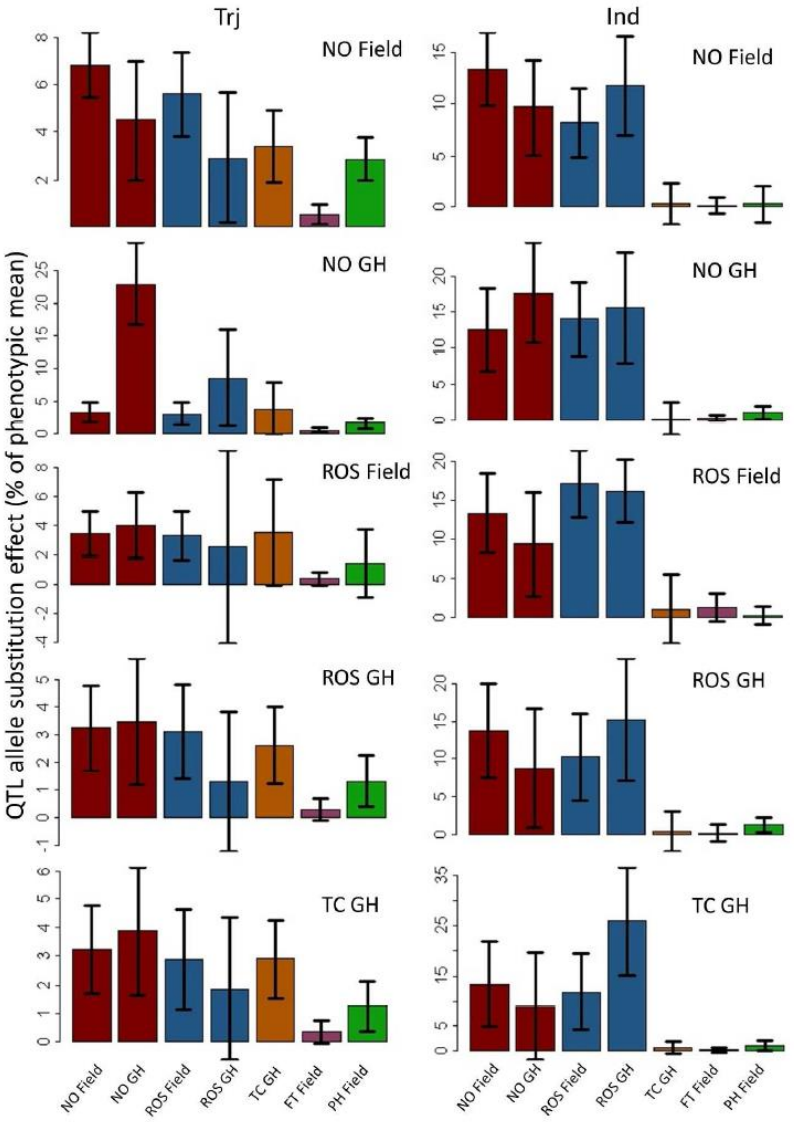

Figure 2. Effects of QTL for resistance to Nakataea oryzae (NO), Rhizoctonia oryzae-sativae (ROS), and Thanatephorus cucumeris (TC) in field and greenhouse (GH) on diseases, flowering time (FT), and plant height (PH) in tropical japonica (Trj) and indica (Ind) populations, estimated with a multi-loci model with all the QTL found in this study for each disease. Allele substitution effect are reported as the percentage of the phenotypic mean.

192x279mm (300 x 300 DPI)

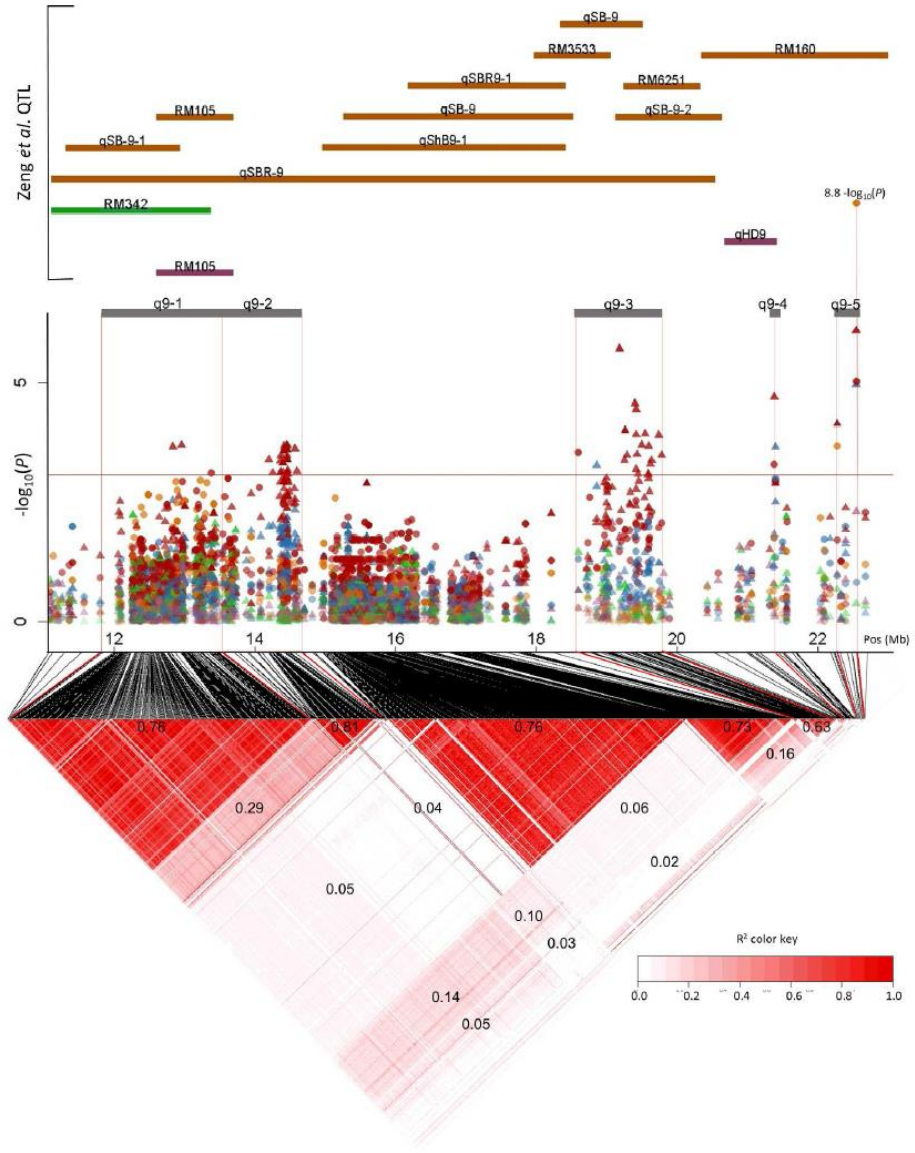

Figure 3. Zoom in positions 11.5 to 23 Mb of chromosome 9 in tropical japonica. The upper section shows the physical positons of previously reported QTL for FT (purple), PH (green) and SB (brown) reviewed by Zeng et al. 2015. QTL found in our study in this region are marked with gray bars. The middle section is the zoomed region of GWAS scan results for NO (red), ROS (blue), SB (orange), FT (green), and PH (purple), in field (triangles) and greenhouse (circles) trials. The lower section is the pairwise R2 between all SNP in the region. Average R2 within and between each LD block are presented.

201x256mm (300 x 300 DPI)

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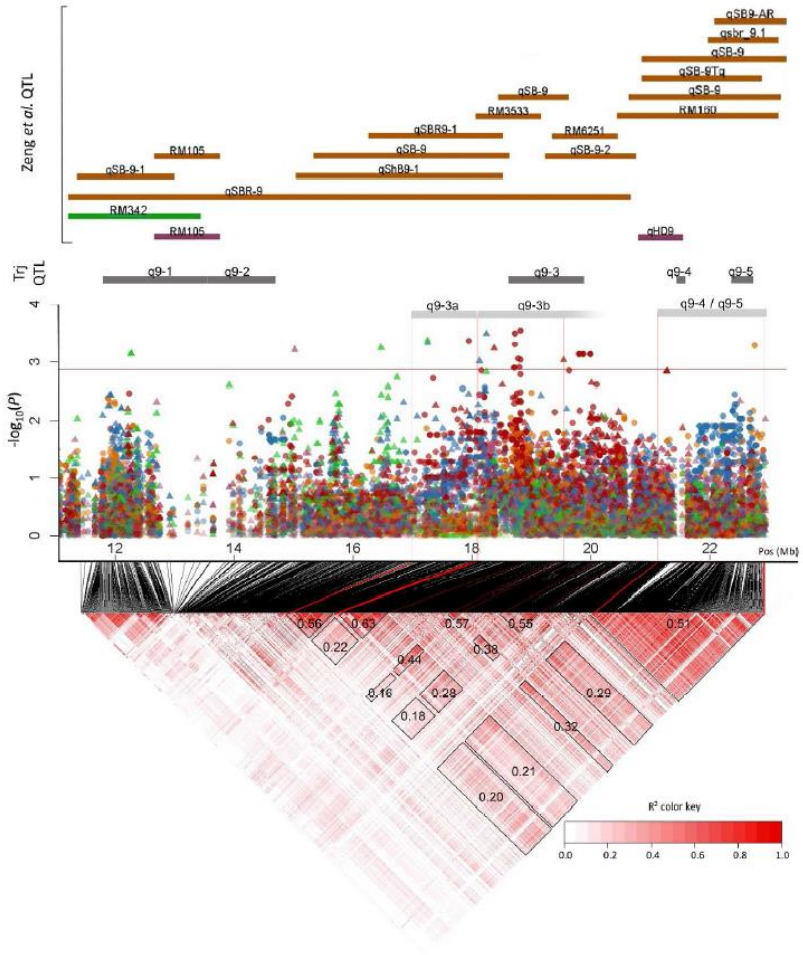

Figure 4. Zoom in positions 11.5 to 23 Mb of chromosome 9 in indica. The upper section shows the physical positons of previously reported QTL for FT (purple), PH (green) and SB (brown) reviewed by Zeng et al. 2015. QTL found in our study in this region in tropical japonica (Trj) are marked in dark gray bars, and QTL found in indica are marked with light gray bars. The middle section is the zoomed region of GWAS scan results for NO (red), ROS (blue), SB (orange), FT (green), and PH (purple), in field (triangles) and greenhouse (circles) trials. The lower section is the pairwise R2 between all SNP in the region. Average R2 within and between each LD block are presented.

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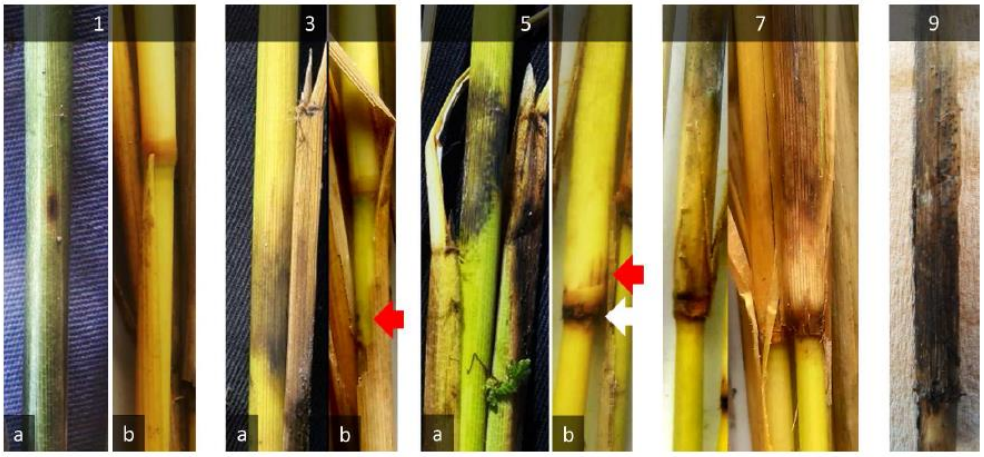

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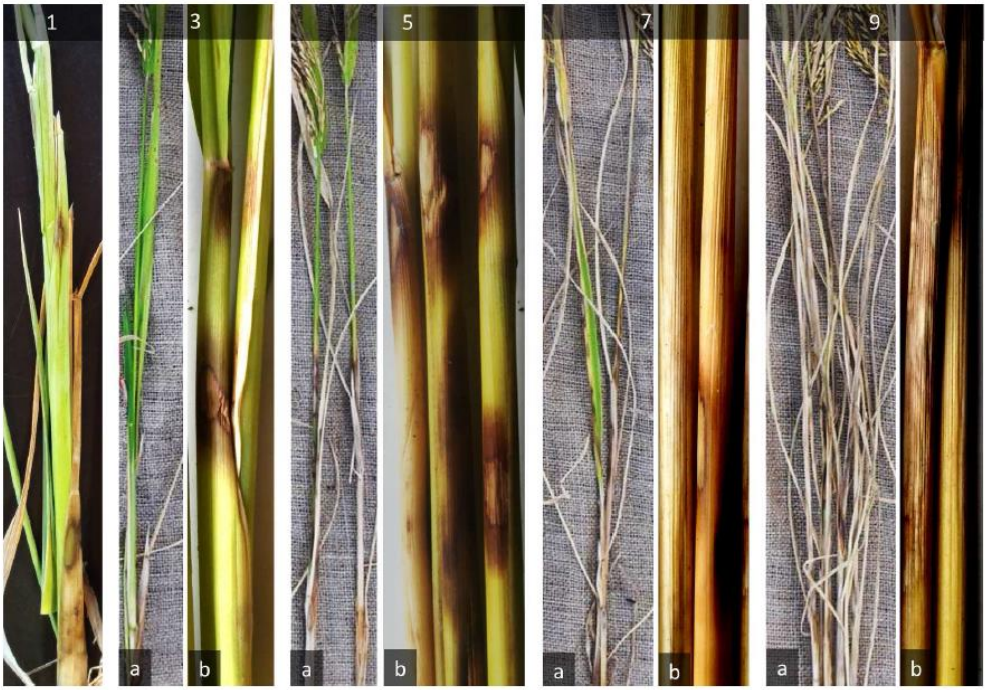

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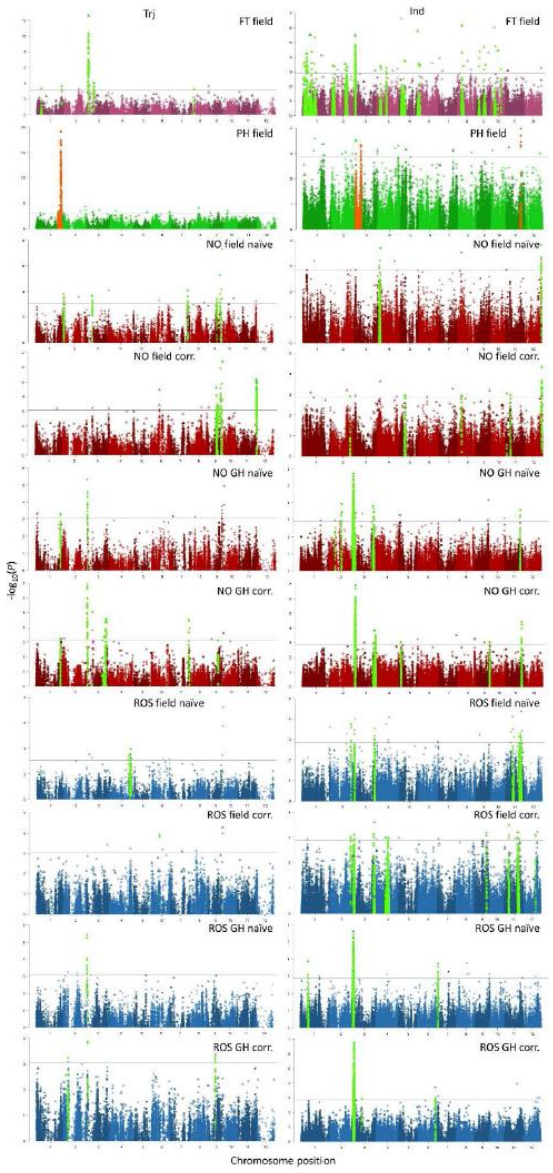

199x426mm (300 x 300 DPI)

4. DISCUSIÓN GENERAL

Este trabajo contribuye sustancialmente a subsanar la escasez de estudios científicos sobre la resistencia del arroz a las enfermedades causadas por NO y ROS. Se demostró que los métodos reportados para evaluar la resistencia a TC en condiciones ambientales controladas (Jia et al. 2013) son adaptables para la evaluación en invernáculo de estos patógenos. Se publicó la primera comparación de métodos de inoculación para estos patógenos basada en parámetros útiles para el mejoramiento genético como la heredabilidad y la capacidad de discriminar entre genotipos (Rosas et al. 2016). También por primera vez, se aplicó un método de invernáculo para el fenotipado masivo de la resistencia a NO y ROS en líneas de arroz (Rosas et al. 2016). Las heredabilidades obtenidas en estos ensayos de fenotipado en invernáculo fueron mayores que las de los ensayos en parcelas de campo utilizados por el programa de mejoramiento genético de arroz de INIA.

El análisis de GWAS permitió identificar regiones cromosómicas asociadas con resistencia a NO, ROS y TC (Rosas et al. 2017). Fue el primer estudio de la genética de la resistencia a ROS, y el primero en utilizar GWAS para estudiar la arquitectura genética de NO y ROS en arroz (Rosas et al. 2017). Muchas de las regiones cromosómicas asociadas con resistencia a NO y ROS identificadas en este trabajo, estaban reportadas para resistencia a TC (Zeng et al. 2015), sugiriendo la existencia de mecanismos comunes de resistencia a estos tres patógenos que atacan tallo y vaina del arroz. En particular, la región identificada en el cromosoma 9 presentó varios SNPs asociados a resistencia a las tres enfermedades, constituyendo uno de los pocos reportes de QTL de resistencia múltiple a enfermedades en plantas (Wiesner-Hanks y Nelson 2016).

En este trabajo se presentó además un procedimiento estadístico general para remover en forma eficiente el efecto de factores de confusión en la evaluación de la resistencia a enfermedades, como PH y FT. Se demostró que la inclusión de estas variables como cofactores en la estimación de las medias fenotípicas ajustadas, y la inclusión de SNPs asociados a PH y FT como cofactores en el análisis de GWAS,

permite identificar QTL de resistencia a enfermedades con poco o ningún impacto en PH y FT.

Esta tesis se inscribe en las líneas de investigación del programa de mejoramiento genético de arroz de INIA. En este sentido, es pertinente evaluar los aportes de este trabajo para el diseño de estrategias de mejoramiento de la resistencia a NO y ROS en el programa de mejoramiento genético de arroz de INIA. En este trabajo se evaluaron nuevos métodos de fenotipado diferentes a los ensayos en parcelas de campo con infección natural que se utilizan actualmente en el programa: los ya mencionados métodos de invernáculo, y los ensayos en campo en *hill plots* con inoculación artificial descriptos en Rosas et al. (2017, pág. 32 de esta tesis). Para evaluar la conveniencia de estos métodos alternativos de fenotipado, es necesario considerar la respuesta a la selección para el fenotipo objetivo que puede esperarse con cada uno de ellos. Falconer (1952) describe la relación entre la respuesta a la selección por un fenotipo alternativo al fenotipo objetivo, y la respuesta a la selección por el fenotipo objetivo, con la ecuación [I]:

$$\frac{\Delta' G}{\Delta G} = \frac{h_2}{h_1}\, r_G \qquad [I]$$

donde $\Delta' G$ y ΔG son respectivamente las respuestas a la selección por el fenotipo alternativo y por el objetivo, h_1 y h_2 son respectivamente la raíz cuadrada de la heredabilidad del fenotipo objetivo y del fenotipo alternativo; y r_G es la correlación entre ambos valores fenotípicos. Para este análisis se consideraron como fenotipos objetivo los ensayos en campo, ya sea con infección natural o con inoculación artificial, y como fenotipos alternativos los ensayos en invernáculo y los ensayos en campo con inoculación artificial. La comparación de fenotipos en base al parámetro expresado en la ecuación [I] muestra comportamientos diferentes entre las poblaciones *indica* y *japonica* tropical. Para *indica*, la mejor estrategia para seleccionar por resistencia a NO y a ROS es con los ensayos en invernáculo (Tabla I). Esto se explica por las bajas heredabilidades y la interacción genotipo por ambiente entre los ensayos de campo para esta población en ambos rasgos. En particular, la correlación negativa entre la resistencia a ROS evaluada en ensayos de campo con

hill plots y la evaluada en ensayos de parcelas en 2011 hace que la selección por uno de estos fenotipos tenga respuesta negativa en el otro. Por otro lado, la mejor estrategia para seleccionar por resistencia a NO y ROS en *japonica* tropical son los ensayos de campo con *hill plots*. Esto se debe a las mayores heredabilidades y/o menor interacción genotipo por ambiente entre ensayos de campo en esta población.

Tabla I. Cocientes de respuesta a la selección en ambiente alternativo vs. ambiente objetivo estimados de acuerdo a Falconer (1952), para resistencia a *Nakataea oryzae* (NO) y *Rhizoctonia oryzae-sativae* (ROS) en las subpoblaciones de tipo *indica* (Ind) y *japonica* tropical (Trj). Se presentan los valores para cada combinación de ambiente alternativo y ambiente objetivo: invernáculo (GH), parcelas y *hill plots*.

Población	Fenotipo Alternativo	Fenotipo Objetivo (resistencia a ROS)					Fenotipo Objetivo (resistencia a NO)				
		Parcelas 2010	Parcelas 2011	Parcelas 2012	Hill Plots 2013	Campo*	Parcelas 2010	Parcelas 2011	Parcelas 2012	Hill Plots 2013	Campo*
Ind	GH Exp. 1	-	0,03	0,44	0,38	0,28	-	0,07	0,27	0,10	0,20
	GH Exp. 2	-	0,09	0,50	0,24		-	0,25	0,21	0,31	
	Parcelas 2011	-	-	0,04	-0,01	0,23	-	-	0,17	0,16	0,16
	Parcelas 2012	-	0,66	-	0,25		-	0,19	-	0,13	
	Hill Plots 2013	-	-0,06	0,25	-	0,10	-	0,18	0,13	-	0,16
Trj	GH Exp. 1	0,08	0,19	0,08	0,01	0,01	0,38	0,24	0,31	0,27	0,29
	GH Exp. 2	-0,08	-0,26	-0,02	0,07		0,33	0,29	0,28	0,20	
	Parcelas 2010	-	0,31	0,02	0,09	0,13	-	0,32	0,32	0,27	0,38
	Parcelas 2011	0,03	-	0,02	0,21		0,41	-	0,38	0,37	
	Parcelas 2012	0,04	0,27	-	0,17		0,48	0,44	-	0,39	
	Hill Plots 2013	0,16	1,16	0,24	-	0,16	0,40	0,41	0,41	-	0,44

*Promedio de los cocientes de respuesta a la selección en ensayos de campo (parcelas 2011 y 2012, y *hill plots* 2013 para *indica*, y parcelas 2010 a 2012 y *hill plots* 2013 para *japonica* tropical).

Por otra parte, los métodos alternativos de selección implementados en este trabajo pueden presentar otras ventajas frente a la selección en ensayos de campo en parcelas. La selección por resistencia a enfermedades suele ser más efectiva en condiciones epidémicas severas que en las leves (Geiger 1989). La distribución de las medias fenotípicas de los ensayos de invernáculo (Figura I) muestra que las condiciones de invernáculo con inoculación artificial favorecen una manifestación más severa de las enfermedades estudiadas. La inoculación artificial utilizada en los ensayos de campo en *hill plots* también tiene como objetivo generar condiciones epidémicas más severas que la infección natural. La mayor severidad permite una mejor discriminación entre genotipos, facilitando la selección. Por otra parte, los ensayos en invernáculo y en *hill plots* permiten seleccionar en etapas más tempranas que en los esquemas actuales del programa de mejoramiento por requerir menos semilla por unidad experimental. Las recomendaciones clásicas para optimizar la ganancia genética indican una intensidad de selección constante a lo largo de los sucesivos ciclos de mejoramiento (Finney 1966). Agregar ciclos de selección en el ambiente de alta expresión de las enfermedades podría acelerar el proceso actual de mejoramiento para resistencia a NO y ROS. En Rosas et al. (2016) mostramos que cualquiera de los métodos de inoculación evaluados para invernáculo (con excepción de los tallos en tubos) puede ser utilizado con similares resultados para la selección por resistencia a NO y ROS. El método de esclerocios esparcidos sobre la lámina de agua sería de muy fácil y rápida implementación para la evaluación masiva de líneas en etapas tempranas.

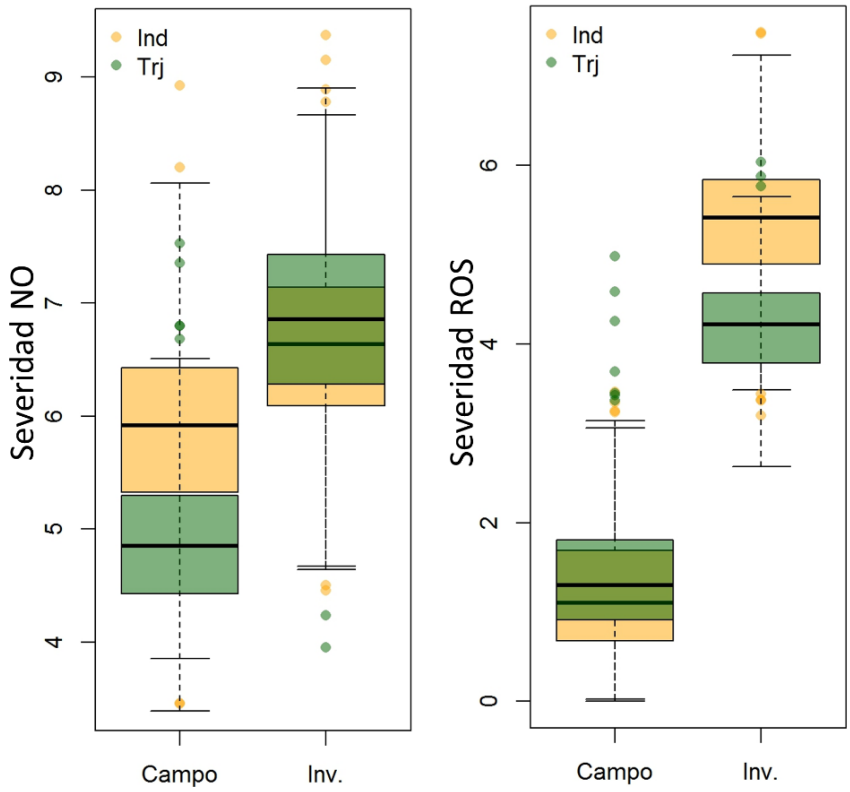

Figura I. Distribución de la severidad de la infección por *Nakataea oryzae* (NO) y *Rhizoctonia oryzae-sativae* (ROS) en las subpoblaciones de tipo *indica* (Ind) y *japonica* tropical (Trj) evaluadas en experimentos de campo e invernáculo (Inv.).

Atendiendo a estos criterios genéticos, puede recomendarse la incorporación de ensayos de invernáculo para selección por resistencia a NO y ROS en *indica*, y ensayos de campo en *hill plots* para selección por resistencia a NO y ROS en *japonica* tropical. Consideraciones logísticas y económicas deben sumarse a estos criterios para determinar la conveniencia de implementar estas metodologías.

La conveniencia de aplicar los marcadores moleculares asociados a resistencia a NO y ROS identificados en este trabajo para selección asistida puede evaluarse comparando la eficiencia genética de la selección asistida por marcadores moleculares, con la eficiencia de la selección fenotípica. Una forma de comparar estas eficiencias de selección es la descripta por Lande y Thompson (1990) y expresada como $\sqrt{PVE/h^2}$, donde PVE es la proporción de la varianza fenotípica explicada por los marcadores moleculares, y h^2es la heredabilidad en los ensayos de selección fenotípica. En la Tabla II se presenta la eficiencia relativa de la selección asistida en comparación con la selección en ensayos de invernáculo, ensayos de campo en parcelas, y ensayos de campo en *hill plots*. La selección asistida presenta una eficiencia similar a la de los ensayos en parcelas, siendo superior para algunos años en ambos patógenos y poblaciones. Desde el punto de vista genético es entonces justificable incluir los SNPs que definen los QTL para resistencia a estos patógenos identificados en esta tesis en un chip de mejoramiento asistido, debiendo estudiarse los aspectos económicos de esta modalidad de selección.

Selección Fenotípica	Eficiencia relativa de selección asistida			
	Trj		Ind	
	NO	ROS	NO	ROS
GH Exp. 1	0,19	0,11	0,39	0,55
GH Exp. 2	0,20	0,11	0,36	0,53
Parcelas 2010	-	0,92	-	1,04
Parcelas 2011	1,90	2,83	0,75	0,91
Parcelas 2012	1,70	0,73	0,59	0,85
Hill Plots 2013	0,74	0,61	0,58	0,83

Tabla II. Eficiencia de la selección asistida por marcadores moleculares asociados a resistencia a *Nakataea oryzae* (NO) y *Rhizoctonia oryzae-sativae* (ROS) comparada con la eficiencia de la selección fenotípica en invernáculo (GH), experimentos de campo en parcelas y *hill plots*, estimados de acuerdo a Lande y Thompson (1990), en las subpoblaciones de tipo *indica* (Ind) y *japonica* tropical (Trj).

5. CONCLUSIONES Y PERSPECTIVAS

Se generaron nuevas alternativas de fenotipado en condiciones de mayor severidad para evaluar masivamente y en etapas tempranas la resistencia a NO y ROS en germoplasma de arroz del programa de mejoramiento de INIA. Asimismo, se identificaron marcadores moleculares de tipo SNPs asociados a resistencia a NO y ROS utilizables en la selección asistida de resistencia a NO y ROS sin alterar el largo de ciclo ni altura de planta.

Están en progreso trabajos para validar las asociaciones encontradas entre los SNPs identificados en este trabajo y las enfermedades del tallo y la vaina, en una nueva población de 1000 líneas avanzadas del programa de mejoramiento genético de arroz de INIA. Esto permitirá determinar la inclusión o no de dichos SNPs en un chip para mejoramiento asistido para los rasgos de mayor interés del programa.

El programa de mejoramiento comenzó en los últimos años a seleccionar por resistencia a *brusone* evaluando líneas en etapas tempranas en ensayos de invernáculo, en estadio de plántula. Estos mismos experimentos pueden adaptarse sin mayores modificaciones para la evaluación en tándem de la resistencia a NO y ROS en las plantas sobrevivientes a la infección por *brusone* usando las metodologías de invernáculo descriptas en esta tesis.

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7. ANEXO: COMUNICACIONES DE ACEPTACIÓN Y PUBLICACIÓN DE ARTÍCULO ”RESISTANCE TO MULTIPLE TEMPERATE AND TROPICAL STEM AND SHEATH DISEASES OF RICE”.

---------- Forwarded message ----------
From: **The Plant Genome** <onbehalfof+muehl003+umn.edu@manuscriptcentral.com>
Date: Tue, May 16, 2017 at 11:31 AM
Subject: The Plant Genome - Decision on Manuscript ID TPG-2017-03-0029
To: Lucia Gutierrez Chacon <gutierrezcha@wisc.edu>

16-May-2017

Dear Dr. Gutierrez:

Manuscript ID TPG-2017-03-0029 entitled "RESISTANCE TO MULTIPLE TEMPERATE AND TROPICAL STEM AND SHEATH DISEASES OF RICE" which you submitted to the The Plant Genome, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

The reviewer(s) have recommended publication, but also suggest some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript.

To revise your manuscript, log into https://mc.manuscriptcentral.com/plantgenome and enter your Author Center, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript using a word processing program and save it on your computer. Please also highlight the changes to your manuscript within the document by using the track changes mode in MS Word or by using bold or colored text.

Once the revised manuscript is prepared, you can upload it and submit it through your Author Center. Your revised manuscript is due on 15-Jul-2017

When submitting your revised manuscript, you will be able to respond to the comments made by the reviewer(s) in the space provided. You can use this space to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please respond to all reviewer comments in a point-by-point fashion. If the AE has made any comments, please be sure to respond to those as well.

---------- Forwarded message ----------
From: **The Plant Genome** <onbehalfof+egebhardt+agronomy.org@manuscriptcentral.com>
Date: Wed, Jul 12, 2017 at 3:09 PM
Subject: The Plant Genome - Manuscript ID TPG-2017-03-0029.R1
To: Lucia Gutierrez Chacon <gutierrezcha@wisc.edu>

12-Jul-2017

Dear Dr. Gutierrez:

Your manuscript entitled "RESISTANCE TO MULTIPLE TEMPERATE AND TROPICAL STEM AND SHEATH DISEASES OF RICE" has been successfully submitted online and is presently being given full consideration for publication in the The Plant Genome.

Your manuscript ID is TPG-2017-03-0029.R1.

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to Manuscript Central at https://mc.manuscriptcentral.com/plantgenome and edit your user information as appropriate.

You can also view the status of your manuscript at any time by checking your Author Center after logging in to https://mc.manuscriptcentral.com/plantgenome.

Thank you for submitting your manuscript to the The Plant Genome.

Sincerely,
The Plant Genome Editorial Office

Resent-From: gutierrezcha@wisc.edu
From: The Plant Genome <onbehalfof+amorrison+sciencesocieties.org@manuscriptcentral.com>
Date: September 22, 2017 at 8:58:34 AM CDT
To: Lucia Gutierrez Chacon <gutierrezcha@wisc.edu>
Subject: TPG-2017-03-0029.R1 | Next steps
Reply-To: "amorrison@sciencesocieties.org" <amorrison@sciencesocieties.org>

Dear Dr. Gutierrez:

We have been notified that your manuscript (TPG-2017-03-0029.R1) has been accepted for publication in The Plant Genome.

Next Steps:

First Look. Your article will be posted online in the next few days on The Plant Genome First Look page (https://dl.sciencesocieties.org/publications/tpg/first-look). You can direct potential readers there while your paper is in production.

Editorial. You will be hearing from a copy editor in the next few weeks with any queries, and a proof will be sent after that.

If you have questions regarding your manuscript, please contact managing editor Liz Gebhardt (egebhardt@sciencesocieties.org).

Thank you for your contribution to The Plant Genome.

Best regards,
Abby Morrison
Publications Systems Assistant
The Plant Genome Editorial Office