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Genetic diversity of Soybean Cyst Nematode (*Heterodera glycines*) populations in Southeastern Goiás state, Brasil

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Abstract

Heterodera glycines, also known as Soybean Cyst Nematode (SCN), is one of the phytosanitary issues that prevents high soybean yields (*Glycine max*) due to its dissemination capacity and high physiological race variability. The first step towards the proper and safe use of resistant cultivars lies on correctly identifying the physiological race of this nematode in the crop in order to avoid new breed-selection pressure. Thus, the aim of the present study was to genetically characterize the races of *Heterodera glycines* populations from soybean-producer counties belonging to the Railroad (Estrada de Ferro) Region (Goiás State, Brazil), as well as to investigate their molecular characterization based on RAPD markers. RAPD data were evaluated in a binary way, in which values (1) and (0) were attributed to the presence and absence of amplified bands for each primer, respectively. The binary matrix was used to estimate the genetic distance between populations, based on the Jaccard dissimilarity index. The clustering analysis was carried out based on the calculated genetic dissimilarity matrix, according to Ward's method. Eight (8) different physiological *Heterodera glycines* races were found in the investigated counties. Only 10 out of 28 RAPD primers were polymorphic among SCN populations, whereas the remaining primers either did not amplify, or presented low amplification of the DNA fragment. Five genetically-different groups were recorded among *H. glycines* population accessions, which presented high genetic variability rate.

Keywords: Characterization; HG Type; polymorphism; race test; RAPD markers; soybean cyst nematode.

Introduction

Heterodera glycines Ichinohe (1952), also known as Soybean Cyst Nematode (SCN) since it was found in the 1991/1992 harvest in Brazil (Lima et al., 1992; Monteiro and Morais, 1992), is one of the phytosanitary issues that make it difficult obtaining high soybean (*Glycine max* (L.) Merril)) yields. Significant crop losses ranging from 10% to 100% were recorded in places where SCN was detected (Sinclair, 1982; Silva, 1998). According to Dhingra et al. (2009), grain yield losses can reach 90% depending on nematode race and infestation degree, as well as on soil fertility and cultivar susceptibility. It is necessary adopting management strategies in crop areas affected by soybean cyst nematodes to help maintaining the economic viability of soybean plantations. Nowadays, the main management strategies are based on crop rotation (Rigss and Schmitt 1993; Yorinori, 2000), soil management (Dhingra et al., 2009; Embrapa, 2015) and on the use of resistant cultivars (Embrapa, 2015). The use of resistant cultivars is the main control method adopted to manage *H. glycines* due to its practicality and economic viability (Niblack et al., 2002). However, we should take the resistant genetic materials into consideration to be used in the management process. These materials present different SCN-resistance levels, which enable nematodes to easily overcome the resistance of these cultivars due to their high genetic variability (Young, 1996; Li et al., 2004; Brucker et al., 2005). This variability is higher in Brazil than other countries. According to Dias et al., (2004) and Asmus et al., (2012), the country has already recorded races 1, 2, 3, 4, 5, 6, 9, 10, 14, 4 +, 9+ and 14+ among the sixteen physiological *H. glycines* races described by Riggs and Schmitt (1988). In addition, races 4+, 9+ and 14+ differ from classic races 4, 9 and 14 due to their ability to parasitize the North American Hartwig cultivar, which was hitherto seen as resistant to all nematode races (Dias et al., 2009). Nematode races with additional parasitism genes have been evolving very rapidly in Goiás, Mato Grosso and Mato Grosso do Sul states (Dias et al., 2010). *H. glycines* reproduces through amphimixia, in which females copulate with several males (Triantaphyllou and Esbenshade, 1990) and enable great genetic variability within species. Thus, controlling this nematode through the use of resistant cultivars, and through the continuous use of the same resistance source, enables the pathogen to easily overcome cultivar resistance (Dias et al., 2009), a fact that does not often prevent the inoculum permanence in the

field. Consequently, it generates selection pressure on the population and enables selecting individuals capable of parasitizing the root system of these cultivars (Dias et al., 1998).

Therefore, the first step towards the proper and safe use of resistant cultivars to avoid selection pressure lies on correctly identifying the physiological *H. glycines* race in the field, since this nematode has high genetic variability (Riggs and Schmitt, 1993). However, physiological race-identification tests are not easily performed, since they can take several months, besides involving soybean differentiator lines (Pickett, Peking, PI 88788, PI 90763, Lee - susceptibility standard, Hartwig - resistance standard) that are not adapted to our soil and climate conditions (Riggs and Schmitt, 1988).

Studies about genetic diversity within *H. glycines* species remain scarce, although they are necessary to investigate the genetic variability of this nematode species. Advances in the molecular biology field enabled developing molecular characterization methods such as the Random Amplified Polymorphic DNA (RAPD) technique, which has been used to analyze the genetic diversity of different phytopathogen types (Cobb and Clarkson, 1993; Faleiro et al., 1998). RAPD is based on the amplification of DNA fragments through the use of arbitrary sequence primers and this technique mainly stands out for enabling simple and easy-to-perform analyses. Abdelnoor et al. (2001) used RAPD markers to investigate the genetic diversity of *H. glycines* populations and found that populations characterized as races 4 and 9 (who did not parasitize Hartwig) were genetically different from races 4+ and 9+ (who parasitized Hartwig). According to the aforementioned authors, RAPD markers allow investigating differences in the gene pool composing different *H. glycines* races. Silva et al. (2000) investigated the genetic variation among sixteen SCN populations and demonstrated that RAPD markers can be used in studies focused on the genetic variability between nematode populations, and within nematode races, besides being used to monitor this pathogen-population dynamics.

In light of the foregoing, the aim of the present study was to genetically characterize (in physiological races) *Heterodera glycines* populations in soybean-producer counties belonging to the Railroad Region in Southeastern Goiás State, as well as to investigate their molecular characterization based on RAPD markers.

Results

A survey about soybean cyst nematode distribution was carried out in counties belonging to the Railroad Region (Goiás State) from 2016 to 2018 (Table 1). Their respective physiological races were identified. The SCN populations belonged to 8 different races (1, 3, 4+, 6, 9, 10, 14 and 14+), as shown in Table 2. In addition, nematode race 3 recorded the highest prevalence rate (50.7% of the samples) in all evaluated counties, except for Campo Alegre de Goiás. It was followed by races 6 and 10 (both representing 10.4% of the samples), races 1, 9 and 14 (representing 6.9% of the sample, each) and, finally, by races 4+ and 14+ (3.5% of the samples, each). Nematode races 3 and HG type 0^- (20% of the samples), 6 and HG type 0^- (20% of the samples), 9 and HG type 1^- (40% of the samples) and 10, HG type 3^- (20% of the samples) were found in Orizona County. Vianópolis County recorded races 3 and HG type 0^- (80% of the samples) and 10 and HG type 3^- (20% of the samples). Silvânia County presented races 3 and HG type 0^- (83.3% of the samples) and 1 and HG type 2^- (16.7% of the samples). Races 3 and HG type 0^- (66.7% of the samples) and 1 and HG type 2^- (33.3% of the samples) were also found in Leopoldo de Bulhões County. Races 3 (HG type 0^-), 6 (HG type 0^-), 10 (HG type 3^-), 4+ (HG type $1.3.8^-$) and 14+ (HG type $1.3.8^-$) (each race accounted for 20% of the samples) were detected in Ipameri County. Campo Alegre de Goiás recorded races 6 and HG type 0^- (33.3% of the samples) and 14 and HG type 1.3^- (66.7% of the samples). Gameleira de Goiás and Catalão counties presented only race 3 and HG type 0^- (100% of the samples) (Table 2).

Eighteen (18) out of the 28 tested RAPD primers (Table 3) did not either amplify, or presented low amplification of, the DNA fragment: OPAC-01, OPA-02, OPAD-11, OPA-17, OPAC-02, OPA-13, OPA-07, A-03, OPAC-14, AT-04, OPAC-04, OPA-10, OPA-08, OPAC-11, OPAC-19, OPAD-02, OPAC-06 and OPAC-09. Only 10 primers showed polymorphism, which resulted in 50 amplification products (bands on the agarose gel); 5 bands per primers, on average; 46 polymorphic bands (Table 4). The remaining 4 bands showed the same standard in all populations.

SCN population accessions per county recorded high genetic isolation rate according to the mean Jaccard dissimilarity value. This value was calculated based on the means of genetic distances, which varied from 0.64 among populations in Vianópolis and Ipameri counties to 0.82 among populations in Silvânia and Catalão counties (Table 5). In addition, there was no positive correlation between the genetic distances among genotypes in the populations and their geographical distances (spatial distribution).

The high dissimilarity degree between *H. glycines* populations in each county could be seen in the cluster analysis (Ward method) due to the formation of two main groups: G1 (lower genetic variability in races) and G2 (higher genetic variability in races), which were different from each other (Figure 1). G1 comprised nematode populations from Silvânia and Leopoldo de Bulhões counties, which only recorded races 1 and 3; whereas G2 comprised nematode populations from Orizona, Vianópolis and Ipameri counties (races 3, 6, 9, 10, 4+ 14+), as well as nematode populations from Campo Alegre de Goiás, Gameleira de Goiás and Catalão counties (races 3, 6 and 14) (Figure 1).

Five (5) different genotypic groups (Figure 2) were formed among *H. glycines* population accessions and presented more than 60% dissimilarity between them. The first group comprised of 3 populations classified as races 4+, 9 and 14. The second group comprised of 6 populations characterized as races 1 and 3; the third group encompassed 7 populations represented by races 3, 6, 10. The fourth group was composed of 7 populations represented by races 3, 9 and 10.; and, finally, the fifth group comprised of 8 populations represented by races 3, 6, 10, 14 and 14+.

We noticed that although genetic groups generated from genetic distances comprised different races at the same level, they shared peculiar characteristics of their parasitism on differentiator lines. All populations in G1 were able to parasitize differentiator lines “Pickett” and “Peking”. Accessions grouped in G2 comprised of populations that did not parasitize Pickett, Peking, PI 90763 and Hartwig. Populations in G3 and G4 did not parasitized PI 88788 and Hartwig. Populations in G5 were unable to parasitize PI 88788 (Table 6).

Table 1. Places where soil samples were collected for *Heterodera glycines* inoculum multiplication, as well as for the quantification of viable cysts to compose the initial population. Urutaí County, GO.

Collected samples	County	Geographic coordinates		Viable cysts*
		W	S	
Orizona 1	Orizona	16º58'39.7"	48º22'59.2"	6
Orizona 2		16º52'16.3"	48º11'51.0"	52
Orizona 3		16º50'06.0"	48º08'59.6"	37
Orizona 4		16º50'06.6"	48º08'59.5"	100
Orizona 5		16º50'53.0"	48º11'77.4"	6
Vianópolis 6	Vianópolis	16º53'53.1"	48º27'16.6"	72
Vianópolis 7		16º34'46.7"	48º18'59.3"	313
Vianópolis 8		16º40'02.7"	48º16'24.7"	169
Vianópolis 9		16º50'59.5"	48º24'55.7"	241
Vianópolis 10[2]		16°46'52.9"	48°29'29.0"	10
Silvânia 11	Silvânia	16°40'27.7"	48°40'40.7"	12
Silvânia 12		16°39'22.3"	48°40'39.9"	96
Silvânia 13[2]		16°39'32.0"	48°39'48.6"	43
Silvânia 14		16°42'50.3"	48°36'54.8"	49
Silvânia 15		16°42'56.7"	48°36'56.6"	45
Silvânia 16		16°42'43.9"	48°36'51.4"	32
Leopoldo de Bulhões 17[1]	Leopoldo de Bulhões	16°40'53.7"	48°48'1.95"	28
Leopoldo de Bulhões 18		16º39'11.4"	48º47'35.4"	194
Leopoldo de Bulhões 19		16°38'21.6"	48°43'45.9"	11
Leopoldo de Bulhões 20		16°39'02.9"	48°43'53.3"	9
Leopoldo de Bulhões 21[1,2]		16°38'36.6"	48°42'56.0"	2
Ipameri 22	Ipameri	17º42'06.0"	48°07'52.6''	59
Ipameri 23		17°35'13.0''	48°12'57.0''	10
Ipameri 24		17º29'18.9"	48º12'53.9"	47
Ipameri 25		17º27'18.0"	48º12'57.6"	85
Ipameri 26		17º42'46.3"	48º07'43.3"	269
Campo Alegre de Goiás 27	Campo Alegre	17º42'14.4"	47º43'20.3"	56
Campo Alegre de Goiás 28		17º36'30.3"	47º49'32.5"	136
Campo Alegre de Goiás 29		17º30'00.3"	47º52'03.3"	27
Campo Alegre de Goiás 30[1]		17º29'14.0"	47º51'51.3"	196
Campo Alegre de Goiás 31[1]		17º36'25.4"	47º48'17.6"	103
Gameleira de Goiás 32	Gameleira de Goiás	16º26'19.6"	48º35'43.1"	90
Gameleira de Goiás 33[1,2]		16º22'14.0"	48º43'29.2"	12
Gameleira de Goiás 34[1,2]		16º27'59.5"	48º38'23.1"	41
Gameleira de Goiás 35[1]		16º31'35.1"	48º39'36.5"	2
Catalão 36[1,2]	Catalão	18°11'12.9"	47°55'36.6"	126
Catalão 38		18°18'19.7"	47°54'01.3"	52
Catalão 39[1,2]		18°18'19.4"	47°54'01.3"	158

* = dead female nematode with eggs inside its body
[1] = population that did not enable race characterization
[2] = sampled population that did not enable molecular characterization

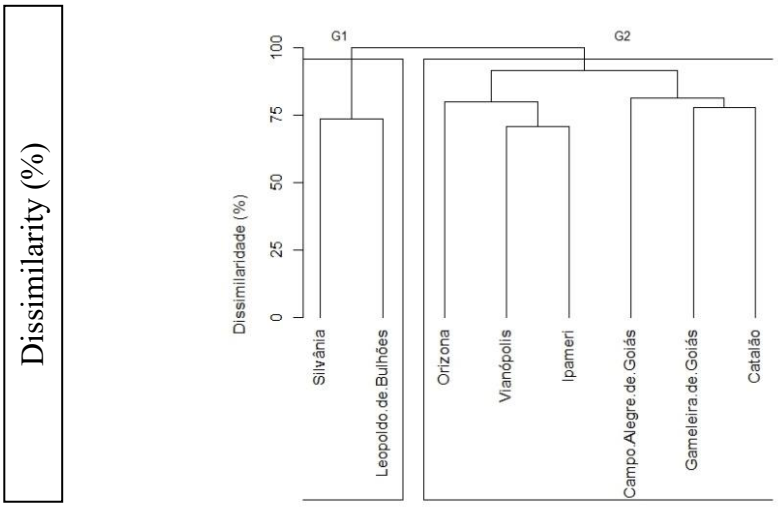

Fig 1. Dendogram based on dissimilarity means of *Heterodera glycines* populations in each evaluated county. Urutaí County, GO.

Table 2. Female Index - FI (%) in *Heterodera glycines* populations in counties belonging to the Railroad Region (GO), which were inoculated in soybean differentiator lines to identify physiological nematode races and HG Type. Urutaí County, GO.

SCN population	County	FI (%) Pickett	FI (%) Peking	FI (%) PI88788	FI (%) PI90763	FI (%) Hartwig	Race	Type HG
1	Orizona 1	3.85(–)	1.92(–)	0.00(–)	0.00(–)	0.00(–)	3	0-
2	Orizona 2	16.96(+)	0.00(–)	0.00(–)	2.68(–)	9.82(–)	6	0-
3	Orizona 3	34.55(+)	10.30(+)	0.00(–)	6.86(–)	0.00(–)	9	1-
4	Orizona 4	27.03(+)	12.61(+)	0.00(–)	6.31(–)	0.00(–)	9	1-
5	Orizona 5	19.07(+)	4.48(–)	0.00(–)	10.82(+)	0.00(–)	10	3-
6	Vianópolis 6	0.00(–)	0.00(–)	0.00(–)	0.00(–)	0.00(–)	3	0-
7	Vianópolis 7	0.00(–)	0.00(–)	0.00(–)	0.00(–)	6.96(–)	3	0-
8	Vianópolis 8	19.22(+)	9.61(–)	3.15(–)	25.68(+)	6.61(–)	10	3-
9	Vianópolis 9	0.00(–)	0.00(–)	0.00(–)	0.00(–)	0.79(–)	3	0-
10	Vianópolis 10	3.49(–)	0.00(–)	2.95(–)	0.54(–)	0.00(–)	3	0-
11	Silvânia 11	0.00(–)	0.00(–)	1.19(–)	0.00(–)	0.00(–)	3	0-
12	Silvânia 12	0.00(–)	0.00(–)	2.63(–)	0.00(–)	0.00(–)	3	0-
13	Silvânia 13	0.00(–)	0.00(–)	2.94(–)	0.00(–)	0.00(–)	3	0-
14	Silvânia 14	0.00(–)	0.00(–)	16.67(+)	0.00(–)	0.00(–)	1	2-
15	Silvânia 15	0.00(–)	0.00(–)	0.66(–)	0.00(–)	0.66(–)	3	0-
16	Silvânia 16	0.00(–)	0.00(–)	1.10(–)	0.00(–)	0.00(–)	3	0-
18	Leopoldo de Bulhões 18	0.00(–)	0.00(–)	10.67(+)	0.00(–)	0.00(–)	1	2-
19	Leopoldo de Bulhões 19	0.00(–)	0.00(–)	0.00(–)	0.00(–)	0.00(–)	3	0-
20	Leopoldo de Bulhões 20	0.00(–)	0.00(–)	0.00(–)	0.00(–)	0.00(–)	3	0-
22	Ipameri 22	11(+)	14.81(+)	3.70(+)	11.11(+)	11.11(+)	4+	1.3.8-
23	Ipameri 23	207.00(+)	7.34(–)	0.15(–)	8.72(–)	0.00(–)	6	0-
24	Ipameri 24	0.95(–)	0.95(–)	0.95(–)	0.00(–)	0.00(–)	3	0-
25	Ipameri 25	407.14(+)	78.57(+)	0.00(–)	100.00(+)	42.86(+)	14+	1.3.8-
26	Ipameri 26	36.52(+)	5.12(–)	0.34(–)	10.92(+)	0.00(–)	10	3-
27	Campo Alegre 27	53.01(+)	42.17(+)	1.20(–)	42.17(+)	0.00(–)	14	1.3-
28	Campo Alegre 28	129.08(+)	22.01(+)	0.82(–)	26.57(+)	0.00(–)	14	1.3-
29	Campo Alegre 29	22.86(+)	9.32(–)	0.00(–)	2.00(–)	0.00(–)	6	0-
32	Gameleira de Goiás 32	0.00(–)	0.00(–)	0.00(–)	0.00(–)	0.00(–)	3	0-
38	Catalão 38	0.00(–)	0.00(–)	0.00(–)	4.95(–)	0.00(–)	3	0-

(–) = soybean differentiator RESISTANT to the evaluated population, (+) = soybean differentiator SUSCEPTIBLE to the evaluated population

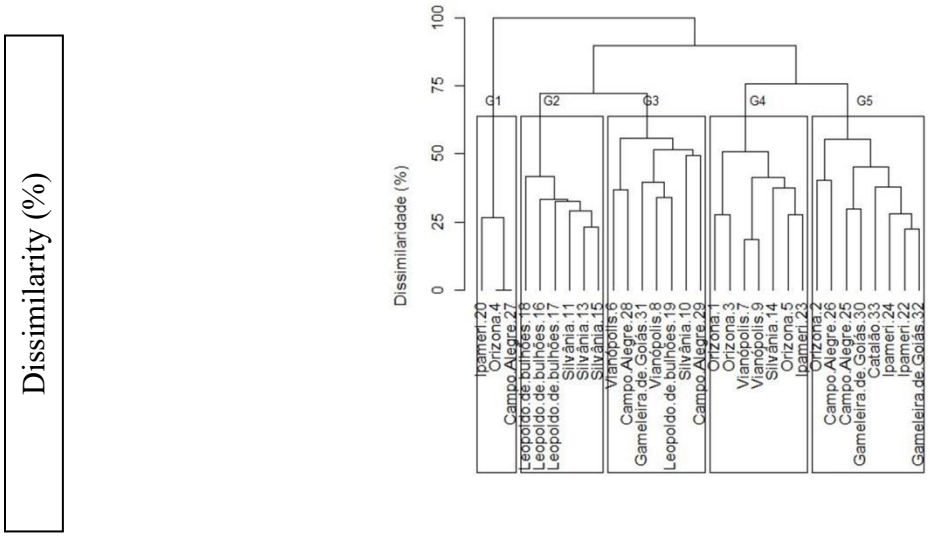

Fig 2. Dendogram presenting the genetic dissimilarity among 31 *Heterodera glycines* populations in counties belonging to the Railroad Region (Goiás State), based on the Jaccard index estimated from 10 primers. Urutaí County, GO.

Table 3. Relation between RAPD primers and their nucleotide sequences used to characterize Heterodera glycines populations in counties belonging to the Railroad Region, Urutaí County, GO.

Primers	Sequence (orientation 5'-3')	Primers	Sequence (orientation 5'-3')
OPAA-02	GAG ACC AGA C	OPAC-02	GTC GTC GTC T
OPAC-12	GGC GAG TGT G	OPA-13	CAG CAC CCA C
A-05	CAC CAG GTG A	OPA-07	GAA ACG GGT G
OPA-06	GGT CCC TGA C	A-03	AAG ACC CCT C
A-01	CCC AAG GTC C	OPAC-14	GTC GGT TGT C
OPAC-07	GTG GCC GAT G	AT-04	AAT CGG CTG
OPA-18	AGG TGA CCG T	OPAC-04	ACG GGA CCT G
OPA-11	CAA TCG CCG T	OPA-10	GTG ATC GCA G
OPAC-03	CAC TGG CCC A	OPA-08	GTG ACG TAG G
OPA-03	AGT CAG CCA C	OPAC-11	CCT GGG TCA G
OPAC-01	TCC CAG CAG A	OPAC-19	AGT CCG CCT G
OPA-02	TGC CGA GCT G	OPAD-02	CTG AAC CGC T
OPAD-11	CAA TCG GGT C	OPAC-06	CCA GAA CGG A
OPA-17	GAC CGC TTG T	OPAC-09	AGA GCG TAC C

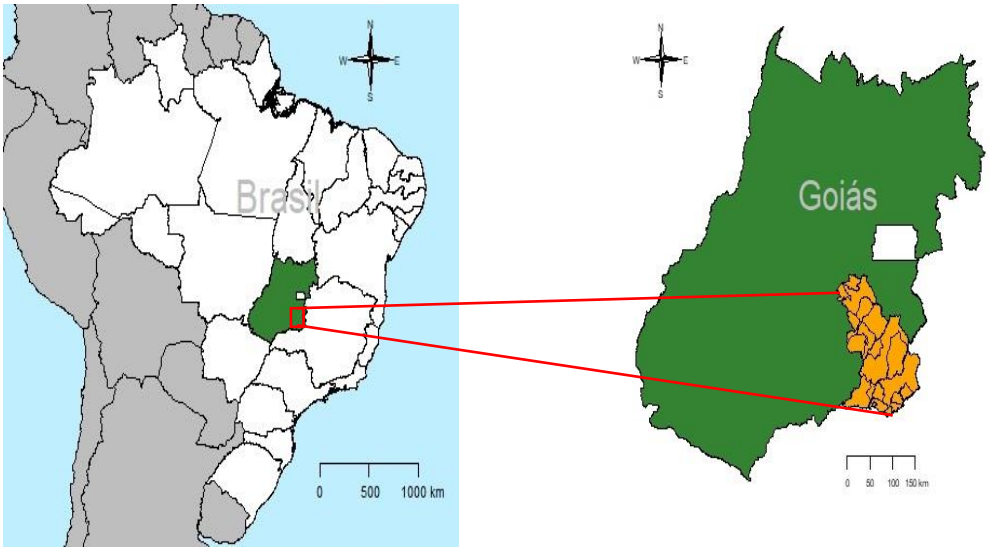

Fig 3. Railroad Region in Southeastern Goiás State. Urutaí County, GO.

Table 4. Relation between RAPD primers and number of bands (polymorphic and monomorphic) amplified and revealed on 1.2% agarose gel, under UV light.

Primers	Sequence 5'-3'	Bands	
		Polymorphic	Monomorphic
OPAA-02	GAG ACC AGA C	8	0
OPAC-12	GGC GAG TGT G	4	1
A-05	CAC CAG GTG A	1	0
OPA-06	GGT CCC TGA C	2	0
A-01	CCC AAG GTC C	5	1
OPAC-07	GTG GCC GAT G	8	1
OPA-18	AGG TGA CCG T	5	0
OPA-11	CAA TCG CCG T	3	0
OPAC-03	CAC TGG CCC A	7	0
OPA-03	AGT CAG CCA C	3	1
Total		46	4
Total of bands		50	

Fig 4. Image showing the agarose gel (0.8%) used to quantify the DNA extracted from *Heterodera glycines* populations in counties belonging to the Railroad Region based on its comparison to phage λ DNA (50, 100 and 200 ng); Urutaí, GO - the "arrow" highlights the phage λ DNA.

Table 5. Matrix of the mean genetic dissimilarity between *Heterodera glycines* populations per collection county, calculated based on the Jaccard coefficient. Urutaí County, GO.

Population origin	Ori.	Via.	Sil.	Leo.	Ipa.	Cam.	Gam.	Cat.
Orizona (Ori.)								
Vianópolis (Via.)	0.74							
Silvânia (Sil.)	0.77	0.73						
Leopoldo de Bulhões (Leo.)	0.81	0.77	0.66					
Ipameri (Ipa.)	0.66	0.64	0.67	0.73				
Campo Alegre de Goiás (Cam.)	0.75	0.79	0.81	0.79	0.70			
Gameleira de Goiás (Gam.)	0.75	0.74	0.76	0.73	0.66	0.71		
Catalão (Cat.)	0.77	0.77	0.82	0.80	0.72	0.74	0.70	

Table 6. Parasitism similarity of *Heterodera glycines* populations in counties belonging to the Railroad Region (Goiás State) over soybean differentiator lines used in the nematode race test by Riggs & Schmitt (1988). Urutaí County, GO.

Genotypic groups		Pickett		Peking		PI 88788		PI 90763		Hartwig		Race
	SCN population	+	-	+	-	+	-	+	-	+	-	
G1	22	22		22		22		22		22		4+
	4	4		4			4		4		4	9
	28	28		28			28	28			28	14
G2	19		19		19		19		19		19	3
	17											-
	18		18		18	18			18		18	1
	12		12		12		12		12		12	3
	14		14		14	14			14		14	1
	16		16		16		16		16		16	3
G3	6		6		6		6		6		6	3
	29	29			29		29		29		29	6
	32		32		32		32		32		32	3
	8	8			8		8	8			8	10
	20		20		20		20		20		20	3
	11		11		11		11		11		11	3
	30		30	30			30		30		30	13
G4	1		1		1		1		1		1	3
	3	3		3			3		3		3	9
	7		7		7		7		7		7	3
	9		9		9		9		9		9	3
	15		15		15		15		15		15	3
	5	5			5		5	5			5	10
	24		24		24		24		24		24	3
G5	2	2			2		2		2		2	6
	27	27		27			27	27			27	14
	26	26			26		26	26			26	10
	38		38		38		38		38		38	3
	25	25		25			25	25		25		14+
	23	23			23		23		23		23	6

+ = differentiator line is SUSCEPTIBLE to the SCN population
– = differentiator line is RESISTANT to the SCN population

Discussion

The existence of different physiological *Heterodera glycines* races indicated different parasitism abilities of these nematodes, as well as their ability to develop and reproduce in susceptible cultivars (Niblack et al., 2002). The high race variability recorded in this study corroborates with the study Dias et al. (2010), who assured that SCN races evolved very rapidly in Brazil.

The SCN race distribution scenario in 1994 only comprised races 3 and 14 in Goiás State (Noel et al., 1994). Races 3, 4, and 6 were identified in Chapadão do Céu County (Goiás State) in 1999 (Silva et al., 1999). Dias et al. (2004) investigated the incidence of SCN in Brazil and recorded races 3 (Ipameri and Rio Verde counties), 9 (Jataí County), 10 (Rio Verde County) and 14 (Chapadão do Céu, Campo Alegre de Goiás, Jataí and Mineiros counties). Races 3, 6, 10 and 14 were reported in Southwestern Goiás State (Rio Verde, Montividiu and Jataí counties) in the 2004/2005 crop (Baumgratz et al., 2005). Besides the previously mentioned nematode races (except for race 10), race 4 was also identified in Mineiros County, Southwestern Goiás State, in the 2006/2007 crop (Ribeiro et al., 2006). Figueiredo (2008) identified races 3, 5, 6 and 15 in Jataí County, Goiás State.

According to Silva et al. (1999), the prevalence of race 3 expresses the wildest character, since most populations presented female index (FI%) equal to zero in all differentiator lines. It is worth emphasizing that races 4+ and 14+ differ from classic races 4 and 14 due to their ability to parasitize the North American Hartwig cultivar, which was considered resistant to all nematode races (Dias et al., 2004; Dias et al., 2009).

According to Figueiredo (2008), the existence of more than one *H. glycines* type and race indicates that soybean cultivars used by farmers in commercial plantations exerted significant selection pressure on the existing SCN populations. In addition, cultivating soybean cultivars presenting similar resistance source for more than 2 years also contributes to the selection of new nematode races. The aforementioned conditions are sufficient to change the gene frequency of the pathogen and turn it into a new race that does not recognize the adopted soybean variety as resistant (Santos, 2014) and overrides the cultivar resistance. Therefore, SCN management in this region should take into consideration adopting crop rotation, as well as nematode control strategies other than just the exclusive use of genetic control techniques.

In the present study, some primes did not amplify, or presented low amplification of the DNA fragment but had recorded DNA amplification with at least 3 polymorphic bands per primer in other studies (Silva et al., 2000; Abdelnoor et al., 2001; Lax et al., 2004). The causes of such low amplification remain unknown, since the extracted DNA presented good quality, as well as no signs of degradation and polysaccharide contamination was present. Only 10 primers showed polymorphism, which resulted in 50 amplification products (bands on the agarose gel); 5 bands per primers, on average 46 polymorphic bands (Table 4). The remaining 4 bands showed the same standard in all populations.

We did not expect to find physiological nematode race variations within genotypic groups. Such assumption was based on Abdelnoor et al. (2001), who found 3 distinct groups: the first group comprised of populations classified as race 4; the second one encompassed populations capable of parasitizing the Hartwig cultivar; and the third group was composed of nematode race 9. However, SCN population accession groups with different races appeared in the same group, and it suggested that they were genetically similar in some *loci* or shared regions. This result is justifiable, since the RAPD technique amplified random differences and it was not sensitive enough to detect such a particular difference in our analysis.

There was no positive correlation between geographical (spatial distribution) and genetic distances (similar genotypes). It was possible understanding that *H. glycines* dissemination mechanisms (assured by cysts), agricultural equipment and implements shared by farmers from different counties without previous cleaning (to prevent clods containing the SCN inoculum to be carried from one place to another), and the use of resistant cultivars for several consecutive years enabled nematode race variability in the Railroad Region.

Materials and methods

The experiments were carried out in protected environment at Instituto Federal Goiano – campus Urutaí (Urutaí County – GO) and they were supported by the Plant Physiology and Parasitism Laboratory and by the Molecular Genetics Laboratory (LaGeM) - both laboratories belong to the aforementioned Institute. First, soil samples (5 kg of soil per sample) were collected (0-20 cm down the ground) in commercial soybean cultivation fields in eight different counties belonging to Railroad Region - GO (Figure 3). At least three different properties per county were sampled. The geographic coordinates of the sampled areas were recorded (Table 1) and soil samples were sent to the laboratory for nematological analysis in order to confirm the presence of the cyst nematode in the inoculum to be multiplied.

Genetic diversity (in race) of H. glycines populations

Nematode populations from eight soybean-producer counties (Gameleira de Goiás, Leopoldo de Bulhões, Silvânia, Vianópolis, Orizona, Ipameri, Campo Alegre de Goiás and Catalão) belonging to the Railroad Region in Southeastern Goiás State were used to determine *H. glycines* races. The experimental design was completely randomized, with factorial arrangement 6 (soybean differentiator lines) x 29 (*H. glycines* populations collected in the aforementioned counties) and ten replicates. Populations that did not enable enough inoculum (4,000 nematode eggs and second-stage juveniles (J2)) were not evaluated (Table 1).

Soybean differentiator lines such as Pickett, Peking (PI 548402), PI 88788 and PI 90763, which can be used to characterize up to 16 nematode races, were adopted in the current experiment (Riggs and Schmitt, 1988). Cultivars Lee 74 and Hartwig were used as susceptibile and resistance standard, respectively (Niblack et al., 2002). Soil samples containing *H. glycines* cysts were collected in naturally infested fields (Table 1), placed in ceramic pots and seeded with nematode-susceptible BRS Valiosa RR soybean seeds (Embrapa, 2014) to enable nematode multiplication for 30 days. Next, based on the methodology by Tihohod (2000), female nematodes and, subsequently, eggs were extracted from the root system of the plants to obtain the nematode eggs + J2 suspension to be inoculated.

Seeds of soybean differentiator lines were pre-germinated in cell trays filled with plant substrate and transplanted to tubes containing only autoclaved sand at 120°C for 20 minutes. *H. glycines* populations were inoculated (Riggs and Schmitt, 1988) using an automatic pipette, in the coolest period of the day, at transplanting time. The suspension containing 4,000 nematode eggs + J2 was inoculated per seedling.

The tubes were kept in greenhouse and, thirty days after inoculation, the number of females in the root system of each soybean differentiator line was evaluated. Plant shoots were discarded in the laboratory, whereas roots were washed with a strong water jet on a set of 20- and 60-mesh sieves (Tihohod, 2000) in order to extract females from the root system. The material retained in the 20-mesh sieve was discarded and the one retained in the 60-mesh sieve was collected in a beaker (using a wash bottle) and filtered on filter paper placed over a netting gutter (Andrade et al., 1995). The filtered material was analyzed in a microscope stethoscope (at 15x magnification) to quantify the number of female nematodes.

According to Riggs and Smith (1988), the designation of nematode races is based on the comparison between the number of females in each soybean differentiator line and the number of females in the standard cultivar Lee 74. Thus, the female index (FI) was calculated for each soybean differentiator line. Soybean differentiator lines presenting FI lower than 10% were classified as resistant (-), whereas the ones presenting FI higher, or equal to 10%, were classified as susceptible (+).

Therefore, FI-based differentiator reactions were determined, wherein FI = (mean number of females in the differentiator line / mean number of females in cultivar Lee 74) * 100%. FI was used to characterize the race of each evaluated nematode population, according to Riggs and Schmitt (1988) and HG Type, according to Niblack et al (2002).

Molecular diversity of H. glycines populations

The molecular characterization of *H. glycines* populations was based on 28 previously selected RAPD primers (Table 3). The large number of fragments amplified per primer was used as primer selection criterion based on the literature (Silva et al., 2000; Abdelnool et al., 2001; Lax et al., 2004).

Genomic DNA was collected from female *H. glycines* specimens belonging to each population extracted from the root systems of soybean plants, based on the methodology by Tihohod (2000). Then, 50-100 white *H. glycines* females from each population were collected in a 2 mL Eppendorf tube and frozen at -20°C until DNA extraction time. Nematode populations that did not present this number of specimens were excluded from the experiment; therefore, only 31 SCN populations were evaluated (Table 1). The DNA extraction procedure used 2% CTAB buffer, according to the methodology by Doyle and Doyle (1990), with modifications: stainless steel beads (6 mm) were used to directly macerate female nematodes in an Eppendorf tube filled with liquid nitrogen (N_2).

DNA extracted from 31 SCN populations was quantified by visually comparing bands generated from a series of known phage λ DNA concentrations (50, 100 and 200 ng) on agarose gels (0.8%) and stained with ethidium bromide (0.5 mgL^{-1}) (Figure 4). Next, DNA was diluted in milliq water at use concentration 10 $ng\mu L^{-1}$ and stored at -20°C.

RAPD reactions were assembled to final volume 25 µl. They comprised of 10 mM of Tris-HCL (pH 8.3), 50 mM of KCl, 2.4 mM of $MgCl_2$, 100 µM of each deoxyribonucleotide, 0.4 µM of "primer", 1 U of Taq DNA polymerase enzyme and 30 ng of DNA. PCR reactions were performed in thermocycler programmed for initial denaturation at 94°C for 5 m, which was followed by 45 cycles consisting of denaturation at 94°C for 15 seconds, annealing at 35°C for 30 seconds, extension at 72°C for 1 minute and a final extension at 72°C for 5 m. Amplification products were separated through horizontal electrophoresis in 1.2% agarose gel containing ethidium bromide and submerged in TBE buffer (0.09 M Tris-Borate, 0.002 M EDTA).

RAPD data were evaluated in a binary way, in which values (1) and (0) were attributed to the presence and absence of amplified bands for each primer, respectively. The binary matrix was used to estimate the genetic distance between populations, based on the Jaccard dissimilarity index. The clustering analysis based on the calculated genetic dissimilarity matrix was performed in the R statistical software version 3.0.3 (R Core Team, 2014), according to Ward's method.

Conclusion

Eight different physiological *Heterodera glycines* races (1, 3, 4, 6, 9, 10, 14 and 14+) were found in counties belonging to the Railroad Region in Goiás State. Five genetically-different groups were recorded among *H. glycines* accessions counties, showing high genetic variability rate.

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Conflict of interest

The authors declare no conflicts of interest.

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