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Molecular diagnostic of both brown and orange sugarcane rust and evaluation of sugarcane brown rust resistance in Tucumán- Argentina using molecular markers associated to *Bru*1, a broad-range resistance allele

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ABSTRACT

Brown rust (*Puccinia melanocephala*), first reported in Tucumán in 1988, and orange rust (*P. kuehnii*), not yet reported in Argentina, cause important yield losses in global sugarcane production. Due to the difficulties in distinguishing these two sugarcane diseases with the naked eye, it is essential to use molecular techniques for an accurate diagnosis. A major gene, *Bru*1, which confers resistance to a broad spectrum of *P. melanocephala* strains in different parts of the world, has been described, and molecular markers closely associated with this allele have been developed. The present study aims to i) optimise a PCR-based method to diagnose and characterise the causal agent population of both types of rust in Tucumán; ii) determine the usefulness of the *Bru*1 gene in the Sugarcane Breeding Program of Estación Experimental Agroindustrial Obispo Colombres (EEAOC), by studying its association with resistant and susceptible phenotypes; and iii) assess the frequency of the *Bru*1 allele in EEAOC sugarcane germplasm. Conditions for both brown and orange rust diagnosis were optimised. When analysing 30 sugarcane samples showing rust symptoms, only *P. melanocephala* DNA was amplified. Sugarcane varieties frequently used in the EEAOC Breeding Program were analysed in order to study the usefulness of the *Bru*1 allele to diagnose brown rust resistance in Tucumán. Out of 129 evaluated genotypes, 49 (38.0%) were found to be resistant to brown rust, but only 8 (16.3%) of these were positive for the *Bru*1 allele. We then analysed the frequency of appearance of the *Bru*1 allele by using diagnostic markers R12H16 and 9020-F4-RsaI, in 191 sugarcane accessions of the EEAOC germplasm. Presence, as determined by the two markers, was detected in only 7% of the evaluated genotypes. In conclusion, although results showed that *Bru*1 markers enable positive selection of this character, additional resistance source(s) are available in the EEAOC Sugarcane Breeding Program.

Key words: marker assisted selection, *Puccinia melanocephala*, *Puccinia kuehnii*.

RESUMEN

Diagnóstico molecular de la roya marrón y naranja de la caña de azúcar y evaluación de la resistencia a roya marrón en Tucumán, R. Argentina, mediante marcadores moleculares asociados a *Bru*1, un alelo de resistencia de amplio espectro

La roya marrón (*Puccinia melanocephala*), reportada por primera vez en Tucumán en 1988, y la roya naranja (*P. kuehnii*), aún no encontrada en la Argentina, causan importantes pérdidas de rendimiento en la producción global de caña de azúcar. Debido a la dificultad de distinguir estas dos enfermedades a simple vista, el empleo de técnicas moleculares es esencial para un diagnóstico preciso. Se ha descripto un gen mayor, *Bru*1, que confiere resistencia contra un amplio espectro de cepas de *P. melanocephala* de diferentes partes del mundo, y se han desarrollado marcadores moleculares estrechamente asociados con él. Los objetivos del presente estudio son: i) optimizar un método basado en PCR para el diagnóstico y la caracterización de poblaciones de los agentes causales de ambas royas en Tucumán; ii) determinar la utilidad del gen *Bru*1 en el Programa de Mejoramiento Genético de la Caña de Azúcar (PMGCA) de la Estación Experimental Agroindustrial Obispo Colombres (EEAOC), estudiando su asociación con fenotipos resistentes y susceptibles; y iii) determinar la frecuencia del alelo *Bru*1 en el germoplasma de la EEAOC. Se optimizaron las condiciones para el diagnóstico de ambas royas. Se analizaron 30 muestras de caña de azúcar con síntomas y solo se amplificó ADN de *P. melanocephala*. Por otra parte, variedades de caña de azúcar frecuentemente utilizadas por el PMGCA fueron analizadas para estudiar la utilidad de los marcadores asociados a *Bru*1 para detectar resistencia a roya marrón. De los 129 genotipos evaluados, 49 (38,0%) resultaron resistentes a la enfermedad y solo 8 (16,3%) de los resistentes fueron positivos para el alelo *Bru*1. También se analizó la frecuencia de aparición de *Bru*1 mediante los marcadores diagnósticos en 191 accesiones de caña de azúcar del germoplasma de la EEAOC. El gen fue detectado en solo 7% de los genotipos evaluados mediante dos marcadores. En conclusión, los resultados demostraron que los marcadores de *Bru*1 posibilitan la selección positiva de este carácter, aun cuando otras fuentes de resistencia están disponibles en el PMGCA de la EEAOC.

Palabras clave: selección asistida por marcadores, *Puccinia melanocephala*, *Puccinia kuehnii*.

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INTRODUCTION

Rusts are fungal diseases caused by obligate pathogens affecting primarily plant foliage. These diseases are characterised by the appearance of small elongated yellowish spots, visible on both sides of the leaves. These spots decrease photosynthetic capacity and increase their length gradually, becoming brown, orange-brown or red-brown over time. *Puccinia melanocephala* is the causal agent of brown rust, an important sugarcane disease in many production areas (Raid and Comstock, 2000). Brown rust has an important economic impact and is associated with significant yield losses (greater than 50%) (Hoy and Hollier, 2009). *Puccinia kuehnii* Butler is the causal agent of orange rust, a less widespread disease than brown rust. The occurrence of this pathogen in the Western hemisphere was confirmed only recently in the United States (Comstock *et al*., 2008) and since then, it has been reported in many countries in Central and South America (Chavarria *et al*., 2009; Barbasso *et al*., 2010), though not in Argentina (Funes *et al*., 2011). Differences in urediniospore coloration and the apical thickening of *P. kuehnii* urediniospores are the only morphological features distinguishing the two pathogens, although they do not constitute a definitive diagnosis method. Since the identification of the two sugarcane rust pathogens in the field is very difficult by simple observation, and due to the fact that orange rust is an important potential threat to the Argentine sugarcane industry, an accurate, fast and robust diagnostic method was developed to allow for a precise and sensitive discrimination between *P. kuehnii* and *P. melanocephala*.

The most effective method for controlling these diseases is the use of resistant varieties. However, resistance durability is affected by a number of factors, including pathogen genetics, plant growth stage, weather conditions, plant nutrition, and soil characteristics (Anderson and Dean, 1986; Anderson *et al*., 1990 and 1991; Raid and Comstock, 2000). Due to this, shifts in cultivar reactions from resistance to susceptibility have been observed (Hoy and Hollier, 2009). Nevertheless, a brown rust resistant sugarcane cultivar, R570, one of the most widely cultivated varieties for the last 20 years on the islands of Reunion and Mauritius, has never lost its resistance to brown rust although it has been challenged with various rust isolates collected from all over the world (Asnaghi *et al*., 2001). The brown rust resistance of this cultivar was studied and results showed a monogenic and dominant control involving a single copy of the resistance allele (Daugrois *et al*., 1996). This major resistance gene, *Bru*1, is the first well-characterised Mendelian trait described in the complex genomic context of sugarcane (Asnaghi *et al*., 2004). Costet *et al*. (2012) reported that brown rust resistance in modern sugarcane cultivars relies essentially on *Bru*1. Le Cunff *et al*. (2008) found that the *Bru*1 gene is included in an unknown size insertion that is specific to the *Bru*1-bearing haplotype, being absent in other hom(e)ologous haplotypes. This insertion induces a reduction of recombination, resulting in strong linkage disequilibrium (LD) in the *Bru*1 region and a complete LD between two flanking molecular markers, R12H16 and 9020-F4. These markers represent good diagnostic markers for *Bru*1 presence, since their detection predicts a resistant behaviour in any modern cultivar. In addition, the absence of these markers in a resistant cultivar indicates the absence of *Bru*1, suggesting the existence of an alternative resistance source (Costet *et al*., 2012).

The work presented here aims to i) optimise a PCR-based method in order to diagnose and characterise the causal agent population of both brown and orange rust in Tucumán; ii) determine the usefulness of applying molecular markers for detecting the *Bru*1 gene in the genetic material used in the Sugarcane Breeding Program of Estación Experimental Agroindustrial Obispo Colombres (EEAOC), by studying their association with resistant and susceptible phenotypes; and iii) assess the frequency of the *Bru*1 allele in EEAOC sugarcane germplasm.

MATERIALS AND METHODS

Detection of *Puccinia kuehnii* and *P. melanocephala* by PCR

To evaluate the presence of brown and orange rust causal agents, 30 young leaf samples (first and second leaves with visible ligules) exhibiting typical rust symptoms were collected from plants in different production areas in Tucumán, Argentina (Cerco Represa, Las Talitas, Santa Ana, Río Seco, La Banda, Mercedes and Fronterita). Plant material with different numbers of uredinial lesions was collected in order to determine the optimal conditions for nucleic acid extraction for rust detection and diagnosis.

Total RNA and DNA were extracted by essentially using the CTAB (cetyl trimethyl ammonium bromide) technique described by Aljanabi *et al*. (1999), optimised for samples with rust pustules.

Ribosomal DNA (rDNA) amplification was performed by PCR, by using five different pairs of primers: two general ones for fungi (ITS1F/ITS4, NL1/NL4), a pair of species-specific primers to differentiate between *P. melanocephala* and *P. kuehnii* by the size of the band amplified (PkPmF: AAGAGTGCACTTAATTGTGGCTC / PkPmR: TCCCACCTGATTTGAGGTCT), one specific for *P. melanocephala* (Pm1F: AATTGTGGCTCGAACCATCTTC / Pm1R: TTGCTACTTT CCTTGATGCTC) and one specific for *P. kuehnii* (PkPmF: AAGAGTGCACTTAATTGTGGCTC / Pk1R: CAGGTAACA CCTTCCTTGATGTG) (Virtudazo *et al*., 2001; Glynn *et al*., 2010). Different concentrations of $MgCl_2$, primers and nucleic acids (mould), as well as annealing temperature of the primers, were analysed. Positive and negative controls were included for *P. melanocephala* and *P. kuehnii* in all cases. The latter control was kindly provided by Centro de Tecnología Canaviera (CTC), Brazil. PCR products were

separated on 1.5% agarose gels, stained with GelRed and visualised on a UV transilluminator. Some amplified bands were removed and purified from agarose gel using the Qiagen Gel Extraction Kit, and sequenced in both directions in the Laboratory of Instituto Nacional de Tecnología Agropecuaria (INTA) (Castelar, Argentina). All DNA sequences obtained were compared with available sequences in the GenBank public data base.

Genotyping for Bru1 presence using molecular markers

To determine the usefulness of *Bru*1 under our local rust conditions, 129 sugarcane accessions frequently used as parents in the EEAOC Breeding Program (Table 1) were analysed in order to study the existence of this gene, by applying molecular diagnostic markers R12H16 and 9020-F4-RsaI. Out of the 129 tested sugarcane cultivars, most were HOCP varieties (46%) obtained in crossings performed at Canal Point and then selected at Houma, and TUC varieties (40%) obtained and selected in the EEAOC Breeding Program.

Then, to determine the frequency of appearance of the *Bru*1 gene in the EEAOC germplasm collection, 191 sugarcane accessions (Table 2) were studied to detect its presence by using diagnostic markers.

Table 1. Brown rust resistance and the presence of molecular diagnostic markers for *Bru*1, from 129 sugarcane accessions studied.

Rust severity[a]	*Bru*1 diagnostic markers presence[b]	Accessions
0	+	HOCP03-714 HOCP03-738 HOCP05-918 HOCP05-920 L79-1002 TUC95-35
	-	HO94-851 HOCP00-961 HOCP05-931 HOCP85-845 HOCP92-631 HOCP95-951 RA87-3 TUC00-56 TUC00-74 TUC94-59 TUC95-24 TUC96-46 TUC97-20 TUC98-44 TUC99-132
1	+	HO94-856 HOCP04-814
	-	HOCP02-640 HOCP03-717 HOCP03-731 HOCP03-744 TUC92-10 TUC99-12
2	-	HO02-653 HOCP01-517 HOCP02-636 HOCP02-652 HOCP03-739 HOCP04-847 HOCP05-961 HOCP94-806 HOCP95-988 TUC00-019 TUC00-165 TUC00-53 TUC01-14 TUC01-23 TUC89-28 TUC95-07 TUC95-37 TUC97-08 TUC98-16 TUC98-24
3	-	CP91-523 HOCP01-523 HOCP03-711 HOCP03-713 HOCP03-730 HOCP03-736 HOCP05-902 HOCP92-645 HOCP92-648 HOCP93-746 HOCP93-750 L89-113 L91-281 L94-424 LCP86-454 TUC00-008 Tuc95-10 TUC95-23 TUC95-34 TUC95-46 TUC96-01 TUC97-30 TUC98-018 TUC98-048 TUC99-05
4	-	CP89-2377 HOCP02-618 HOCP02-622 HOCP03-718 HOCP03-720 HOCP05-903 HOCP91-555 HOCP92-624 L94-428 LHO83-153 TUC94-47 TUC96-24 TUC96-60 TUC98-01 TUC98-20 TUC99-10 TUCCP77-42
5	-	HO95-985 HOCP00-950 HOCP01-551 HOCP02-610 HOCP02-625 HOCP03-704 HOCP03-708 HOCP03-719 HOCP92-618 HOCP93-754 HOCP96-540 TUC01-24 TUC95-18 TUC95-25 TUC95-36 TUC96-52 TUC97-07 TUC98-02 TUC98-13
6	-	HOCP01-561 HOCP01-564 HOCP02-632 HOCP03-725 HOCP05-923 HOCP05-937 HOCP92-675 TUC97-101
7	-	CP65-357 HOCP03-749 TUC01-11
8	-	HOCP03-743 HOCP05-904 HOCP91-552 TUC01-29 TUC98-108 TUC99-125
9	-	LCP85-384 TUC95-22

[a] Rust severity scored on a 0 (the most resistant) to 9 (the most susceptible) scale, according to Amorin *et al*. (1987).
[b] *Bru*1 presence detected by molecular diagnostic markers R12H16 and 9020-F4-RsaI: (+) indicates the presence of both markers and (-) indicates the absence of both markers.

To genotype for the presence of *Bru*1 gene, three young leaves from each genotype were collected and stored at -70ºC until further processed. Before DNA extraction, plant tissue was kept frozen by using liquid nitrogen, and then ground to a fine powder with a mortar and pestle. Genomic DNA was extracted following the CTAB method, essentially as described by Aljanabi *et al*. (1999). Two different molecular diagnostic markers, R12H16 and 9020-F4, associated with *Bru*1 and strongly related to brown rust resistance (Costet *et al*., 2012) were used in this study to test for the presence of the *Bru*1 gene. All PCR reactions were carried out using the same set-up: 20 µL final volume containing 50 ng template DNA; 0.4 µM of each primer; 0.4 mM of each dNTP; 2.5 mM $MgCl_2$; and 0.5 units Taq Polymerase (Invitrogen LifeTech) with 1X PCR buffer, provided with the enzyme. Cycling was performed in MyCycler thermocycler (Bio-Rad) as follows: 4 min denaturation at 94°C, followed by 35 cycles of 94°C for 30 s, 55.5°C for 45 s, 72°C for 72 s, and final elongation for 8 min at 72°C. All amplifications were repeated at least three times. Fifteen microlitres of the 9020-F4 PCR products was digested with restriction enzyme Rsa I to visualise the presence of a specific band associated with the *Bru*1 allele. The R12H16 PCR marker corresponds to a specific fragment whose presence is associated with the *Bru*1 gene. R12H16 PCR products and 9020-F4-RsaI restriction fragments were visualised following electrophoresis on a 1.5% and 3% agarose gel, respectively, and stained with Gelred.

Field evaluation of brown rust resistance

In order to determine the usefulness of the *Bru*1 gene under our local conditions, field-testing for rust susceptibility and resistance of 129 sugarcane cultivars was determined by using natural infections in plant-cane in an unreplicated design, consisting of individual 3-metre-long row plots. The trial was planted in Cerco Represa (Tucumán, Argentina) in July 2011 and evaluated for brown rust presence in February 2012, i.e. in a high pressure inoculum locality and in the most favourable period for rust development. Brown rust reaction was scored in each plot on a 0 (totally resistant) to 9 (very susceptible) scale, according to Amorin *et al*. (1987), by two independent observers. This scale is based on visual assessment of the disease symptoms. A score 0 to 2 indicates resistant plants with no fungus pustules, though some necrotic or chlorotic spots may appear on the leaves. A

Table 2. Sugarcane accessions studied for the presence of *Bru*1 gene.

*Bru*1 presence [a]	Accessions
+	CO290 CO419 CO421 CP52-68 CP53-17 POJ2878 R570 TUC01-45 TUC02-38 TUC 02-41 TUC03-32 TUC03-36 TUC04-4 TUC94-58
-	CO281 CP33-224 CP36-105 CP44-155 CP48-103 CP48-126 CP52-1 CP53-16 CP57-614 CP61-37 CP67-411 CP72-370 HO94-850 HOCP95-931 L65-69 LCP82-89 NA56-79 TUC00-15 TUC00-16 TUC00-24 TUC00-26 TUC00-27 TUC00-33 TUC00-36 TUC00-5 TUC00-55 TUC00-68 TUC00-72 TUC00-9 TUC01-17 TUC01-22 TUC01-3 TUC01-38 TUC01-39 TUC01-40 TUC01-41 TUC01-42 TUC01-43 TUC01-44 TUC01-46 TUC01-47 TUC01-48 TUC01-49 TUC01-50 TUC01-51 TUC01-52 TUC01-53 TUC01-54 TUC01-55 TUC01-56 TUC02-27 TUC02-28 TUC02-29 TUC02-30 TUC02-31 TUC02-32 TUC02-33 TUC02-34 TUC02-35 TUC02-36 TUC02-37 TUC02-39 TUC02-40 TUC02-42 TUC02-43 TUC02-44 TUC02-45 TUC02-46 TUC02-47 TUC02-48 TUC02-49 TUC02-50 TUC02-51 TUC02-52 TUC02-53 TUC02-54 TUC02-55 TUC02-56 TUC02-57 TUC02-58 TUC02-59 TUC02-60 TUC02-61 TUC02-62 TUC02-63 TUC02-64 TUC02-65 TUC02-66 TUC02-67 TUC02-68 TUC02-69 TUC02-70 TUC02-71 TUC02-72 TUC03-17 TUC03-18 TUC03-19 TUC03-20 TUC03-21 TUC03-22 TUC03-23 TUC03-24 TUC03-25 TUC03-26 TUC03-27 TUC03-28 TUC03-29 TUC03-30 TUC03-31 TUC03-33 TUC03-34 TUC03-35 TUC03-37 TUC03-38 TUC03-39 TUC03-40 TUC03-41 TUC03-42 TUC03-43 TUC04-1 TUC04-2 TUC04-3 TUC04-5 TUC04-6 TUC04-7 TUC69-2 TUC71-7 TUC78-17 TUC79-9 TUC90-14 TUC92-3 TUC93-104 TUC93-116 TUC93-58 TUC93-89 TUC94-55 TUC94-61 TUC95-02 TUC95-1 TUC95-17 TUC95-26 TUC95-30 TUC95-33 TUC95-39 TUC95-41 TUC95-59 TUC95-65 TUC96-17 TUC96-19 TUC96-21 TUC96-23 TUC96-41 TUC96-43 TUC96-53 TUC96-55 TUC96-59 TUC97-1 TUC97-19 TUC97-21 TUC97-22 TUC97-23 TUC97-24 TUC97-25 TUC97-26 TUC97-27 TUC97-4 TUC97-9 TUC98-19 TUC98-21 TUC98-36 TUC98-38 TUC98-49 TUC98-5 TUC98-54 TUC99-11 TUC99-17 TUC99-3

[a] *Bru*1 presence detected by molecular diagnostic markers R12H16 and 9020-F4-RsaI: (+) indicates the presence of both markers and (-) indicates the absence of both markers.

score of 3 to 4 indicates moderately resistant plants, with very rare pustule lesions on old leaves, but with no sporulating pustules. From grade 5 to 9, plants are considered susceptible as they show an increasing lesion density, lesions on younger leaves, and extensive leaf necrosis.

The LCP 85-384 variety, which is very susceptible to brown rust, was planted on the border of the trial, as well as repeatedly inside the plot in order to maximise infection.

RESULTS

Diagnosis of *Puccinia kuehnii* and *P. melanocephala* using PCR

Good quality nucleic acid was obtained by using samples with a reduced number of pustules and one additional round of phenol treatment in the extraction procedure. All 30 sugarcane leaf samples that exhibited rust symptoms were found to be positive for *P. melanocephala*, generating a 670 base pairs (bp) product with primer set ITS1F/ITS4, a 608 bp product with the NL1/NL4 pair, a 585 bp product with PkPmF/PkPmR, and finally a 480 bp product with Pm1F/Pm1R (Figure 1). No band was observed at 527 bp in any of the samples when using the primer pair specific for *P. kuehnii*.

Comparisons between sequences obtained and sequences deposited in the GenBank database confirmed brown rust presence in the tested samples.

Frequency of brown rust resistance and *Bru*1 markers in the breeding population of EEAOC

Reaction to brown rust for 129 sugarcane parents frequently used in the EEAOC Breeding Program was evaluated in the field under natural infestation conditions. Rust severity was scored on a 0 (most resistant) to 9 (most susceptible) scale, according to Amorin *et al*. (1987). Genotypes were considered as resistant when they got a score of 2 or lower, and as susceptible when their score was equal to or higher than 3. Out of 129 evaluated genotypes, 49 (38%) were found to be resistant to brown rust. Both molecular markers associated with the *Bru*1 gene, R12H16 and 9020-F4-*Rsa*I, were detected in 8 out of the 49 resistant genotypes tested (16.3%). Both markers were present in all positive samples, suggesting a high linkage grade between them, and were absent in all the 80 susceptible accessions. Interestingly, 83.7% of all clones considered to be resistant to rust, as seen in our field trial, did not contain *Bru*1 as inferred from analyses with the two diagnostic markers. This result clearly indicates that resistance to brown rust in the EEAOC Breeding Program depends mostly on one or more alternative resistance sources.

Screening EEAOC sugarcane germplasm for rust resistance gene *Bru*1 with molecular markers

One hundred and ninety-one sugarcane accessions from EEAOC sugarcane germplasm were analysed to study the frequency of appearance of the *Bru*1 gene, through molecular diagnostic markers R12H16 and 9020-F4-*Rsa*I. Only 7% (14/191) of the evaluated genotypes, namely CO290, CO419, CO421, CP52-68, CP53-17, POJ2878, R570, TUC01-45, TUC02-38, TUC02-41, TUC03-32, TUC03-36, TUC04-4 and TUC94-58 were found to contain the *Bru*1 gene allele.

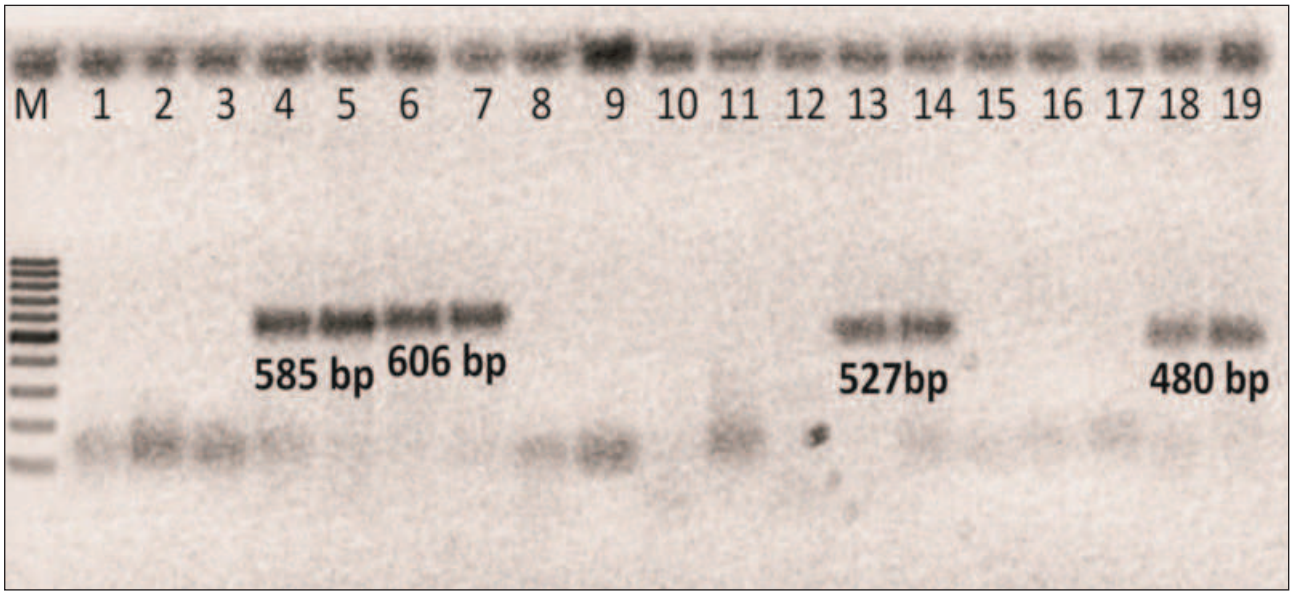

Figure 1. Amplification products with primers PkPmF/PkPmR: lanes 1 to 3, negative controls; lanes 4 and 5, *P. melanocephala* positive controls, and lanes 6 and 7, *P. kuehnii* positive controls. Amplification products with PkPmF/Pk1R primers: lanes 8 to 10, negative controls; lanes 11 and 12, *P. melanocephala* samples, and lanes 13 and 14, *P. kuehnii* positive controls. Amplification products with Pm1F/Pm1R primers: lane 15, negative control; lanes 16 and 17, *P. kuehnii* samples, and lanes 18 and 19, *P. melanocephala* positive controls. M: ladder molecular Cien Marker, Promega (100 to 1000 pb).

CONCLUSIONS

A rapid and robust nucleic acid extraction protocol was optimised for the detection of fungi causing sugarcane rust disease. This enabled a simple and quick detection, especially when assessing a large number of plant samples, since it was not necessary to separate plant tissue from fungus spores.

Protocol optimisation for detecting *P. melanocephala* and *P. kuehnii* by PCR allowed developing a robust and specific detection procedure for both rust agents. It is also important to highlight that *P. kuehnii* was not detected in commercial sugarcane fields in Argentina during this study. Therefore, this protocol for orange rust detection will allow monitoring both crops and imported plant materials continuously, thus helping to prevent the outbreak of this disease in Argentina. Moreover, it is always important to have access to precise diagnostic methods to enable the design of management strategies for potential diseases, whose consequences are still unknown.

The usefulness of the molecular markers associated with the *Bru*1 gene was clearly demonstrated, since all genotypes having these markers were resistant to brown rust under our local conditions. However, it must be pointed out that 83.7% of all the clones considered as resistant tested negative for *Bru*1 presence, which indicates that resistance to brown rust in the EEAOC Breeding Program depends mostly on different resistance sources.

After screening 191 accessions of EEAOC sugarcane germplasm for rust resistance gene *Bru*1, it was found that this gene was only present in 7% of the evaluated clones, which included clones from the last evaluation stages of the EEAOC Breeding Program, as well as CP varieties ancestors (from Canal Point, USA). The program constantly exchanges breeding parents with the US (Canal Point, Louisiana and Houma), due to similar climate and growth conditions. As a consequence, breeding programs from these countries share a narrow gene pool (Perera *et al*., 2012). The low frequency of *Bru*1-positive genotypes found is in agreement with the results obtained by Glynn *et al*. (2012): they found that the frequency of *Bru*1 in parental clones used in Louisiana sugarcane crossing programs was 6%.

The alternative sources of resistance available in our germplasm could be important assets, as they can help widen the dangerously narrow genetic basis for brown rust resistance, which is a common feature of genetic resources in other parts of the world (Costet *et al*., 2012).

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