MLST reveals a separate and novel clonal group for *Acidovorax avenae* strains causing red stripe in sugarcane from Argentina.

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ABSTRACT

Acidovorax species cause a wide range of economically important diseases in monocotyledonous and dicotyledonous plants, including sugarcane, corn, rice, oats, millet, foxtail watermelon and orchids. In Argentina, the red stripe disease of sugarcane caused by *A. avenae* affects 30% of the milling stems with important economic losses. To explore the genetic diversity of this bacterium associated with red stripe in Argentina, MLST was applied. This study included 15 local strains isolated from four different sugarcane planting regions and selected after RAPD analysis and reference strains of *A. citrulli*, *A. avenae*, and *A. oryzae* to investigate their phylogenetic relationships. MLST analysis resulted in five sequence types (STs) among the sugarcane *A. avenae* strains which constitute a clonal complex, meaning a common and close origin. Sugarcane strains were related to *A. avenae* from other hosts and distant to *A. citrulli.* Signals of frequent recombination in several lineages of *A. avenae* was detected and we observed that *A. oryzae* is closely related to *A. avenae* strains. This study provides valuable data in the field of epidemiological and evolutionary investigations of novel clone of *A. avenae* strains causing sugarcane red stripe. The knowledge of the genetic diversity and the specificity strain-host are important to select the genotypes with the best response to the red stripe disease.

Keywords: sugarcane, red stripe, *Acidovorax*, MLST, genetic diversity

Sugarcane is an important commercial crop worldwide, and one of the main sources of sugar and ethanol (FAO 2017). Due to the increasing demand to its use as biofuel, the sugarcane has a great potential for expansion to new cropping areas (de Vries et al. 2010). In Argentina, sugarcane production is geographically distributed in three regions: Tucumán, Northern (Salta and Jujuy) and Littoral (Santa Fe and Misiones), extending in a 365.000 ha approximate area (Wallberg and Minetti 2015). Tucumán is the main sugarcane production province of Argentina, with 68% of total national production (Perez et al. 2007). Sugarcane diseases have caused significant direct and indirect losses to sugar industry (Rott et al. 2013). Pathogenic bacteria such as, *Leifsonia xyli* subsp. *xyli*, *Xanthomonas albilineans* and *Acidovorax avenae* are the etiologic agents of the three most important bacterial sugarcane diseases: ratoon stunting, leaf scald and red stripe, respectively (Rott et al. 2000). Sugarcane red stripe, also known as “polvillo”, affects sugarcane crop practically worldwide. Symptoms appear on the leaves as water-soaked stripes that gradually turn reddish, and may extend to the plant apical meristem which becomes wet, resulting in top rot in severe infections (Rott and Davis 2000). New agricultural techniques implemented in Argentina, such as green-cane harvesting and crop rotation with soybean, resulted in a significant increase of the red stripe disease incidence. Severe symptoms occurrence in commercial varieties of the Northwest production areas was observed in the last 15 years. Causal agent of this infective outbreak in sugarcane was identified for the first time by Fontana et al. (2013) as *Acidovorax avenae*. In addition, the whole genome sequence of a virulent strain, *A. avenae* T10_60 for sugarcane, has been recently announced (Fontana et al. 2016). Currently, ongoing studies are focused in providing information on the molecular mechanisms involved in the pathogenesis of this sugarcane pathogen.

Acidovorax species cause a wide range of economically important diseases in monocotyledonous and dicotyledonous plants (Giordano et al. 2012). According to Willems and Gillis (2015), three subspecies for *A. avenae* were described*: A. avenae* subsp. *cattleyae, A. avenae* subsp. *citrulli* and *A. avenae* subsp. *avenae*. The three subspecies have different host ranges: *A.*

avenae subsp. *citrulli* infects *Cucurbitaceae* family members; *A. avenae* subsp. *cattleyae* infects only *Cattleya* and *Phalaenopsis* species and *A. avenae* subsp. *avenae* infects *Poaceae* family members, including maize, rice, sorghum, corn, oats, barley, rye, various millets, vasey grass and sugarcane (Martin and Wismer 1989; Song et al. 2003; Fontana et al. 2013; Willems and Gillis 2015). However, even now, several authors adopted the reclassification up to species level proposed formerly by Schaad et al. (2008) as *A. avenae*, *A. cattleyae*, *A. citrulli* and *A. oryzae* sp. nov. (for the rice isolates). According to phylogenetic analysis based on 16S rRNA gene sequences, the plant pathogenic *Acidovorax* species cluster together and the non-plant pathogenic strains cluster together as a separate clade (Giordano et al. 2012). The ability to accurately identify and differentiate *Acidovorax* pathogenic strains causing disease is of critical importance for epidemiological surveillance and for designing efficient crop management procedures. The development of molecular typing methods based on nucleic acid fingerprint has contributed to distinguish accurately *Acidovorax* strains; among these RAPD (Random Amplification of Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), RFPL (Restriction Fragment Length Polymorphism) and PFGE (Pulsed-field gel electrophoresis) have been largely applied (Stead, 1995; Walcott et al. 2000; Fontana et al. 2013; Pulawska et al. 2013; Yan et al. 2013, Silva et al. 2016; Li et al. 2017; Dhkal et al. 2018). Moreover, the combination of these methods with techniques based on sequence analysis such as MLST (Multilocus Sequence Typing) introduced valuable information in the field of epidemiological investigation of these bacterial pathogens (Feng et al. 2009; Yan et al. 2013; Silva et al. 2016).

In this study, MLST was applied to explore genetic diversity among *A. avenae* strains from sugarcane associated with red stripe disease and to understand phylogenetic relationships with other *Acidovorax* strains from different hosts and geographical origins.

MATERIALS AND METHODS

Plant material. Leaf samples from sugarcane exhibiting red stripe symptoms were collected from 2008 to 2014 in Tucumán, Salta, Santa Fe and Misiones provinces, representing the main sugarcane production areas from Argentina (Fig.S1). Young plants (50), of less than four months after harvesting, were sampled starting when the initial symptoms were more easily identified. In this study, samples collected from Salta, Santa Fe and Misiones were placed on filter paper into ziplock plastic bags; one portion of these was placed at 4-7ºC, 24 to 48 hours and then used for the isolation of *A. avenae*. Samples remaining were kept at -20ºC for long preservation time. Five sugarcane *A. avenae* strains previously isolated from Tucuman (T10_61; T8_45; T6_50; T4_53)) and Salta (S11_3) were also included in this work. Sample codes, sugarcane genotype, cultivation regions and strains used in this study, are indicated in Table 1**.**

Isolation, identification and typing of *A. avenae* strains. Leaves stored at 4-7ºC were cut into small pieces (approximately 1 cm), disinfected twice with 70 % ethanol (1 min) and rinsed with sterile water (1 min). Leaf material (approximately 0.5 g) was manually macerated with pellet pestle-polypropylene (Sigma, Argentina) in sterile 2 ml tubes using 1 ml of saline solution (0.9 g/l NaCl), the supernatant was used to prepare decimal; 0.1 ml of each dilution was plated on the surface of nutritive agar (NA), prepared using: peptone 5.0 g/l, meat extract 3.0 g/l, NaCl 3.0 g/l and agar 17.0 g/l. After incubation for 48 h at 37 °C, colonies with distinct morphologic characteristics (circular, translucent, white-cream colored colonies with entire margins) were selected, streaked onto YDC agar (yeast extract 10.0 g/l, glucose 20.0 g/l calcium carbonate, 20.0 g/l and agar 15.0 g/l) and incubated for 48 h at 37 °C. Typical *Acidovorax* colonies, circular, translucent, beige colored with entire margins were retained. Taxonomic identification was achieved by species-specific PCR according to Fontana et al. (2013) from a pure culture grown on Lysogeny Broth (Bertani 2004) overnight at 30 ºC in a shaking incubator. For this PCR and other molecular testing, total genomic DNA was extracted and purified according to the CTAB method described by Ausubel et al. (1992). The bacterial DNA was quantified with Qubit® (Invitrogen, Argentina), visualized by electrophoresis through 0.7 % (w/v) agarose gel and stained with Gel Red (Genbiotech, Argentina). RAPD reactions were carried out using primer M13 GAGGGTGGCGGTTCT (Huey and Hall, 1989), according to Fontana et al. (2005) in 50 µl of reaction volume containing 3 mM $MgCl_2$, reaction buffer (1x), deoxynucleoside triphosphate (200 µM each), 1 µM of each primer, 20 ng of DNA and 0.5 U of Taq polymerase (Promega, Italy). PCR products were electrophoresed at 100 V on 2.5 % agarose gel and stained with Gel Red (Genbiotech, Argentina). RAPD profiles were normalized and submitted to Cluster Analysis with BioNumerics software version 5.0 (Applied Maths, Belgium) Dice similarity coefficient was used for similarity matrix calculation and dendrograms were obtained by the un-weighted pair group method with arithmetic averages (UPGMA).

MLST analysis

PCR amplification and sequencing. Fragments of seven housekeeping genes (Table 2), representing a total of 3,247 bp, were used for the MLST analysis as previously described (Feng et al. 2009). PCR amplifications were carried out in a final volume of 25 µl containing 1x Master Mix PCR (Promega, Italy), 0.8-1.0 µM of each primer and 10-20 ng of sample DNA. Reaction conditions included an initial denaturation step at 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 60°C for 30 s for primer annealing, and an extension step at 72°C for 30 s. The final step was an extension period at 72°C for 5 min. Purification of the PCR products was performed with the ExoSap-IT Clean-up system (USB Co., Cleveland). Sequencing with forward and reverse primers was performed in a 3130xl Genetic Analyzer (INTA Castelar, Buenos Aires, Argentina).

MLST data analysis. MLST analysis included sequences downloaded from GenBank from strains of *A. avenae* (9), *A. citrulli* (93) and *A. oryzae* (1) and from 15 strains isolated from sugarcane (Table 2). The analyzed housekeeping gene sequences are available under GenBank accession numbers MF623064 to MF623168 and EU928004 to EU928726 for *Acidovorax* strains isolated from sugarcane in Argentina and other hosts, respectively. Sequences were aligned with MEGA7.0.26 (http://www.megasoftware.net/); allelic profiles for each strain were calculated using MLSTest software (Tomasini et al. 2013). Based on the allelic profile a Sequence Type (ST) was assigned to each strain (McCombie et al. 2006). A BURST analysis (Feil et al. 2004) was performed using MLSTest to identify clonal complexes with a group definition of at least six shared alleles (Tomasini et al. 2013). In addition, to build a Neighbor-joining (NJ) tree, with different node support measures MLSTest was used. Consensus trees summarizing the information of individual fragment trees (based on branch frequency into the NJ tree for each *locus*) were also built. Multidimensional scaling plots from pairwise distance matrices were created. Topological incongruence between *locus* trees and consensus networks were calculated by MLSTest to address recombination into the *Acidovorax* species and the statistical significance was addressed using the Templeton test (Tomasini et al. 2013).

Seedling virulence assays. The virulence of sugarcane *A. avenae* strains representing the five ST determined by MLST analysis on a susceptible sugarcane variety TucCP 77-42 was evaluated (Rago 2005). *A. avenae* strains, T10_61, S11_3. S22_3, SF17_4 and SF18_1 (ST5, ST1, ST4, ST2 and ST3 respectively), were used to inoculate young plants (less than 2 months). *A. avenae* T10_61 (Fontana et al. 2016), was also used as virulent positive control. Inoculum was prepared from a pure bacterial culture grown on Lysogeny Broth on shaking incubator for 48 h at 30°C. Bacterial suspensions, adjusted to around 10^8 CFU/ml, were applied on adaxial and abaxial surfaces by rubbing the leaves manually. Plants used as control were inoculated in an identical way with sterile water. A total of 20 biological replicates (potted plants) were assessed for each treatment and the experiment was carried out once. Plants were placed in 300 ml pots with a mixture of non-pasteurized soil and substrate (INTA, Famaillá-Tucumán) in a ratio of 70/30 and were maintained under high relative humidity (> 90 %) in plastic tunnels at constant temperature (30°C). A completely randomized experimental design was used. Red stripe occurrence on leaves from seedlings was evaluated every day up to 10 days postinoculation (dpi). The severity was evaluated once on day 10 dpi as follows: 0 = no symptom, 1 = localized infection and less than three red stripes per leave; 2 = advanced infection and more than three red stripes per leave; 3 = severe infection with red stripe that reaches the apical bud; 4 = apical top rot and/or death of the apical top. This scale was developed by Fontana (2010) based on a similar scale described by Rott et al. (1994) with minor modifications and adapted to the red stripe disease characteristics. Data was used to calculate the mean of severity for each plant. One-way analysis of variance (ANOVA) was performed for severity data analysis using the InfoStat software (Di Renzo et al. 2018). Leaves showing red stripe were subjected to microbiological and molecular analysis as described above, to confirm that red stripe symptoms were caused by the inoculated *A. avenae* strains (data not shown).

RESULTS

Identification and differentiation of *A. avenae* isolates. One hundred colonies exhibiting the typical morphology of *Acidovorax* on NA (circular, translucent, white-cream colored and entire margin) were isolated. After a first characterization by microscopy examination and Gram staining, only Gram negative, typical colonies with single or two- or three-rods chains morphology were selected for molecular assays. The species-specific PCR (s-sPCR) reaction from all white creamy colonies showed that approximately 50% of the isolates exhibited a positive signal for a specific product of 550 bp size. This result indicated the presence on the plates of other bacterial groups with morphology and color like *A. avenae* colonies. The isolates identified as *A. avenae* by means of s-sPCR were analyzed by RAPD to investigate their genetic relatedness. Figure 1 shows the dendrogram drawn by the cluster analysis performed based on RAPD profiles of 31 strains. At a similarity level of ~75% three main clusters were observed: Cluster I include 5 strains isolated from Santa Fe (sugarcane genotype INTA 04-1604 and INTA CP 98-828) and 4 from Salta (sugarcane genotype NA 02-2320) provinces; the *A. avenae* strains isolated from an "unknown" sugarcane variety cultivated in Misiones were only allocated in Cluster II together with 5 strains from Santa Fe isolated also from an "unknown" genotype of sugarcane, while Cluster III contains the only one strain from Tucumán (sugarcane genotype INTA NA 89-686), one strain from Salta and one from Santa Fe isolated from the sugarcane genotype NA 85-1602 and the rest of ten Santa Fe strains that were obtained from NA 85-1602 and INTA 04-1604. Since the number of genotypes of sugarcane sampled in the province of Santa Fe was higher compared to the other provinces (Table 1), the number of strains isolated was also higher being these strains placed in the three clusters according to the sugarcane genotype from which they were isolated. Regarding to the year of sampling, Cluster I and II contained only isolates obtained in 2014 while cluster III grouped strains in 2008, 2013 and 2014 years of sampling. *A. avenae* strains (3, 4 and 5 strains from cluster 1, 2 and 3 respectively), isolated from different sugarcane genotypes from different production regions were subjected to MLST analysis. The *A. avenae* strains T4_53; T6_50; T8_45, T10_61 and S11_3 isolated in previous work were also included in the MLST (Table 2).

Sugarcane strains have a recent clonal origin. MLST allelic profiles are reported in Table 2. Five Sequence Types (STs), not previously described, were defined among the fifteen *A. avenae* strains from sugarcane analyzed in this study; most of them were typed as ST1 or ST2 (each ST composed by six strains), whereas ST3, ST4 and ST5 were singletons. As indicated by the allelic profiles analysis, the greatest variability for *A. avenae* sugarcane strains corresponded to *lepA* gene (Table 2). The BURST algorithm clustered such sequences in a single clonal complex meaning a common and close origin for all of them (Fig. S2). In addition, NJ-tree was made to analyze the relationships with other *A. avenae* strains. Sugarcane strains were clustered together and separated of other strains with a high bootstrap value and four loci supporting the split, suggesting a possible host-specificity (Fig. 2). Topological incongruence between trees for each *locus* was not detected in these strains supporting the clonal behavior (Fig. S3). A Fisher exact test showed that no significant association was found between the strains analyzed and their geographic origin.

Genetic exchange in *A. avenae.* Sugarcane strains and *A. citrulli* conformed different clonal complexes, while other strains were not clustered together by a BURST analysis (i.e. singletons). In addition, the NJ analysis showed that such singletons were clustered in branches with low support (Fig. 2) and with high and statistically significant topological incongruence (Fig S3). These results indicate frequent recombination among strains (Tomasini et al. 2014). Additional information about the recombination for *A. avenae* strains was obtained by building a consensus network (Fig. 4). The network shows several square patterns indicating recombination. From the NJ-tree (Fig. 2), incongruence tests (Fig. S3) and the multidimensional scaling plot (Fig. 3), it was possible to observe that of *A. oryzae* grouped with the *A. avenae* from rice.

Seedling virulence assays. Sugarcane strains T10_61, S11_3. S22_3, SF17_4 and SF18_1 successfully reproduced the red stripe symptoms on sugarcane leaves. Significant differences in the severity of symptoms were observed among strains from different STs (F=520.82; P <0.0001). As shown in Table 3 strains S22_3 and S11_3 were more virulent (mean severity ratings of 3.65 and 3.11, respectively) than strains SF17_4 and SF18_1 (mean severity ratings of 2.20 and 2.30, respectively). The strains S22_3 and S11_3 developed lesions on leaves considered as severe and generalized striations, affecting apical bud in some cases. Strains SF17_4 and SF18_1 exhibited an intermediate virulence developing typical red stripe lesions on leaves. The positive control, *A. avenae* T10_61 showed a lower level of symptom severity compared with the rest of the strains (mean severity ratings of 1.60). In all cases, first symptoms were observed after 48 hours of inoculation, but the severity was more evident for *A. avenae* S22_3 and S11_3 strains. Seedling death by apical bud rot (top rot) due to infection was not observed up to 10 dpi. *A. avenae* was successfully re-isolated from sugarcane leaves inoculated.

DISCUSSION

Red stripe of sugarcane is a bacterial disease distributed among most sugarcane producing areas in the world. In Argentina, for the last 15 years red stripe has become the most serious plant disease causing industrial losses of 30% due to the occurrence of severe infections on susceptible sugarcane genotypes. Fontana et al. (2013), reported for the first time the isolation and identification of *A. avenae* as the causal agent of red stripe affecting sugarcane in Argentina. The main strategy adopted currently to manage this disease, after repeated infection cycles, is the replacement of the susceptible sugarcane variety by a resistant one. Due to this, the knowledge on the genetic diversity among *A. avenae* is an important factor to be considered for improving an accurate diagnosis and/or for the selection of sugarcane tolerant varieties. To investigate their genetic similarity, *A. avenae* isolated from different sugarcane varieties infected with red stripe in 2008, 2013 and 2014, in four provinces of Northern Argentina, were analyzed by RAPD. The clusters analysis grouped 31 strains (29 isolated in this study and two previously isolated by Fontana et al 2013) in three main clusters. No association was observed with years of sampling and geographical origin of the strains. Based on RAPD profiles, intra species diversity among *A. avenae* strains isolated from sugarcane commercial varieties was observed. In accordance with Fontana et al. (2013), the presence of *A. avenae* strains adapted to sugarcane genotypes was detected. Fontana et al (2013) analyzed by RAPD *A. avenae* strains from Tucumán and Salta (Northwest region), being these strains grouped in two main cluster by their geographical origin. Northwest region is the bigger sugarcane producers, containing the 98% of total ha of cultivation from Argentina (Benedetti 2018). Due to the increasing demand to use sugarcane as biofuel, the Northeast region (Santa Fe and Misiones), is an expanding production area with great potential (Wallberg and Minetti 2015), having small growers that cultivate often different sugarcane varieties, as a way to select the best adapted, representing a source different and more diverse of strain. In the present work, not a clear geographical association was observed, maybe due to the greater and different area of sampling.

A. avenae strains representative from different sugarcane genotypes covering all the sampling production areas were selected to explore their genetic diversity applying a MLST scheme already described by Feng et al. (2009). MLST databases for other *Acidovorax* strains from different hosts and geographical origins was also included to understand the phylogenetic relationships. The MLST analysis showed that strains from sugarcane clustered together and they have a relatively recent origin and clonal behavior suggesting host specificity. Such host specificity in different clades of *A. avenae* was also observed for other groups (Yan et al. 2017). It was already demonstrated that there is a strong association of *A. avenae* more with the host than with the geographical origin (Feng et al. 2009; Yan et al. 2013), In this study, *A. avenae* strains from sugarcane, were clustered separately from *A. citrulli* from watermelon and melon strains, and closer to *A. avenae* from *Poaceae* origin (millet, rice, corn, vasey grass and sorghum).

Since, we applied a MLST scheme design by Feng et al. (2009), in accordance with their finding, the presence of two clonal complexes grouping the *A. citrulli* was observed with a clear separation from the other *A. avenae* strains and *Acidovorax* spp. Similarly, MLST analysis of 118 strains of *A. citrulli* from Chinese watermelon resulted in 73 STs that were typed into three clonal groups (Yan et al. 2013). Even if, new taxon for the *A. avenae* from rice: *A. oryzae*, was proposed by Schaad et al. (2008), we observed that *A. oryzae* is closely related to other *A. avenae* strains from rice. We also detected phylogenetic incongruence in *A. avenae* suggesting frequent recombination in some clades. Recombination between different lineages has been described for virulence genes in some *A. avenae* that share the same host (Zeng et al. 2017). This is relevant because new highly virulent strains may originate in such clade, where recombination is frequent (Feil et al. 1999).

Recombination in other plant pathogens was also reported. Timilsina et al. (2015) found evidence of multiple recombination events between *Xanthomonas euvesicatoria* and *X. perforans,* which indicate that there have been shifts in the species composition of bacterial spot pathogen populations due to the global spread of dominant genotypes and that recombination between species has generated genetic diversity in these populations.

It is important to highlight that despite their close relationships by MLST, the sugarcane strains showed virulence differences when virulence assays were performed. However, this is not contradictory because virulence factors are codified by genes that mutate faster than housekeeping genes (Moxon et al*.* 1994). Consequently, there is much more relevant genetic diversity that is hidden to the resolution power of MLST.

In this study, based on allelic profile analysis of seven housekeeping genes, five ST were defined among the fifteen sugarcane *A. avenae* strains analyzed; most of them were typed as ST1 (containing strains from Misiones, Tucumán, Salta and Santa Fe) and the ST2 and its derivatives (ST3, ST4 and ST5) that are in Santa Fe, Tucumán and Salta (Fig S2). It could be inferred that the dominant ST are ST1 and ST2, however, for more conclusive information about more predominant ST in Argentina, more isolates are necessary to be analyzed.

The most virulent *A. avenae* strains on sugarcane genotype TucCP 77-42 were the strains S22_3 (ST4) and S11_3 (ST1) from Salta while strains SF17_4 (ST2) and SF18_1 (ST3) from Santa Fe exhibited an intermediate virulence being just the T10_61 strain (ST5) of Tucumán, the less virulent. Similar results were reported by Fontana et al. (2013) when investigated *A. avenae* cross pathogenicity, observing that red stripe symptoms developed earlier in Tucumán sugarcane variety (TucCP 77-42) inoculated with a pathogenic strain from another province. Recently, Silva et al. (2016) reported high variability in disease severity when selected *A. citrulli* strains representing the most abundant PFGE-determined haplotypes observed in Brazil were used to infect watermelon seedlings.

Molecular typing methods are powerful tools to differentiate between genetically near related organisms with acceptable reproducibility, good performance and easy interpretation. The MLST data reported in this study provide invaluable platform for epidemiological and evolutionary investigations of novel clone of *A. avenae* strains. The knowledge of genetic diversity and specificity strain-host has great value at the time of select the genotypes with the best response to the red stripe disease.

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FIGURE LEGENDS

Fig. 1. Dendrogram obtained from RAPD-PCR patterns of sugarcane *A. avenae* strains generated with M13 primer and analyzed by *BioNumerics* software. Similarity matrix was calculated using Dice coefficient and the dendrogram was constructed by UPGMA analysis. Letters on the strain code represent the sugarcane producing province as following: Salta (S), Santa Fe (SF), Misiones (M) and Tucumán (T). For example, T10_61 represents the strain number 61 isolated from the sample numbers 10 (INTA NA 89-686 sugarcane genotype) from Tucuman, while SF20_1 represents the strain number 1 isolated from the sample numbers 20 (INTA CP 98-828 sugarcane genotype) from Santa Fe.

Fig. 2. Neighbor-joining (NJ) tree for analyzed sugarcane strains and other *Acidovorax* strains. The tree was build based on nucleotide p-distance of seven concatenated loci. Support values (based on 1000 bootrstrap replications) are shown at each branch.

Fig. 3. Multidimensional Scaling of *Acidovorax* strains based on the concatenated sequences. The two axes represent more than 90% of the variability into the data.

Fig. 4. Consensus network of seven loci showing possible genetic Exchange. Each split in the network is shown if at least two trees had such split. Network regions with square patterns indicates probable recombination. Sugarcane *A. avenae* strains are encircled on the right side.

Fig. S1. Sugarcane production areas from Argentina.

Fig. S2. BURST analysis of sugarcane *A. avenae* strains showing clonal complexes.

Fig. S3. Topological incongruence against the tree based on concatenated loci. Numbers above branches indicates the number of individual locus trees that are incompatible with such branch. Colored branches indicate that topological incongruence is statistically significant with $p < 0.01$ (orange) and $p < 0.001$ (red) according to Templeton test.

Fig. S4. Severity differences of red stripe symptoms on sugarcane cultivar TucCP 77-42 used for the virulence assays. According to 0–4 rating scale: a) 0= no symptoms, b) 1 = localized infection and less than three red stripes per leaves; c) 2 = advanced infection and more than three red stripe per leaves; d) 3 = severe infection with red stripe that reaches the apical bud; e) 4 = apical top rot and/or death of the apical top.

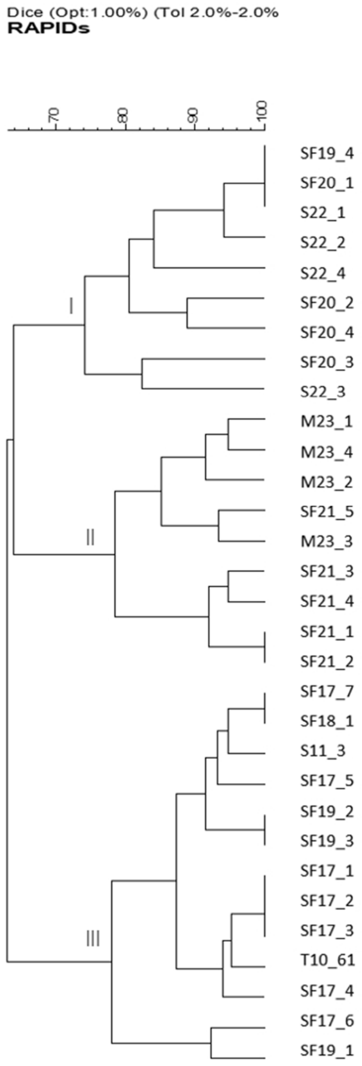

Fig. 1. Dendrogram obtained from RAPD-PCR patterns of sugarcane A. avenae strains generated with M13 primer and analyzed by BioNumerics software. Similarity matrix was calculated using Dice coefficient and the dendrogram was constructed by UPGMA analysis. Letters on the strain code represent the sugarcane producing province as following: Salta (S), Santa Fe (SF), Misiones (M) and Tucumán (T). For example, T10_61 represents the strain number 61 isolated from the sample numbers 10 (INTA NA 89-686 sugarcane genotype) from Tucuman, while SF20_1 represents the strain number 1 isolated from the sample numbers 20 (INTA CP 98-828 sugarcane genotype) from Santa Fe.

140x185mm (150 x 150 DPI)

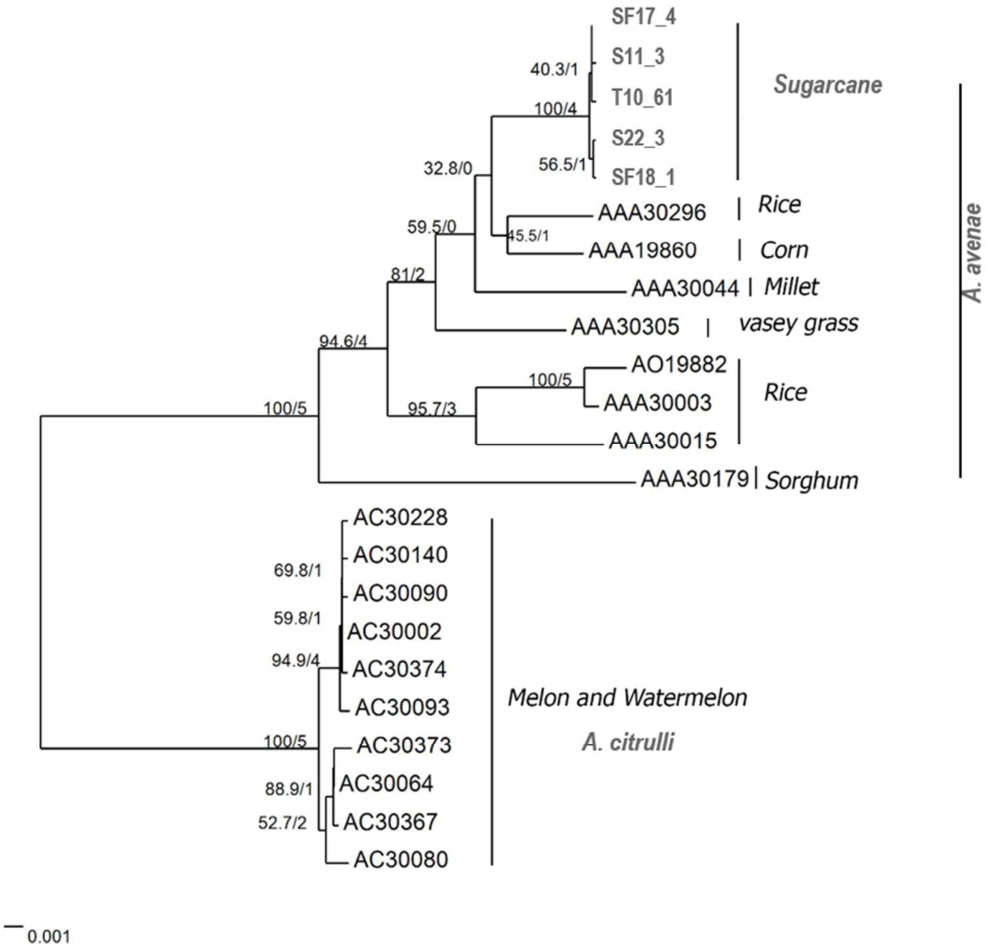

Fig. 2. Neighbor-joining (NJ) tree for analyzed sugarcane strains and other Acidovorax strains. The tree was build based on nucleotide p-distance of seven concatenated loci. Support values (based on 1000 bootrstrap replications) are shown at each branch.

141x142mm (150 x 150 DPI)

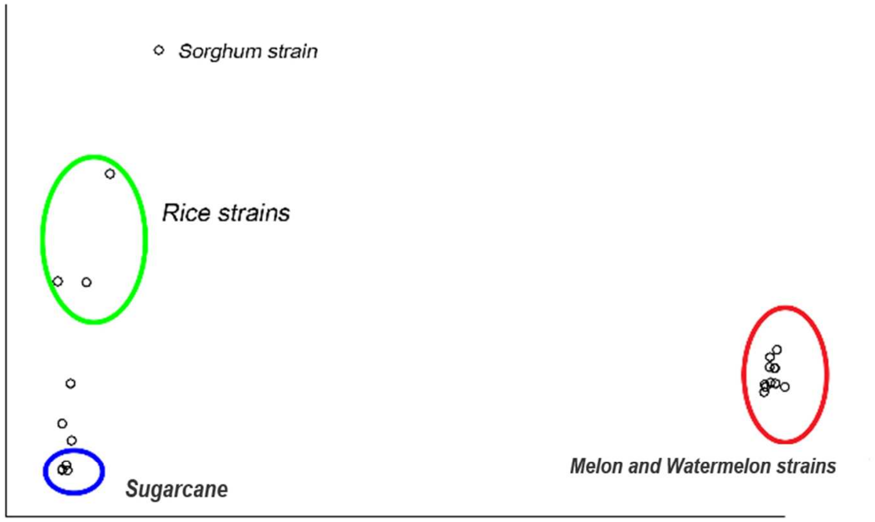

Fig. 3. Multidimensional Scaling of Acidovorax strains based on the concatenated sequences. The two axes represent more than 90% of the variability into the data.

172x106mm (150 x 150 DPI)

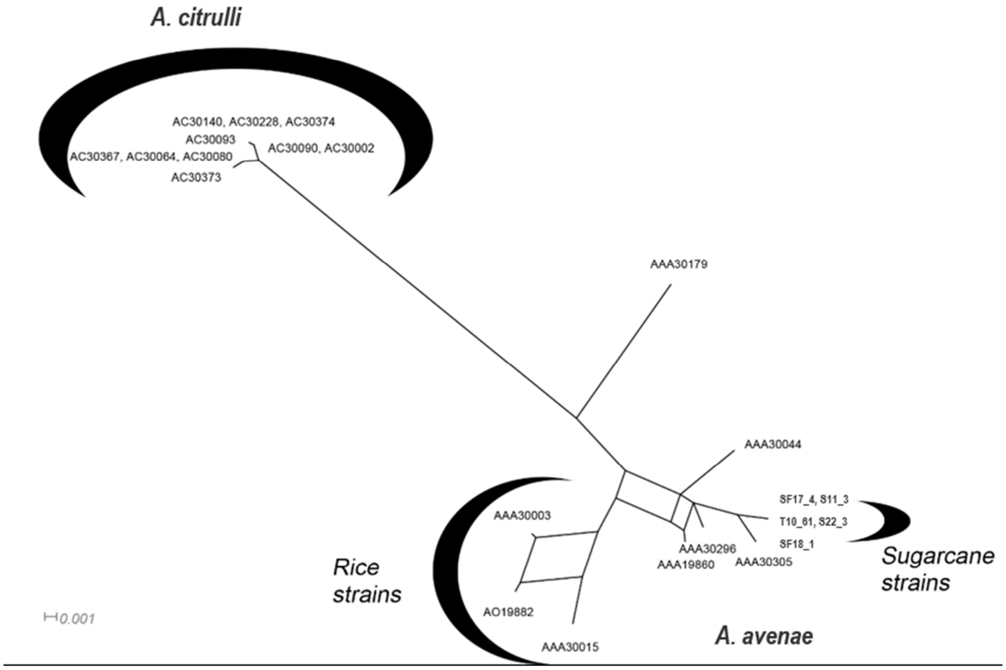

Fig. 4. Consensus network of seven loci showing possible genetic Exchange. Each split in the network is shown if at least two trees had such split. Network regions with square patterns indicates probable recombination. Sugarcane A. avenae strains are encircled on the right side.

144x101mm (150 x 150 DPI)

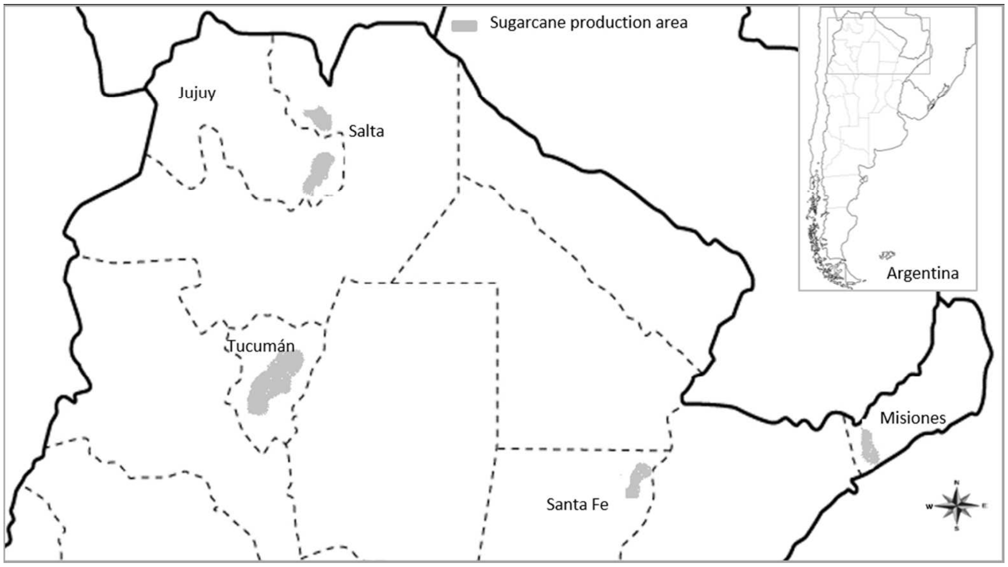

Fig. S1. Sugarcane production areas from Argentina.

175x98mm (150 x 150 DPI)

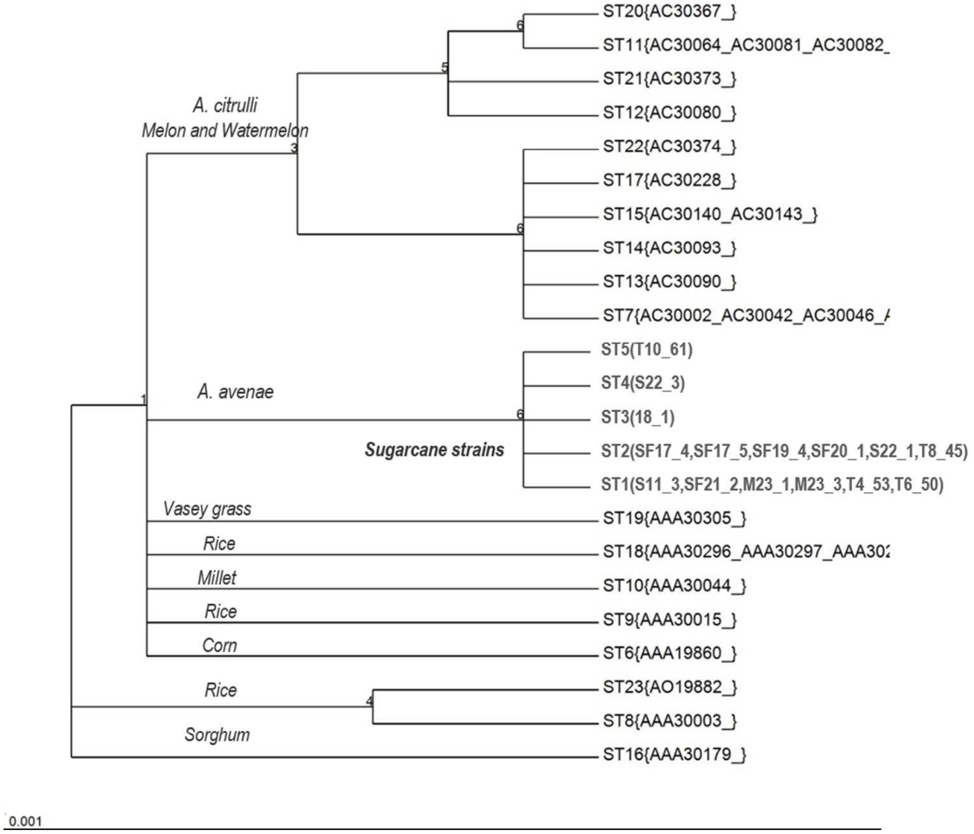

Fig. S2. BURST analysis of sugarcane A. avenae strains showing clonal complexes.

178x155mm (150 x 150 DPI)

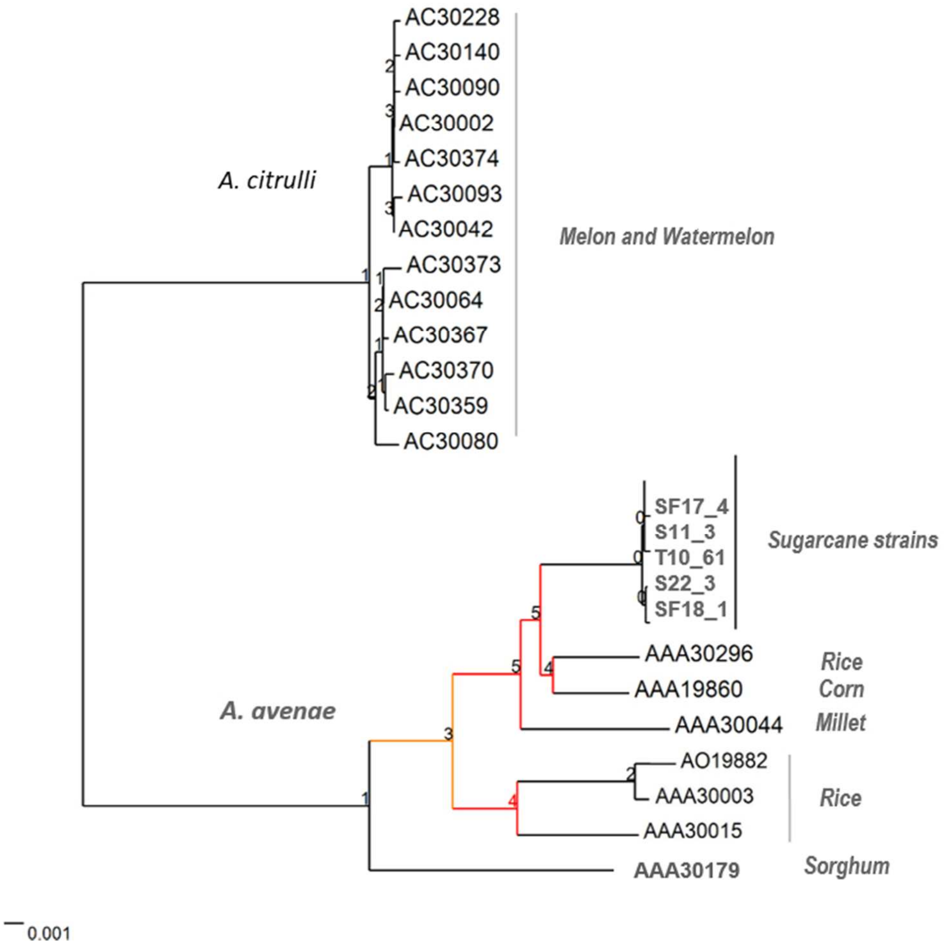

Fig. S3. Topological incongruence against the tree based on concatenated loci. Numbers above branches indicates the number of individual locus trees that are incompatible with such branch. Colored branches indicate that topological incongruence is statistically significant with $p < 0.01$ (orange) and $p < 0.001$ (red) according to Templeton test.

132x133mm (150 x 150 DPI)

Fig. S4. Severity differences of red stripe symptoms on sugarcane cultivar TucCP 77-42 used for the virulence assays. According to 0–4 rating scale: a) 0= no symptoms, b) 1 = localized infection and less than three red stripes per leaves; c) 2 = advanced infection and more than three red stripe per leaves; d) 3 = severe infection with red stripe that reaches the apical bud; e) 4 = apical top rot and/or death of the apical top.

161x135mm (150 x 150 DPI)

Table 1. Samples description and strains used in this study.

Samples ID	Sugarcane genotypes	Strains	Cultivation region	Province	Date
4	INTA NA 89-686	T4_53	La Trinidad-south	Tucumán	2008
6	INTA NA 91-209	T6_50	Cruz Alta-central	Tucumán	2008
8	TucCP 77-42	T8_45	Las Piedritas-central	Tucumán	2008
10	INTA NA 89-686	T10_61	Famaillá-central	Tucumán	2008
11	NA 85-1602	S11_3	Colonia Santa Rosa	Salta	2008
17	NA 85-1602	SF17_1; SF17_2; SF17_3; SF17_4; SF17_5; SF17_6 SF17_7	Tacuarendí	Santa Fe	2013
18	NA 85-1602	SF18_1	Tacuarendí	Santa Fe	2014
19	INTA 04-1604	SF19_1_SF19_2 SF19_3; SF19_4	Tacuarendí	Santa Fe	2014
20	INTA CP 98-828	SF20_1; SF20_2 SF20_3; SF20_4	Villa Ocampo	Santa Fe	2014
21	unknown	SF21_1; SF21_2 SF21_3; SF21_4 SF21_5	Las Toscas	Santa Fe	2014
22	NA 02-2320	S22_1; S22_2 S22_3:S22_4	Tabacal	Salta	2014
23	unknown	M23_1; M23_2; M23_3; M23_4	San Javier	Misiones	2014

Table 2. Allelic profiles and sequence types (ST) obtained by MLST analysis in this study.

ST	**Strains***	***glt*A**	***gmc***	*lep***A**	***pha*C**	***pil*T**	***trp*B**	***ugp*B**	**Host**	**Geographic origin**	**Reference**
1	S11-3	1	1	1	1	1	1	1	Sugarcane	Argentina	Fontana et al. 2013
1	SF21-2	1	1	1	1	1	1	1	Sugarcane	Argentina	In this study
1	M23-1	1	1	1	1	1	1	1	Sugarcane	Argentina	In this study
1	M23-4	1	1	1	1	1	1	1	Sugarcane	Argentina	In this study
1	T4-53	1	1	1	1	1	1	1	Sugarcane	Argentina	Fontana et al. 2013
1	T6-50	1	1	1	1	1	1	1	Sugarcane	Argentina	Fontana et al. 2013
2	SF17-4	1	1	1	1	2	1	1	Sugarcane	Argentina	In this study
2	SF17-5	1	1	1	1	2	1	1	Sugarcane	Argentina	In this study
2	SF19-4	1	1	1	1	2	1	1	Sugarcane	Argentina	In this study
2	SF20-1	1	1	1	1	2	1	1	Sugarcane	Argentina	In this study
2	S22-1	1	1	1	1	2	1	1	Sugarcane	Argentina	In this study
2	T8-45	1	1	1	1	2	1	1	Sugarcane	Argentina	Fontana et al. 2013
3	SF18-1	1	1	2	1	2	1	1	Sugarcane	Argentina	In this study
4	S22-3	1	1	3	1	2	1	1	Sugarcane	Argentina	In this study
5	T10-61	1	1	4	1	2	1	1	Sugarcane	Argentina	Fontana et al. 2013, 2016
6	AAA19860	2	2	5	1	3	2	2	Maize	USA	Lucas et al. 2011
7	AC30002	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30042	3	3	6	2	4	3	3	Watermelon	Japan	Feng et al. 2009
7	AC30046	3	3	6	2	4	3	3	Watermelon	Nigeria	Feng et al. 2009
7	AC30073	3	3	6	2	4	3	3	Melon	Korea	Feng et al. 2009
7	AC30084	3	3	6	2	4	3	3	Watermelon	Nigeria	Feng et al. 2009
7	AC30087	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30091	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30092	3	3	6	2	4	3	3	Watermelon	Brazil	Feng et al. 2009
7	AC30107	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30119	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30120	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30121	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30137	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30139	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30142	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30144	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30146	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30147	3	3	6	2	4	3	3	Watermelon	China	Feng et al. 2009
7	AC30248	3	3	6	2	4	3	3	unknown	China	Feng et al. 2009
7	AC30249	3	3	6	2	4	3	3	unknown	China	Feng et al. 2009
7	AC30287	3	3	6	2	4	3	3	Melon	China	Feng et al. 2009
7	AC30288	3	3	6	2	4	3	3	Watermelon	Japan	Feng et al. 2009
7	AC30290	3	3	6	2	4	3	3	Melon	China	Feng et al. 2009
7	AC30293	3	3	6	2	4	3	3	Watermelon	Malaysia	Feng et al. 2009
7	AC30294	3	3	6	2	4	3	3	Watermelon	Malaysia	Feng et al. 2009
7	AC30353	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30354	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30355	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30356	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30358	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30372	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30375	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30376	3	3	6	2	4	3	3	Melon	USA	Feng et al. 2009
7	AC30377	3	3	6	2	4	3	3	Watermelon	USA	Feng et al. 2009
7	AC30381	3	3	6	2	4	3	3	Watermelon	Australia	Feng et al. 2009
7	AC_W1	3	3	6	2	4	3	3	Watermelon	Australia	Feng et al. 2009

7	AC_W2	3	3	6	2	4	3	3	Watermelon	Brazil	Feng et al. 2009
7	AC_W4	3	3	6	2	4	3	3	Watermelon	China	Feng et al. 2009
7	AC_W6	3	3	6	2	4	3	3	Watermelon	China	Feng et al. 2009
8	AAA30003	4	4	7	3	5	4	4	Rice	China	Feng et al. 2009
9	AAA30015	5	5	8	4	3	5	5	Rice	China	Feng et al. 2009
10	AAA30044	6	6	9	5	6	6	2	Millet	China	Feng et al. 2009
11	AC30064	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30081	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30082	7	7	6	2	7	3	6	Melon	China	Feng et al. 2009
11	AC30118	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30123	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30145	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30148	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30150	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30151	7	7	6	2	7	3	6	Rockmelon	China	Feng et al. 2009
11	AC30152	7	7	6	2	7	3	6	Rockmelon	China	Feng et al. 2009
11	AC30224	7	7	6	2	7	3	6	Melon	China	Feng et al. 2009
11	AC30226	7	7	6	2	7	3	6	Melon	China	Feng et al. 2009
11	AC30229	7	7	6	2	7	3	6	Melon	China	Feng et al. 2009
11	AC30231	7	7	6	2	7	3	6	Melon	USA	Feng et al. 2009
11	AC30235	7	7	6	2	7	3	6	Melon	USA	Feng et al. 2009
11	AC30237	7	7	6	2	7	3	6	Melon	USA	Feng et al. 2009
11	AC30238	7	7	6	2	7	3	6	Melon	China	Feng et al. 2009
11	AC30240	7	7	6	2	7	3	6	Melon	China	Feng et al. 2009
11	AC30243	7	7	6	2	7	3	6	Melon	Japan	Feng et al. 2009
11	AC30250	7	7	6	2	7	3	6	Melon	Japan	Feng et al. 2009
11	AC30251	7	7	6	2	7	3	6	Melon	Japan	Feng et al. 2009
11	AC30254	7	7	6	2	7	3	6	Melon	USA	Feng et al. 2009
11	AC30289	7	7	6	2	7	3	6	Melon	China	Feng et al. 2009
11	AC30291	7	7	6	2	7	3	6	Melon	China	Feng et al. 2009
11	AC30292	7	7	6	2	7	3	6	Melon	China	Feng et al. 2009
11	AC30357	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30359	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30360	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30361	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30362	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30363	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30364	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30365	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30366	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30370	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30371	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30378	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30379	7	7	6	2	7	3	6	Melon	China	Feng et al. 2009
11	AC30380	7	7	6	2	7	3	6	unknown	China	Feng et al. 2009
11	AC30382	7	7	6	2	7	3	6	Melon	China	Feng et al. 2009
11	AC30383	7	7	6	2	7	3	6	Watermelon	China	Feng et al. 2009
11	AC30384	7	7	6	2	7	3	6	Watermelon	USA	Feng et al. 2009
11	AC30385	7	7	6	2	7	3	6	Watermelon	USA	Feng et al. 2009
11	AC_M1	7	7	6	2	7	3	6	Melon	China	Feng et al. 2009
11	AC_M6	7	7	6	2	7	3	6	Melon	Turkey	Feng et al. 2009
12	AC30080	2	7	6	2	8	3	6	Watermelon	China	Feng et al. 2009
13	AC30090	8	3	6	2	4	3	3	Watermelon	China	Feng et al. 2009
14	AC30093	9	3	6	2	4	3	3	Watermelon	China	Feng et al. 2009
15	AC30140	3	3	6	6	4	3	3	Watermelon	China	Feng et al. 2009
15	AC30143	3	3	6	6	4	3	3	Watermelon	USA	Feng et al. 2009
16	AAA30179	10	8	10	7	9	7	7	Sorghum	USA	Feng et al. 2009
17	AC30228	3	3	6	8	4	3	3	Melon	USA	Feng et al. 2009

18	AAA30296	11	9	11	9	10	8	2	Rice	USA	Feng et al. 2009
18	AAA30297	11	9	11	9	10	8	2	Rice	Israel	Feng et al. 2009
18	AAA30298	11	9	11	9	10	8	2	Rice	Israel	Feng et al. 2009
19	AAA30305	12	10	12	10	11	3	8	Vasey grass	Israel	Feng et al. 2009
20	AC30367	13	7	6	2	7	3	6	Melon	Israel	Feng et al. 2009
21	AC30373	14	7	6	11	7	3	6	Melon	Israel	Feng et al. 2009
22	AC30374	15	3	6	2	4	3	3	Watermelon	Israel	Feng et al. 2009
23	AO19882	16	11	13	3	5	4	4	Rice	USA	Kyrpides et al. 2014

Note: *Letters on the strains names represent the sugarcane producing province as following: Salta (S), Santa Fe (SF), Misiones (M) and Tucumán (T). For example, *A. avenae* T10_61 represents the strain number 61 isolated from the sample numbers 10 (INTA NA 89-686 sugarcane genotype) from Tucuman.
AAA: *A. avenae* from other hosts (9 strains), AC: *A. citrulli* (93 strains) and AO: *A. oryzae* (1 strain)

Table 3. Mean severity and standard error (SE) values for each strains are reported. The values followed by different letters are significantly different according LSD Fisher test ($P < 0.05$).

Strains	Means Severity ± SE
Control	0.00 ± 0.05a
T10_61	1.61 ± 0.05b
SF17_4	2.20 ± 0.05c
SF18_1	2.30 ± 0.05c
S11_3	3.11 ± 0.05d
S22_3	3.56 ± 0.05e