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LEVELS OF REGIONAL PHENOTYPIC ADAPTATION (Q_{ST}) INDICATE THAT NEUTRALITY HAS SHAPED THE POPULATION STRUCTURE OF THE SOYBEAN-INFECTING PATHOGEN *Rhizoctonia solani* AG-1 IA[1]

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ABSTRACT - Populations of the soybean leaf blight pathogen (*Rhizoctonia solani* AG-1 IA) are highly genetically differentiated along a latitudinal gradient in the major soybean growing regions of Brazil. However, the evolutionary processes leading to regional adaptation are still unknown. The objective of this study was to evaluate the relative importance of neutral genetic variation and natural selection on the divergence and regional adaptation of populations of the soybean-infecting pathogen *R. solani* AG-1 IA. Therefore, we compared the phenotypic differentiation in quantitative traits (Q_{ST}) and the neutral genetic differentiation (F_{ST}, based on microsatellites data) among three pairs of populations. As measures of phenotypic responses of the fungus (quantitative traits), we estimated the tolerance to temperature stress and the tolerance to a broad-spectrum fungicide (copper oxychloride) under optimal (25 °C) and high temperature conditions (33.5 °C). In general there was an increase in genetic variance with a positive effect on the heritability for tolerance to copper fungicide under temperature stress. The genetic differences among populations were the main determinants of thermal adaptation in *R. solani* AG-1 IA ($h2 \geq 0.70$). The analysis of neutral genetic structure (F_{ST}) indicated subdivision between the three pairs of populations. Although population pairwise comparisons between F_{ST} and Q_{ST} values did not follow a single pattern, the majority of Q_{ST} values did not differ significantly from F_{ST}, indicating that, for the quantitative characters studied, neutrality (or neutral evolution) had a major role in the regional adaptation of *R. solani* AG-1 IA populations.

Keywords: Q_{ST}. F_{ST}. Directional selection. Neutrality. Stabilizing selection.

NÍVEIS DE ADAPTAÇÃO FENOTÍPICA REGIONAL (Q_{ST}) INDICAM QUE ANEUTRALIDADE MOLDOU A ESTRUTURA DE POPULAÇÕES DE *Rhizoctonia solani* AG-1 IA DA SOJA

RESUMO - Populações do patógeno da mela da soja (*Rhizoctonia solani* AG-1 IA) são altamente diferenciadas geneticamente ao longo de um gradiente latitudinal nas mais importantes regiões de cultivo de soja do Brasil. Entretanto, os processos evolutivos que guiaram a adaptação regional são ainda desconhecidos. O objetivo deste trabalho foi avaliar a importância relativa da variação genética neutra e da seleção natural sobre a divergência e adaptação regional de populações de *R solani* AG-1 IA da soja. Para tanto, comparou-se a diferenciação fenotípica por caracteres quantitativos (*Q*ST) e a diferenciação genética neutra (baseada em dados de microssatélites) entre três pares de populações (*F*ST). Como medidas de respostas fenotípicas do fungo (caracteres quantitativos), estimou-se a tolerância ao estresse de temperatura e a tolerância a um fungicida cúprico de amplo espectro de ação (oxicloreto de cobre) sob condições ótimas (25 °C) e de temperatura elevada (33,5 °C). No geral houve aumento da variância genética com um efeito positivo sob a herdabilidade para tolerância ao fungicida cúprico na temperatura de estresse. As diferenças genéticas entre populações foram os principais determinantes da adaptação térmica em *R. solani* AG-1 IA ($h2 \geq 0.70$). A análise da estrutura genética neutra (*FST*) indicou subdivisão entre os três pares de populações. Embora a comparação de *FST* com os dados *QST* entre pares de populações não tenha seguido um único padrão, a maioria dos valores de *QST* estimados não diferiram significativamente de *FST*, indicando que, para os caracteres quantitativos estudados, a neutralidade (ou evolução neutra) teve importante papel na adaptação regional das populações do patógeno.

Palavras-chave: *QST*. *FST*. Seleção direcional. Neutralidade. Seleção estabilizadora.

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INTRODUCTION

Genetic structure is the amount and distribution of genetic diversity or variation within and between populations and can provide the basis for inferences about the life history and evolutionary processes that shaped the structure of these populations in agroecosystems (MCDONALD; LINDE, 2002; TYAGI et al., 2014; IQBAL; RAHMAN, 2017).

For the characterization of genetic diversity, phenotypic markers were used for a long time (TOPPA; JADOSKI, 2013). In recent decades, empirical studies related to the genetic structure of fungal populations have focused on the use of discrete genetic molecular markers (BUSH; MOORE, 2012). The association between these two categories of markers allows for inferences about the effect of natural selection on the divergence between populations (ARRAOUADI et al., 2009; WHITLOCK; GUILLAUME, 2009; PRICE et al., 2010; EDELAAR; BJÖRKLUND, 2011).

As a study model to measure the effect of natural selection on the divergence between populations of a plant pathogen, we used the basidiomycete fungus *Rhizoctonia solani* (sexual stage = *Thanatephorus cucumeris*) anastomosis group (AG) 1 IA, the causal agent of soybean leaf blight in the Amazon. *Rhizoctonia solani* AG1 IA is a necrotrophic soilborne plant pathogen of worldwide importance that infects both Fabaceae and Poaceae hosts in tropical and subtropical climate region, causing web blight on cowpea, sheath blight on rice, banded leaf and sheath blight on maize, besides the soybean leaf blight (CHAVARRO-MESA et al., 2020; GONZÁLEZ-VERA et al., 2010). The pathogen survives through resistance structures (sclerotia) that can be locally disseminated by water and dispersal of contaminated soil particles. The spread over long distances occurs by infected seeds, contributing to the clonal spread of the fungus (CHAVARRO-MESA et al., 2020).

Specifically about the soybean leaf blight, the disease causes yield losses between 31 and 60% when the conditions are favorable for the development of the disease as in crops cultivated in the Amazon agro-ecosystem (MEYER et al., 2006). Controlling the soybean leaf blight is very difficult. Firstly, because *R. solani* AG-1 IA can attack several distinct hosts such as cowpea, rice, and signal grass, therefore making the adoption of crop rotation unfeasible (CHAVARRO-MESA et al., 2020). Secondly, because there are still no commercial varieties of soybean resistant to leaf blight, which is essential for maintaining the soybean's cropping system in the Amazon. However, there are few resistant genotypes identified so far that can be included in breeding programs for disease resistance (NECHET et al., 2008). Thirdly, because the management of soybean leaf diseases depends mainly on systemic fungicides spray programs, although the efficacy of fungicides has steadily decreased over the past 17 years in Brazil, from full control to only 20% effectiveness (MEYER et al., 2006). This decrease in fungicide efficacy was probably due to the emergence of resistance to the two major fungicide classes over two decades, i.e. QoI – strobilurins and DMI - triazoles (MEYER et al., 2006).

The pioneering study by Ciampi et al. (2008) with populations of the leaf blight pathogen from the most important soybean cropping regions in Central-western and Northern Brazil indicated evident population differentiation. Selection and genetic drift may have enhanced genetic differentiation, as many of these populations are genetically isolated, following the isolation-by-distance model.

Population differentiation may result from local adaptation due to spatial and temporal heterogeneity in the selection pressure or in the random genetic drift due to the finite size of small populations. One way to distinguish natural selection from genetic drift is to look at the distribution of genetic variation among loci. Genetic drift must affect the entire genome equally. As a result, neutral loci are expected to show similar levels of population differentiation in the genome (JARAMILLO-CORREA; BEAULIEU; BOUSQUET, 2001). On the other hand, natural selection affects only loci (or loci strongly linked) directly or indirectly involved in the nature of a selected phenotype, resulting in varying degrees of population differentiation between loci under selection and neutral loci (ZHAN et al., 2005).

Due to the fact that genetic variation is considered the basis for future adaptation to a changing climate (PAULS et al., 2013), we postulated in this study that the highly different genetic nature of *R. solani* AG-1 IA confers the pathogen high potential for adaptation, by natural selection, to thermal stress (BERNARDES DE ASSIS et al., 2009).

The objective of this study was to evaluate the relative importance of neutral genetic variation (followed by genetic drift) and natural selection on the divergence and regional adaptation of populations of the soybean-infecting *R. solani* AG-1 IA. Therefore, we compared the phenotypic differentiation in quantitative traits (Q_{ST}) and the neutral genetic differentiation (F_{ST}, based on microsatellites data) among three pairs of populations. As measures of phenotypic responses of the fungus (quantitative traits), we estimated the tolerance to temperature stress and the tolerance to a broad-spectrum fungicide (copper oxychloride) under optimal and high temperature conditions. It was hypothesized that (H0), in populations of *R. solani* AG-1 IA, characters associated with the response to environmental conditions (such as stress tolerance) do not show any evidence of selection

(directional or stabilizing) for local or regional adaptation, i.e. $Q_{ST} = F_{ST}$. An alternative hypothesis was that, considering that the three populations analyzed are genetically differentiated along a latitudinal gradient axis, they possibly diverged under the effect of directional selection (i.e. $Q_{ST} > F_{ST}$) imposed by the temperature stress along this latitudinal gradient. As a result, if ecotypic adaptation was occurring, we expected that populations from these three distinct areas would also exhibit phenotypic and heritability differences in the tolerance to temperature stress and fungicide under high temperature conditions.

MATERIALS AND METHODS

Rhizoctonia solani AG-1 IA isolates were obtained in 2006 from infected soybean plants sampled from cropping areas in Maranhão (MA), Mato Grosso (MT) and Tocantins (TO) States, Brazil (Table 1).

Table 1. Characterization of the *Rhizoctonia solani* AG-1 IA soybean populations from Brazil used in this study and measures of genotypic diversity*.

Population[1]	Geographic coordinates	Original sample size (N)	Number of genotypes detected	Genotypic diversity[2,3]	Genotypes used
MA	-47.05 E -6.52 S	91	15	0.47 c	12
MT	-55.75 E -12.60 S	55	22	0.73 b	12
TO	-48.17 E -8.97 S	22	16	0.95 a	12
Total		250	53	0.92	36

*Adapted from Ciampi et al. (2008).
[1]Historical annual means between 1982 and 2012 of minimum (T_{min}), average (T_{ave}) and maximum (T_{max}) temperatures in the sampled locations (Source: https://pt.climate-data.org/):
Tupirama, TO – T_{min} = 20.66; T_{ave} = 26.74 and T_{max} = 32.88;
Balsas, MA – T_{min} = 20.47; T_{ave} = 26.37 and T_{max} = 32.30;
Sorriso, MT – T_{min} = 18.03; T_{ave} = 24.98 and T_{max} = 32.01; the distribution of historical monthly temperature averages is shown in (Figure 1).
[2]Genotypic diversity of Stoddart, calculated according to Stoddart and Taylor (1988).
[3]Means followed by the same letter are not significantly different ($p \geq 0.05$), according to the bootstrap test between pairs of populations.

These fungal isolates were genotyped by Ciampi et al. (2008), with 10 highly polymorphic microsatellite markers (ZALA et al., 2008). From these, 12 genetically distinct isolates were selected from each population (Table 1). Fungal sclerotia from isolates stored on silica gel at -20 °C were transferred to 90 mm Petri dishes containing 15 mL potato-dextrose agar medium, PDA (18.5 g potato dextrose broth, 15 g agar, supplemented with 50 µg mL^{-1} of chloramphenicol and streptomycin). Once the original stock cultures were reactivated starting from sclerotia, to standardize the growth rate of the cultures, a second subculturing was carried out using mycelium discs from the margins of the colonies. Soon after, the inoculum was prepared for the experimental phases. Mycelium discs of 4 mm diameter were cut from the margin of colonies with active mycelial growth and transferred to the experimental plates. A single mycelial disc was transferred to the center of each plate.

Half of the plates were amended with 0.5 g L^{-1} of the copper fungicide Recop (containing 84% copper oxychloride) and the other half corresponded to the treatment without the addition of fungicide, containing only the PDA medium. The copper fungicide, based on copper oxychloride, has multiple sites of action and a wide spectrum of activity (AL-ASSIUTY et al., 2014) interfering non-specifically with the general enzymatic activity of the pathogen. The fungi were incubated at 25.0 °C, as optimal temperature for *R. solani* AG-1 IA growth and at 33.5 °C, as stress temperature (COSTA-SOUZA et al., 2007). After 48 hours of incubation, the mean radial mycelial growth was measured along the line with the largest diameter and at the perpendicular line, in units of cm / 48 hours. The experiments of this study were conducted in 2012.

To determine the values of heritability and phenotypic differentiation by quantitative Q_{ST} characters, three phenotypic responses were measured: (i) Tolerance to stress temperature (ratio between growth at 33.5 °C and the average growth of the isolate at 25 °C, both in the absence of fungicide); (ii) Tolerance to copper fungicide at 25.0 °C (ratio between the growth at the dose of 0.5 g L^{-1} of Recop and the average growth in the absence of fungicide, both at 25.0 °C); and (iii) Tolerance to copper fungicide at 33.5 °C (ratio between the growth at the dose of 0.5 g L^{-1} of fungicide and the average growth in the absence of fungicide, both at 33.5 °C).

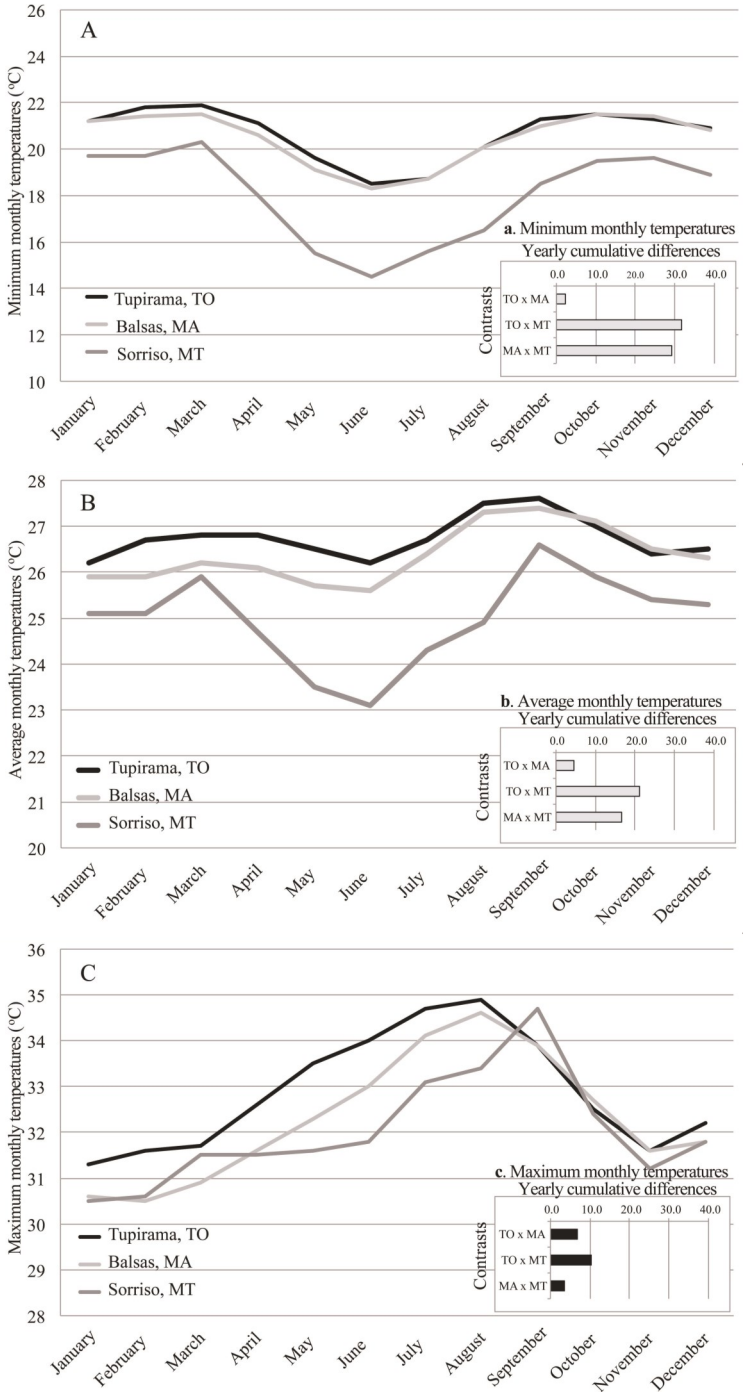

Figure 1. Historical monthly averages between 1982 and 2012 of minimum (A), average (B) and maximum (C) temperatures in the sampled locations from Tocantins, Maranhão and Mato Grosso (Source: https://pt.climate-data.org). The internal tables indicate the yearly cumulative differences, between minimum (a), average (b) and maximum (c) temperatures, contrasting geographic populations (TO x MA, TO x MT and MA x MT).

The experimental design used was a completely randomized, with nine replications, in two different schemes: (i) Measurement of tolerance to stress temperature at 33.5 °C: three populations of the pathogen (MA, MT and TO); (ii) Measurement of copper fungicide tolerance: three populations of the pathogen (MA, MT and TO) and two temperatures (25.0 and 33.5 °C). Each population consisted of 12 fungal isolates. The isolates were considered as a random effect and populations and temperature as fixed effects. The experiment was carried out twice. The phenotypic response data (x) was transformed into a logarithm of ($x + 1$). Analysis of variance to test the effect of population and temperature was performed using the PROCGLM statistical procedure in the SAS program

(SAS_INSTITUTE, 2010). The means of phenotypic responses for each population were compared using the *t* test at $p \leq 0.05$. For the analysis of the effects of populations and / or temperature on the components of variance and the heritability of the response to the conditions tested, the variance explained by the factor isolated within each population was interpreted as genetic variance (V_G) and the experimental error was considered as environmental variance (V_E). Heritability (*h2*) was calculated as the ratio between the genetic variance and the phenotypic variance. The estimated values of heritability and the 95% confidence interval for these estimates were determined in the statistical program R, using bootstrap with 1,000 resamples of the data. The level of population differentiation in quantitative characters *(i.e.* Q_{ST}, which is analogous to F_{ST}) was estimated based on components of additive genetic variance "between" and "within" populations, as described by Zhan and McDonald (2011) in a study conducted with the plant pathogenic fungus *Mycosphaerella graminicola*. For diploid species (or dikaryotic organisms such as *R. solani*, *i.e.* containing two independent nuclei in each cell), the level of population differentiation in quantitative characters is expressed by: $Q_{ST}=\delta^2_{AP}/(\delta^2_{AP}+2\delta^2_{WP})$ where: δ^2_{AP} is the additive genetic variance between populations for tolerance to each condition studied and δ^2_{WP} is the additive genetic variance within populations.

The components of variance "between pairs" and "within" populations were obtained using PROC VARCOMP in the SAS program (SAS_INSTITUTE, 2010). The genetic diversity for microsatellite loci was estimated according to NEI (1973) by partitioning the components of variance "within" and "between" populations to measure the degree of differentiation (F_{ST}) between pairs of the three chosen populations (MA, TO and MT). The genotypic data used were those generated by Ciampi et al. (2008). The program FSTAT version 2.9.3 (GOUDET, 1995) was used to determine the pairwise F_{ST} values. The 95% confidence interval of the F_{ST} estimates was obtained by bootstrapping the original data, with 1,000 re-samplings of the data.

Finally, the patterns of genetic variation in microsatellite markers (F_{ST}) were compared with variation in quantitative characters (Q_{ST}) to infer the importance of the effects of natural selection acting upon populations of *R. solani* AG-1 IA from soybean used in this study.

RESULTS AND DISCUSSION

In the first part of this study, the three populations of *R. solani* AG-1 IA from soybean were analyzed for differences in phenotypic responses and heritability for tolerance to temperature stress and copper fungicide (copper oxychloride) under optimal conditions (25.0 °C) and high temperature (33.5 °C).

The population from Tocantins showed a higher tolerance to the temperature of 33.5 °C than the populations from Maranhão and Mato Grosso, which did not differ significantly from each other by the *t* test ($p \leq 0.05$). In fact, historical differences in maximum temperatures (Figure 1 c) indicated that the highest maximum temperatures were recorded in Tocantins (T_{max} annual average = 32.88 ± 1.22 °C) over a period of 20 years (Figure 1 C and c), which may justify the higher stress temperature tolerance of the pathogen population originally sampled there. At 25 °C the population from Tocantins also showed higher tolerance to the copper fungicide than the two other populations. However, at 33.5 °C, the population from Mato Grosso showed the highest tolerance to copper fungicide. In general, the three populations of *R. solani* AG-1 IA showed higher tolerance to copper fungicide at 33.5 °C (Figure 2).

When the parameter under evaluation was the heritability (*h2*) for temperature tolerance, the population from Maranhão showed a higher average heritability (0.86) than the population from Mato Grosso, although it did not differ from the population from Tocantins. There was no significant difference between the three populations with respect to heritability for fungicide tolerance at both 25 °C and 33.5 °C. However, for the populations from Mato Grosso and Tocantins, the heritability for fungicide tolerance was higher at 33.5 °C temperature (Figure 2).

In general there was an increase in the genetic variance with a positive effect on heritability for tolerance to fungicide at stress temperature. On average, at 33.5 °C there was an increase of 0.39 ± 0.14 units in *h2* for fungicide tolerance, which represented an increase of 1.4 times in comparison to *h2* at the optimum temperature (25 °C). This is in line with the first set of hypotheses by Hoffmann and Merilä (1999) regarding the effect of environmental stress on genetic variation and heritability in populations of various organisms. The authors associated the increase in heritability under stress conditions to the mechanism they called decanalization. Under the decanalization mechanism, there is the expression of genetic variants that normally only manifest in stressful environments or because of insufficient temporal selection to remove harmful alleles in new and stressful environments. In contrast, Willi et al. (2011) in a study under conditions of optimal and high temperature and three concentrations of fungicide (absent, low and high concentration), with the plant pathogenic fungus *R. solani* AG-3 from potatoes, found that the heritability for mycelial growth was not significantly high due to these stress conditions, perhaps because the increase in genetic variance was offset by the increase in environmental variance.

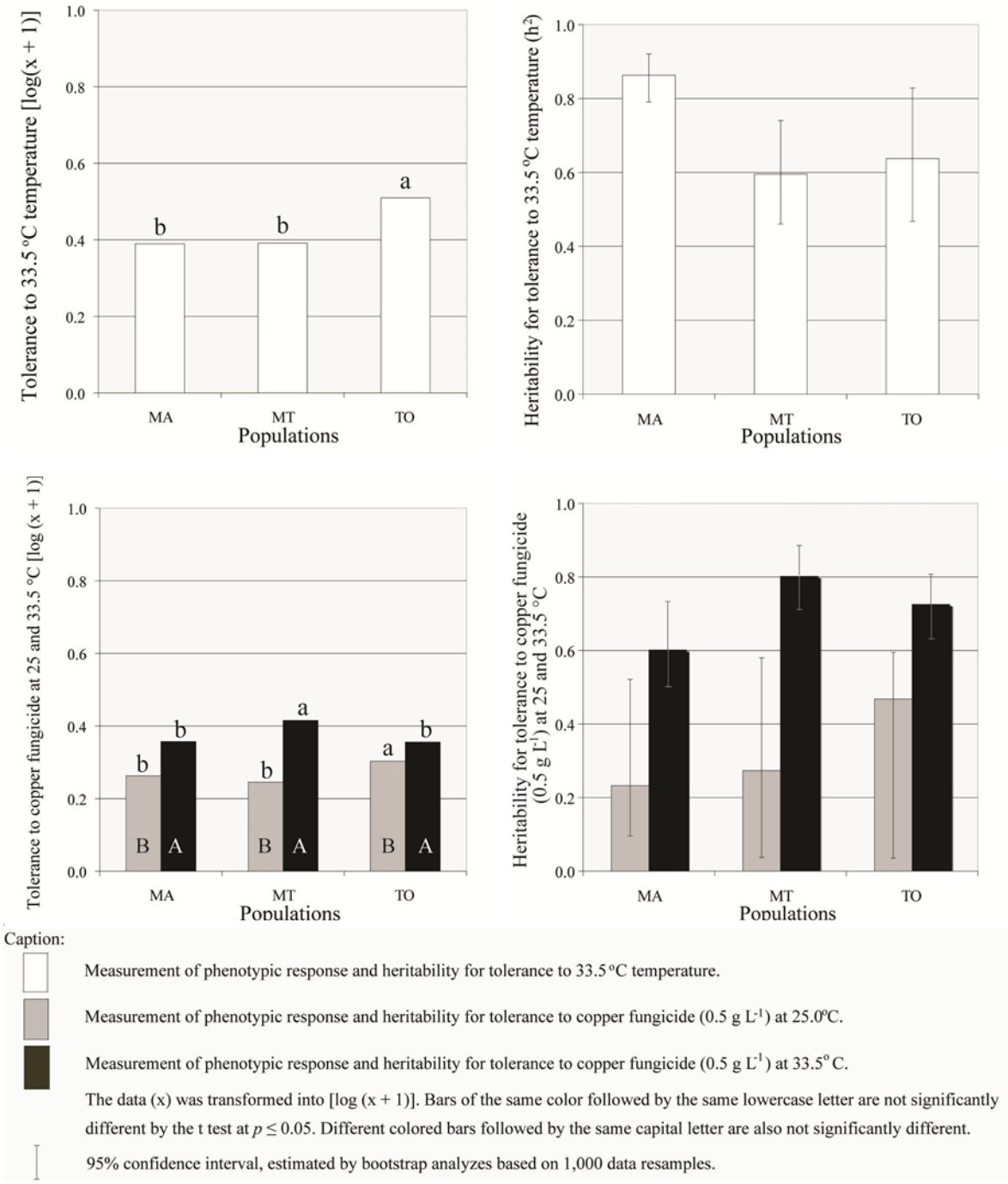

Figure 2. Tolerance to 33.5 °C temperature, tolerance to copper fungicide (0.5 g L^{-1}) at 25 °C and at 33.5 °C and heritability in each condition, in populations of *Rhizoctonia solani* AG-1 IA from Maranhão (MA), Mato Grosso (MT) and Tocantins (TO).

In the second part of our study, a combination of molecular genetic markers was used to investigate the genetics of thermal adaptation in *R. solani* AG-1 IA from soybean seeking to discern between the relative importance of neutral genetic variation (followed by genetic drift) and natural selection on the divergence between the populations.

Thermal adaptations can occur in organisms due to phenotypic plasticity and/or genetic differentiation. Phenotypic plasticity is the phenomenon by which a genotype produces different phenotypes in response to different environmental conditions. In contrast to phenotypic plasticity, genetic differentiation is an intrinsic property of organisms that produces permanent genetic adaptations, allowing them to face fluctuations in local temperature (YAMORI et al., 2010). In our study, the relative contribution of plasticity to tolerance to stress temperature (33.5 °C) was inferred from the additive genetic variance. The average additive genetic variance (measured as average heritability, or the fraction of the total

phenotypic variance that is additive) was responsible for 70% of the total phenotypic variation in temperature tolerance, indicating that the average contribution of plasticity must be ≤ 30%. The genetic contribution was 1.5 to 6.0 times higher than that of plasticity, depending on the individual population. Thereby, heritability is a common measure of evolutionary potential. Therefore, understanding variations in heritability for thermal tolerance could help predict the future responses of a species to climate change (FANG-ZHOU et al., 2014).

Our results indicated that genetic differences were determinant for thermal adaptation in populations of *R. solani* AG-1 IA from soybean. Thermal adaptation to local or regional temperature can be caused by antagonistic pleiotropy or by accumulation of mutation (COOPER; BENNETT; LENSKI, 2001). These two distinct processes have been recognized for a long time, but their contributions in relation to the thermal adaptation of species are rarely examined, due to the difficulty in distinguishing them (SGRO; PARTRIDGE, 1999). Antagonistic pleiotropy results from the directional selection for high fitness of organisms under different temperature conditions while the accumulation of mutations results from genetic drift (COOPER; BENNETT; LENSKI, 2001). According to the antagonistic pleiotropy theory, thermal adaptation to a temperature regime and poor adaptation to other temperature regimes are caused by the same mutations, which lead to a trade-off between performances at different temperatures. If antagonistic pleiotropy is the main mechanism responsible for thermal adaptation, it is expected that directional selection in different environments will increase the differentiation of populations for temperature-related characteristics, giving rise to a significantly higher Q_{ST} for thermal tolerance than the F_{ST} for neutral loci. If the accumulation of mutations is the main mechanism responsible for thermal adaptation, it is expected to find similar levels of population differentiation for thermal tolerance and neutral loci, assuming that genetic drift has affected the entire genome equally (JARAMILLO-CORREA; BEAULIEU; BOUSQUET, 2001).

To determine the relative importance of neutral genetic variation and natural selection on regional divergence and adaptation between populations of *R. solani* AG-1 IA from soybean, neutral genetic differentiation between populations (F_{ST}) was compared with the phenotypic differentiation by quantitative characters (Q_{ST}) between three pairs of populations.

The analysis of the neutral genetic structure (F_{ST}) indicated subdivision between the three pairs of populations, consistent with divergence due to the geographical distance between populations previously detected by Ciampi et al. (2008). The pairwise F_{ST} values did not differ significantly from one another, indicating that populations had similar levels of subdivision (CI95% for F_{ST} (MA x MT) = 0.042 – 0.128; for F_{ST} (MA x TO) = 0.040 – 0.231 and for F_{ST} (MT x TO) = 0.030 – 0.133) (Figure 3).

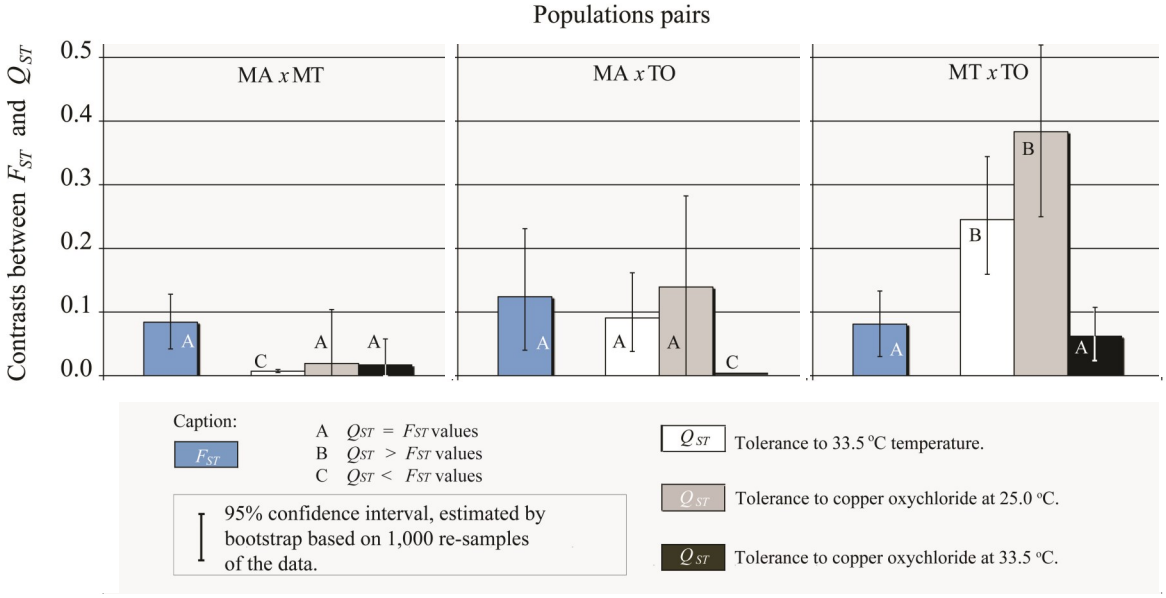

Figure 3. Contrast between F_{ST} and Q_{ST} values for temperature tolerance at 33.5 °C and tolerance to copper fungicide (0.5 g L^{-1}) at 25 °C and at 33.5 °C, between pairs of populations of *Rhizoctonia solani* AG-1 IA [Maranhão (MA), Mato Grosso (MT) and Tocantins (TO)]

The estimated values of Q_{ST} were compared to the F_{ST} to test the null hypothesis of genetic divergence of the quantitative characters due to the neutral phenotypic evolution (*i.e.*, by neutral mutation followed by differentiation by genetic drift, mainly). If the Q_{ST} value does not differ significantly from the F_{ST}, the hypothesis of population differentiation caused by genetic drift cannot be rejected. Q_{ST} values significantly higher than the F_{ST} distribution indicate directional selection action on the character. If the Q_{ST} is significantly lower than the F_{ST}, the selection must be restricting population differentiation, that is, it is stabilizing (MERILÄ; CRNOKRAK, 2001).

The first group of contrasts of F_{ST} versus Q_{ST} values between the populations from Maranhão and Mato Grosso (MA x MT) indicated a value of Q_{ST} lower than that of F_{ST} for the tolerance to 33.5 °C. In this case, the stabilizing selection must have restricted the population differentiation in quantitative characters (Figure 3). The documented cases of $Q_{ST} < F_{ST}$ show high population differentiation in neutral genes (MERILÄ; CRNOKRAK, 2001; MCKAY; G. LATTA, 2002). However, for the other two phenotypic responses measured, the Q_{ST} values were similar to the F_{ST}, indicating neutrality.

The second group of contrasts between the populations from Maranhão and Tocantins (MA x TO) resulted in Q_{ST} values within the F_{ST} distribution, which indicated a tendency towards neutrality for tolerance at 33.5 °C and for tolerance to copper fungicide at 25 °C (Figure 3). Under neutrality, an agreement is expected between genetic differentiation for phenotypic traits - Q_{ST} and measures of neutral genetic diversity - F_{ST} (SPITZE, 1993). In this situation, the accumulation of neutral mutations affecting the entire genome equally (JARAMILLO-CORREA; BEAULIEU; BOUSQUET, 2001) followed by genetic drift is possibly responsible for the regional adaptation. In contrast, the Q_{ST} value for fungicide tolerance at 33.5 °C was lower than that of F_{ST}, indicating stabilizing selection.

In the third pair of comparisons, between the populations from Mato Grosso and Tocantins (MT x TO), for fungicide tolerance at 33.5 °C, the Q_{ST} value was similar to F_{ST}, also indicating a tendency to neutrality. As for tolerance to temperature and copper fungicide at 25 °C, it was observed, in a peculiar way, a Q_{ST} value higher than the F_{ST} distribution (Figure 3). This observation supports the hypothesis that the directional selection for local or regional adaptation acted in the divergence between these two populations. Although these were the only two observations of Q_{ST} higher than F_{ST} in our study, this has been the common pattern reported in the literature. The common occurrence of directional selection (*i.e.*, when Q_{ST} is higher than F_{ST}) was reported by MERILÄ; CRNOKRAK (2001) in an extensive review of eighteen published papers comparing Q_{ST}s (of morphological characters, of life history or of the mixture of the two types of characters, in plants, vertebrates and invertebrates) with F_{ST} data (based on allozymes, microsatellite markers, ribosomal DNA, mitochondrial DNA, RAPD and RFLP loci) and by Mckay and G. Latta (2002) in a study about hereditary variation of adaptable characteristics with 29 plant species. Zhan and McDonald (2011) also detected directional selection for thermal adaptation in five world populations (about 140 isolates) of the plant pathogenic fungus *M. graminicola*.

The divergence between the populations from Mato Grosso and Tocantins regarding the tolerance to copper fungicide at 25 °C ($Q_{ST} \sim 0.4$) may have resulted both from the effect of natural selection acting on the pre-existing genetic variation (since the two populations were distinct under this condition) (Figure 2) and from new mutations that emerged independently in each population. The regional adaptation for these populations is probably due to the effect of geographic isolation between them, since the genetic differentiation observed between these two populations ($F_{ST} = 0.081$) is considered a requirement for local or regional adaptation (LAZZARO et al., 2008). Although accurately determining the relative contribution of these two sources of genetic variation to the observed adaptation may sound impossible, the distinction between these sources does not affect what was found because genetic variation from any of the sources would still be subject either to antagonistic pleiotropy or to accumulation of mutations.

In general, most of the Q_{ST} values estimated in this study (5 out of 9 pairs of comparisons), did not differ significantly from F_{ST}, tending to neutrality. These results are in accordance with the H_0 hypothesis raised in our study, which asserted that characters associated with the response to environmental conditions (such as tolerance to thermal stress) in populations of *R. solani* AG-1 IA did not show evidence of directional selection for regional adaptation, *i.e.* $Q_{ST} = F_{ST}$.

The fact that there was no significant difference between F_{ST} and Q_{ST} did not necessarily indicate an absence of selection, but rather that the hypothesis that the effects of selection are indistinguishable from those caused by drift in a given character is plausible (MERILÄ; CRNOKRAK, 2001).

The absence of genetic differentiation for temperature tolerance between pairs of populations of the pathogen (especially between MA and MT) may be related to the fact that differences in regional temperatures were not sufficient for thermal adaptation to occur. In fact, the historical differences in maximum temperatures indicated higher similarity between the MA and MT populations but differentiation from TO (Figure 1 C-c). This TO

population showed a higher tolerance to 33.5 °C temperature than the MA and MT populations (Figure 2).

Consequently, the conclusions of this study must be interpreted within limits, considering: a) The reduced number of populations analyzed. According to O'hara and Merilä, (2005), Q_{ST} estimates are intrinsically biased and their accuracy is low for data sets typically analyzed (<20 populations); b) The number of markers used (n = 10), although allowed to discriminate the differentiation between the three populations analyzed, may not have been sufficient to accurately portray the levels of population divergence; and c) The number of quantitative factors analyzed (two: temperature and fungicide tolerance), may have been insufficient to infer, in general, how evolutionary forces have shaped populations of the pathogen to regionally adapt.

CONCLUSION

The mycelial growth heritability (h^2) values were high for all three populations of *R. solani* AG-1 IA from soybean.

Under conditions of thermal stress, there was an increase in the genetic variance for tolerance to the fungicide copper oxychloride in all three populations of the pathogen, with a positive effect on heritability.

Genetic differences were the main determinants of tolerance to copper oxychloride under thermal stress in *R. solani* AG-1 IA from soybean.

Most of the estimated Q_{ST} distributions did not differ from F_{ST}, indicating neutrality for population divergence considering temperature tolerance and tolerance to copper oxychloride under thermal stress.

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