SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Characterization and Genetic Relationships Among Brazilian Biotypes of *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) Using RAPD Markers

MARCELO LOPES-DA-SILVA[1,2], GABRIELA E.L. TONET[3] AND LUIZ G.E. VIEIRA[4]

[1]*Depto. Zoologia, Curso de Pós-Graduação em Entomologia, Universidade Federal do Paraná, C. postal 19020, Curitiba, PR*
[2]*Depto. Ciências Agrárias, Campus Universitário, Parada Benito, 98025-810, Universidade de Cruz Alta, Cruz Alta, RS*
[3]*Área de Entomologia, EMBRAPA-Trigo, Passo Fundo, RS*
[4]*Lab. Biotecnologia Vegetal, Instituto Agronômico do Paraná, IAPAR, C. postal 481, 86001-970 Londrina, PR*

Neotropical Entomology 33(1):043-049 (2004)

Caracterização e Similaridade Genética Entre Biótipos de *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) que Ocorrem no Brasil Usando Marcadores Moleculares RAPD

RESUMO - O pulgão-verde-dos-cereais, *Schizaphis graminum* Rondani, é uma das mais importantes pragas de cereais no mundo. Nas populações dessa espécie, podem ser separados vários biótipos, que são clones que compartilham a mesma relação de virulência com plantas cultivadas. Visando diminuir custos e aumentar a rapidez na identificação de biótipos, marcadores moleculares têm sido bastante utilizados para caracterizar geneticamente populações. Com o propósito de encontrar marcadores RAPD para identificar biótipos de *S. graminum*, dezenove populações clonais de três biótipos (B, C e E) do Brasil e três populações clonais oriundas dos Estados Unidos foram examinadas. Dezoito oligonucleotídeos iniciadores foram utilizados para a análise dos clones coletados para este estudo. Apenas seis oligonucleotídeos iniciadores revelaram polimorfismos e dentre estes, apenas três foram diagnósticos para discriminar os três biótipos. Utilizando o índice de similaridade de Jaccard e agrupamento por UPGMA, foi demonstrado que o biótipo B é geneticamente distinto de C e E, e estes são muito relacionados entre si. O biótipo C foi o que apresentou maior diversidade, enquanto que o biótipo E foi o menos diverso. Análise da Variância Molecular (AMOVA) mostrou que populações clonais pertencentes à mesma categoria biotípica têm menor variância genética do que populações clonais agrupados conforme a similaridade geográfica.

PALAVRAS-CHAVE: Variabilidade genética, pulgão-verde-dos-cereais, relação inseto-planta

ABSTRACT - The greenbug *Schizaphis graminum* (Rondani) is one of the most important cereals pests in the world. Within populations of this species, several biotypes, which are clones that share same virulence relationships with cultivated plants, can be distinguished. Molecular markers have been used to genetically characterize insect populations because they are fast and cost effective. In order to find RAPD markers to identify Brazilian *S. graminum* biotypes, nineteen clonal populations of three biotypes (B, C and E) from Brazil and three clonal populations from the U.S. were examined. Eighteen primers were used to analyze the material, but only six primers revealed polymorphisms and among those, three produced diagnostic band profiles that allowed biotype characterization. Using Jaccard Similarity Index and UPGMA clustering method it was possible to show that biotype B is genetically very distinct from C and E, which are closely related to each other. The biotype C showed the greatest genetic diversity, while biotype E was the least diverse. Analysis of Molecular Variance (AMOVA) showed that genetic variance among clonal populations belonging to the same biotype is smaller than among clonal populations grouped according to their geographical similarity.

KEY WORDS: Genetic variability, molecular marker, greenbug, insect-plant relationship

The host-races and biotypes within pest populations are sometimes obstacles to pest management by plant breeding. Biotypes are considered different forms of the same species and represent a genetic variation that is focus of some important questions about evolutionary mechanisms (Futuyma & Peterson 1985). Parthenogenesis reproduction may be related with biotypes occurrence, particularly within aphids (Saxena & Barrion 1987). Differences in survival and

growth are noticeable among biotypes, especially in host resistant strains (Futuyma & Peterson 1985). Different parthenogenetic biotypes have either different geographic distribution or they coexist on a same local (Lowe 1974). In practical terms, biotype usually designates a group of organisms within a species that is distinguished by a particular trait of interest, usually non-morphological, such as adaptation to plant genotypes (Wilhoit & Mittler 1991).

Biotype identification of *S. graminum* is the first step for any cereal-breeding program to obtain resistant cultivars to this pest. Greenbugs are identified and classified as biotypes based on host plant response. Greenbug biotypes are defined by their abilities to damage different plant species and cultivars (Shufran *et al.* 1992). At present, there are ten officially accepted biotypes which are designed by letters: A, B, C, E, F, G, H, I, J and K. The biotype D was differentially assigned based on insecticide resistance, but such designation has been discarded (Puterka & Peters 1990). In Brazil, until 1993, only biotype C had been reported on wheat. Biotypes E and B were first recorded in 1994 and 1996, respectively (Tonet 1999).

Damage in differential hosts is still considered the only reliable way to identify greenbug biotypes. However, this method is difficult to be adapted for Brazilian cropping system, because it depends on growing different winter wheat differential varieties, which are not adapted to tropical and subtropical climates.

Morphometric patterns, cytological studies and isozymes markers have been used as alternative methods to discriminate biotypes. Morphometric variation among biotypes B, C and E was assessed by Fargo *et al*. (1986) and Inayatullah *et al*. (1987). They found that alate and apterous *S. graminum* biotypes form distinct groups when multivariate analysis was applied to a large number of morphological measurements. Isozymes profiles for biotypes B, F and C/E showed distinctive patterns (Abid *et al*. 1989). Measurements of total chromosome length in meiotic metaphase found that biotype differed significantly from biotypes B and C, but B and C did not differ (Mayo & Starks 1972).

Using COI and COII (Cytochrome oxidase subunits I and II) genes of mtDNA, Shufran *et al.* (2000) inferred that *S. graminum* biotypes are a mixture of genotypes belonging to three clades that may have diverged as host-adapted races on wild grasses. Aikhionbare *et al*. (2000), using mtDNA genes, revealed variation in nucleotide sites among seven greenbug biotypes. There was not any nucleotide variation within seven clones of biotype E. Using RAPD (Randomly Amplified Polymorphism DNA), Black IV *et al.* (1992) found distinctive patterns in some biotypes, with exception of C and E. Aikhonbare *et al.* (1998) also reported that is possible to identify *S. graminu*m biotypes using these molecular markers.

Based on historic records, Porter *et al*. (1997) proposed that there is no relationship between the use of resistant varieties and development of new biotypes. Therefore, greenbug biotypes probably are more pre-adapted opportunists than individuals selected by resistant cultivars.

The objective of this work was to select RAPD markers that could be used for quick identification of *S. graminum* biotypes as an alternative to host plant response tests. Also, the genetic variability within each biotype and the genetic relationships among biotypes B, C and E were also assessed.

Material and Methods

***S. graminum* Clones.** Twenty-two clonal populations from biotypes B, C and E were used for this research (Table 1). Clonal populations of biotypes B, C and E collected on wheat fields from the State of Mato Grosso do Sul (MS), Paraná (PR) and Rio Grande do Sul (RS) were obtained from EMBRAPA-Trigo (Passo Fundo, RS). One clonal population of biotype C, obtained from EMBRAPA Milho e Sorgo (Sete Lagoas, MG), was collected from sorghum. Also, clones of biotypes B, C and E were received from ARS-USDA (Agriculture Research Service-USDA, Stillwater, Oklahoma-USA).

Table 1. Collection data of *S. graminum* biotypes from Brazil and United States for biotype characterization.

Collection sites	Host	Biotype codes
Passo Fundo RS	Wheat	B3, C1
Santa Rosa RS	Wheat	C3
Carazinho RS	Wheat	C2
Santo Ângelo RS	Wheat	C4
Cascavel PR	Wheat	C7
Tapera RS	Wheat	C6
São Borja RS	Wheat	C5
Seberi RS	Wheat	E1
Mal. Cândido Rondon PR	Wheat	E2
Tibagi PR	Wheat	C8
Itaporã MS	Wheat	C9
Londrina PR	Wheat	E3
Medianeira PR	Wheat	E6
Panambi MS	Wheat	E4
Caarapó MS	Wheat	B1,B2
Dourados MS	Wheat	E5
Sete Lagoas MG	Sorghum	C10
Stillwater, Oklahoma	-	B4, C11, E7

Biotype Identification. Lesion technique as described by Puterka & Peters (1988), with some modifications, was carried out for biotype identification. The species and cultivars used in this experiment are presented on Table 2. Ten to fifteen seeds of each resistance source were sown on each vase. The vases were transferred to a chamber with controlled conditions (21ºC, 70% relative humidity and 12h photophase). After seven days of sowing, ten one-week old *S. graminum* individuals were placed on the seedlings leaves and allowed to feed for 96h. The insects were eliminated and the plant tissues were inspected for assessing the damage. Plants were considered susceptible when brown necrotic lesions were present and resistant when only chlorosis was observed at the insect feeding site. Each bioassay result represents a mean of four independent assessments.

Table 2. Plant species and genotypes used for biotype characterization of *S. graminum.*

Plant species / genotype	B	C	E
Wheat/DS28A	S *	S	S
Wheat/Amigo	R	R	S
Wheat/Largo	R	R	R
Rye/Insave	R	R	R
Oats/PF91582	R	R	R
Wheat/CI17882	R	R	R
Wheat/BR36	R	R	S
Wheat/BR-1	-	S	S
Barley/Will	S	R	R
Barley/Omugi	S	R	S
Sorghum/KS-30	S	R	S

* R, resistant; S, susceptible; -, no information available

DNA Extraction. DNA was extracted using a protocol suggested by Carvalho & Vieira (2001). Five clones of each population were put in microcentrifuge tubes and frozen in N_2 liquid. The insect bodies were grounded manually and 200 ml buffer extraction (200 mM Tris-HCl pH 8.0; 2 M NaCl; 70 mM EDTA pH 8.0) and 50 ml of sarcosyl were added. After incubation for 30 min. at 65ºC with occasional mixing the extract was centrifuged at 10000 rpm for 15 min. and the supernatant recovered to a new microtube. DNA was precipitated by adding 110 µl ammonium acetate and 250 ml of cold isopropanol to the aqueous supernatant. The solution was left overnight at -20ºC, and then centrifuged for 15 min. at 10000 rpm. The pellet was washed with 70% ethanol, air dried and and resuspended in 25 µl of TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) containing RNase (10 mg/ml), and stored at -20ºC. DNA concentration was estimated by fluorescence using a DyNA Quant 200 minifluorimeter (Hoefer Instruments).

RAPD Reactions. RAPD reactions were performed in 25 µl aliquots containing 25 ng genomic DNA, 2.5 mM buffer, 20 mM Tris (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 100 mM of each dNTP, 0.2 mM primer and 1 U Taq polymerase. The amplifications were performed in a PTC-100™ (MJ Research, Inc.) using the following temperature program: 5 min. at 94º followed by 40 cycles of 1 min. at 94ºC, 90 s at 40ºC and 2 min. at 72ºC, with a final extension of 5 min. at 72ºC. Amplification products were analyzed by electrophoresis at 5V/cm in agarose gel (1.5%). The gel was stained in 0.5 mg/ml ethidium bromide solution and photographed with the KODAK EDAS 120 system.

Data Analysis. Only RAPD fragments (bands) less than 2.5 kb fragments and reproducible in two or more gels were scored and considered for the analysis. A matrix of Jaccard's distances (Sneath & Sokal 1973) were estimated between all pairs of the twenty-one clones of the *S. graminum* biotypes as follows: $S = a / (a + b + c)$ where a was the number of bands present for both clonal population; b the number of bands present for a clonal population 1 but not for clonal population 2, and c, the number of bands present for clonal population 2, but not for clonal population 1. The clustering procedure - UPGMA - Unweighted Pair Group with Arithmetic Mean (Sneath & Sokal 1973) was used to study the genetic relationships among the clones based upon the distance matrix. Analysis of Molecular Variance (AMOVA) (Excoffier *et al.* 1992) was used in order to investigate the magnitude of genetic variation among the biotypes. We excluded USA clonal populations (B4, C11, E7) which only biotype identification data was available and C10 because it was only one population collected in Minas Gerais (MG). Two AMOVA analyses were performed at two-level (clonal populations within regions and clonal populations within biotypes) to assess the contribution of geographical origin to the population genetic variance. We used AMOVA-PREP (Miller 1998) to prepare files to perform AMOVA analyses utilizing the WINMOVA software (Excoffier *et al.* 1992). This analysis produced values for index Φst (Excoffier *et al.* 1992), which is correlated with fixation index (Wright's F-statistics) to quantify the genetic differentiation level derived from population division.

Results and Discussion

Biotype Identification. Nineteen arbitrarily chosen primers were tested. Most of them yielded identical RAPD profiles among all populations and were therefore not useful to identify the different biotypes. Six primers provided polymorphisms, but only primers OPA-3, OPA-7 and OPC-2 were diagnostic to discriminate biotypes B, C and E. OPA-3 primer was able to discriminate biotype B by the presence of a 550 bp fragment, which is absent in C and E, and by the presence of 750 bp fragments, which are present in C and E and absent in B (Fig.1). OPA-7 allowed to discriminate all three Brazilian biotypes, but was not able to distinguish the C and E clonal populations from U.S. (Fig. 2). The primer OPC-2 was able to discriminate biotype C from E by the presence of a 1000 bp DNA fragment in all clones of biotype E that was absent in all C clonal populations examined (Fig.3). Black *et al*. (1992) reported that RAPD markers were not able to distinguish biotypes C and E, what contrasts with the Aikhonbare *et al.* findings (1998), which demonstrated that RAPD was capable to produce distinct polymorphisms for C and for E. The results showed that these biotypes of *S. graminum* from Brazil can be readily identified, even with diagnostic primers different from those used by Aikhonbare *et al*. (1998). This study demonstrated the usefulness of RAPD for discriminating *S. graminum* biotypes B, C and E occurring in Brazil. Compared with the traditional host-plant trial method for biotype identification, RAPD is faster, cheaper and less labor intensive.

Genetic Relationships. The clustering analysis showed three groups: Group I, formed by biotype B clonal populations; Group II, constituted exclusively by biotype C and Group III, formed by clonal populations of biotypes C and E (Fig. 4).

The biotype B was recorded for the first time in Brazil in 1998 (Tonet 1999) and since then, only three clones were observed in 2000 (the same three clones used in this work). These three clones were collected in two very distant collection sites (Caaporá-MS and Passo Fundo-RS). RAPD

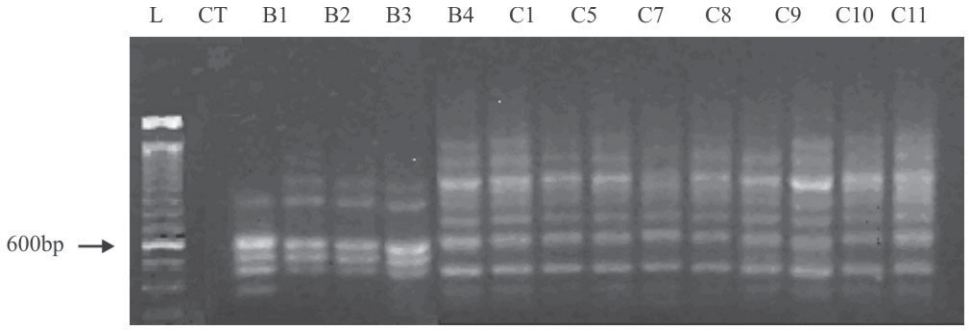

Figure 1. RAPD profile of *S. graminum* biotypes clones using primer OPA-03. White arrows show discriminant marker. L: ladder 100bp, CT: negative control; B1-B4, biotype B clones; C1-C11, biotype C clones; E1- E7, biotype E clones.

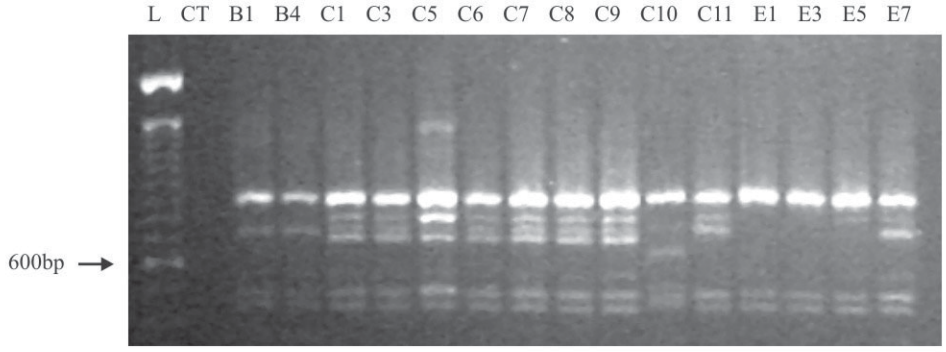

Figure 2. RAPD profile of *S. graminum* biotypes clones using primer OPA-07. White arrow shows discriminant markers. (L) ladder 100bp; (CT) negative control; B1, B4 biotype B clones; C1-C11) biotype C clones; E1-E7 biotype E clones.

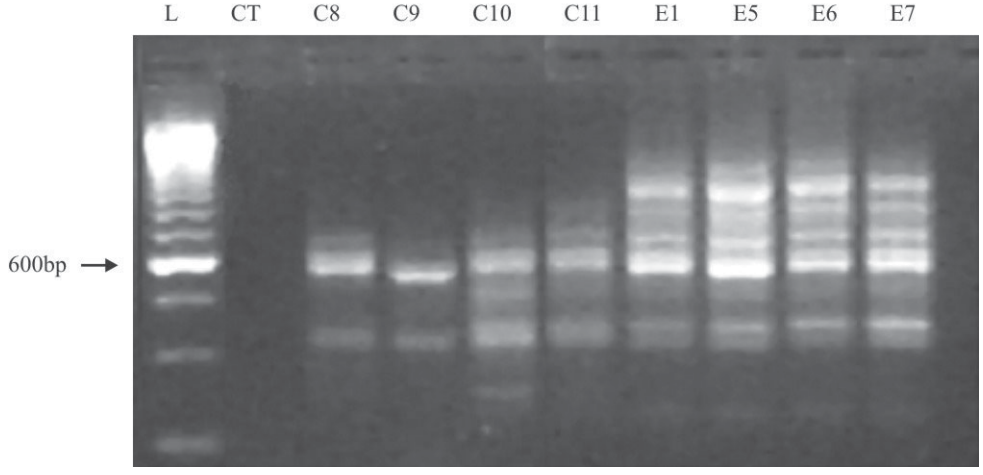

Figure 3. RAPD profile of *S. graminum* biotypes clones using primer OPC-2. White arrows show discriminant markers. (L) ladder 100bp; (CT) negative control; C1-C11 biotype C clones; E1- E7 biotype E clones.

profiles were able to distinguish biotype B from C and E. The clonal populations of biotype B had a small genetic variability. Despite its presence in cereal crops, B biotype is associated mainly with wild hosts (Anstead *et al*. 2002). It has been reported that biotype B is exclusively anholocyclic (Puterka & Peters 1990), which may have contributed for the small genetic variability among biotypes from unrelated geographical origin. In molecular phylogenetic studies (Shufran *et al*. 2000) biotype B is in different clade of the agricultural biotypes (biotypes C-E-I-K).

The biotype C had the largest intra-biotype genetic variability. The biotype C clones used in this study were collected mainly from high latitutes. Association of the band profiles with the geographical origin of the clones was

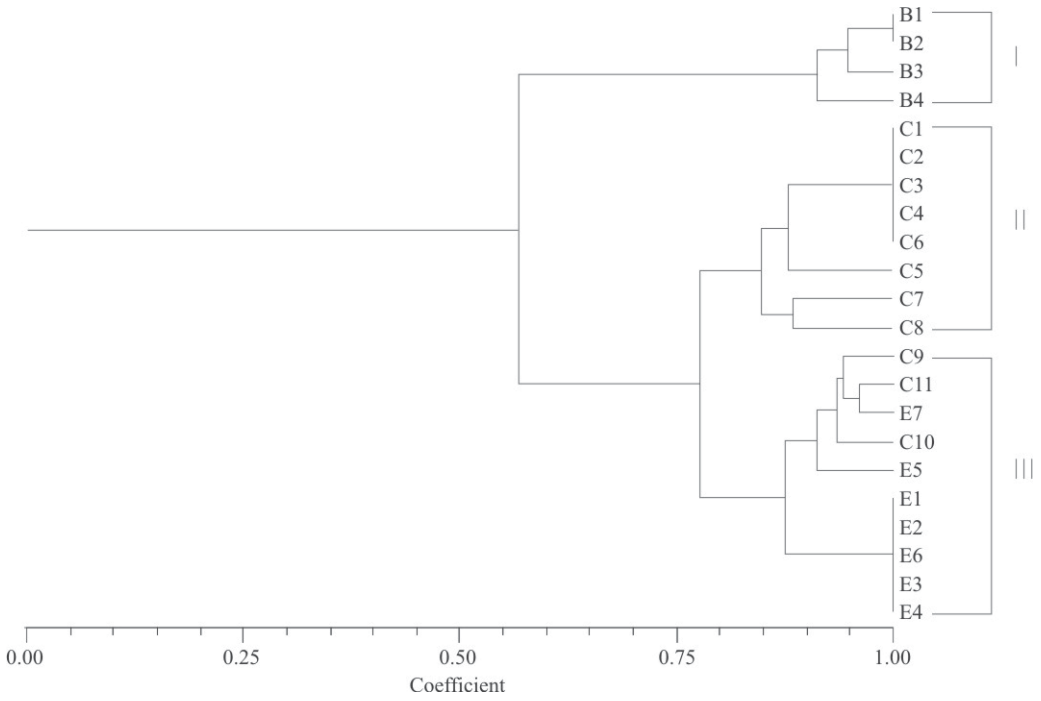

Figure 4. Dendogram of *S. graminum* biotypes B, C and E clustered by UPGMA method based on RAPD bands that was generated using six primer. Scale value of 1.00 indicates 100 % of genetica similarity. Biotype codes according to Table 1.

observed. All clones collected in RS (C1, C2, C3, C4 and C6) had identical RAPD profile, except clone C5. This last clone was collected from a site distant from others. Clones C7 and C8, collected in PR, showed the largest divergence among the C clonal populations within the group II.

All clonal populations of the biotype E and three clonal populations of the biotype C formed the group III. With exception of E5 and E7, all other clonal populations had the same RAPD profile. The C10 clone (Sete Lagoas, MG) was collected from sorghum fields and has a distinctive genetic profile within this group. C11 and E7 clones are populations from laboratory-raised colonies at ARS-USDA (Stillwater, OK) and are close genetically related to each other. Also, C9 from wheat (Itaporã, MS), C10 from sorghum (Sete Lagoas, MG) and C11 from USA showed more genetic similarity with clonal populations of biotype E than with others clones classified as biotype C. As would be expected, the clone of biotype E from USA (E7) presented a small but significant divergence from all Brazilian clones of biotype E. These results are in agreement with previous reports that are showing the lack of genetic differentiation among E clones using RAPD (Black *et al*.1992, Aikhonbare *et al.* 1998). Despite the high similarity, it was possible to find a specific marker for biotype E from USA (Fig. 2) even using a limited number of primers. These results suggest the possibility of finding greater variability in clonal populations of biotype E from different geographical origins with RAPD analysis with increase number of primers. Previously, genetic differentiation among E clones was only obtained using rDNA intergenic spacers, which are extremely variable DNA regions (Shufran *et al*. 1992). The clustering analysis showed that some clonal populations of biotype C are closely related to those of biotype E. Using isozyme analysis, Abid *et al*. (1989) reported indistinctive patterns between C and E. Despite the easy identification of biotypes B, C and E by RAPD, the overall genetic similarities between C and E do not support these biotypes as evolutionary divergent groups.

There was an agreement between these RAPD results and among those obtained with mtDNA studies in regarding relationships to these three biotypes. Shufran *et al*. (2000) using partial sequences of cytochrome oxidase subunits I and II genes of mtDNA obtained evidence for host-adapted races existence within in *S. graminum* with biotype B positioned in a different clade from C and E. Also, Aikhonbare & Mayo (2000) using four other mtDNA genes found that biotype B is less related to C and E. The genetic relationships analysis showed that biotype B seems to be a natural group diverging from biotypes C/E and presents low intra-biotype variation. This is a consequence from genetic isolation, because biotype B is exclusively parthenogenetic (Puterka & Peters 1990).

Molecular Variance Analysis. The analysis of molecular variance at two-level is summarized on Table 3. The component of genetic variance within biotypes was smaller than within states (16,12% and 79,37%, respectively). This implies that biotype, regardless the geographical origin, was the most important factor contributing to genetic similarity among clonal populations. In statistical terms, this could be explained as a consequence of the genetic similarity within clones of biotypes B and E. Despite geographical origins, biotypes B and E have low intra-biotype variability and they are genetically distant from each other. The high value of Φst obtained in the AMOVA when the clonal populations were grouped by biotype indicates high genetic divergence

Table 3. AMOVA for clones of *S. graminum* grouped by collection site within states and biotypes.

Grouping	Genetic variance component (%)		Φ*st*	P value
	Among	Within		
States	20.3	79.4	0.203	< 0.001
Biotype	83.9	16.1	0.839	< 0.0659

between B and C/E group, supporting the observation made by Puterka & Peters (1990) regarding absence gene flow between B and other two biotypes. Although, presenting a smaller Φ*st* value when the clones were arranged within states of origin, a relative geographical population structure was observed, mainly caused by absence of genetic variation within clonal populations of biotype C from RS.

Based on the assumption that majority of cereals aphids populations of RS are originated from northern region, the low genetic variability of biotype C from RS could be a result from population subdivision in the migration process. When a population is divided, each resulting population becomes more homogeneous and divergent from original population due to the founder effect, differential selection or random genetic drift (Hartl & Clark 1997).

Other hypothesis that should be considered is the natural selection caused by natural enemies at local level, which may be responsible for differences in genetic composition of local resident population that subsists in wild hosts or spontaneous crops. Until 1992, according to Salvadori & Tonet (2001), 74% of all populations of parasitoids reared in EMBRAPA for the Brazilian Wheat Aphids Biological Control Program were released in RS. In New York, Henter & Via (1995) reported selection caused by biological control agents in pea aphid, *Acyrtosiphon pisum* (Harris) Great selection pressure by natural enemies would be one of some explanations for this genetic homogeneity of C biotype from RS.

In this paper, we demonstrated that RAPD analysis is useful to identify *S. graminum* biotypes from Brazil using only a limited set of primers. Confirming the results obtained by Aijkhonbare *et al.* (1998) with USA populations, greenbug clones of biotype B occurring in Brazil are genetically very distinct from C and E, and these two biotypes are close related to each other. Analysis of Molecular Variance (AMOVA) showed that *S. graminum* clones belonging to the same biotype have greater genetic similarity than clones grouped by their geographical origin.

Acknowledgments

We are indebted with Dr. David Porter from ARS-USDA for providing wheat seed lines used for biotype discrimination. We also thank Dr. Kevin Shufran, Dr. John Burdon and Melissa Burrows (ARS-USDA) for sending USA biotype clones and Dr. José M. Waquil (Embrapa Milho e Sorgo) for providing biotype C collected from sorghum. A special thanks to Rafael Bonfante (Embrapa-Trigo) for collecting and maintaining the clones and to Dr. Nelson Fonseca Júnior (IAPAR) for his contribution on data analysis.

Literature Cited

Abid, H.S., S.D. Kindler, S.G. Jensen, M.A. Thomas-Crompton & S.M. Spomer. 1989. Isozyme characterization of sorghum aphid species and greenbug biotypes (Homoptera: Aphididae). Ann. Entomol. Soc. Am. 82: 303-306.

Aikhionbare, F.O., K.P. Pruess, K.P & Z.B. Mayo. 1998. Greenbug (Homoptera: Aphididae) biotypes characterized using random amplified polymorphic DNA. Genetic Analysis: Biomolecular Engineering 14: 105-108.

Aikhionbare, F.O. & Z.B. Mayo. 2000. Mitochondrial sequences of greenbug (Homoptera: Aphididae) biotypes. Genetic Analysis: Biomolecular Engineering 16: 199-205.

Anstead, J.A., J.D. Burdon & K.A. Shufran. 2002. Mitochondrial DNA sequence divergence among *Schizaphis graminum* (Hemiptera: Aphididae) clones from cultivated and non-cultivated hosts: haplotype and host associations. Bull. Entomol. Res. 92: 17-24.

Black IV, W.C., N.M. DuTeau, G.J. Puterka, J.R. Nechols & J.M. Pettorini. 1992. Use of random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to detect DNA polymorphisms in aphids (Homoptera: Aphididae). Bull. Entomol. Res. 82: 151-159.

Carvalho, A.O.C. & L.G.E. Vieira. 2001. Determinação das condições ótimas para análises de PCR-RAPD em *Atta sexdens rubropilosa* Forel. (Hymenoptera: Formicidae) Neotrop. Entomol. 30: 593-600.

Excoffier, L., P.E. Smouse & J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction sites. Genetics 131: 479-491.

Fargo,W.S., C. Inayatullah, J.A. Webster & D. Holbert. 1986.. Morphometric variation within apterous females of *Schizaphis graminum* biotypes. Res. Pop. Ecol. 28: 163-172.

Futuyma, D.J. & S.C. Peterson. 1985. Genetic variation in the use of resources by insects. Ann. Rev. Entomol. 30: 217-238.

Hartl, D.L. & A.G. Clark. 1997. Principles of population genetics. 3rd edition. Sunderland (MA), Sinauer Associates, 481p.

Henter, H. & S. Via. 1995. The potential for coevolution I. Genetic variation in susceptibility of pea aphids to a wasp parasitoid. Evolution 49: 427-438.

Inayatullah, C., J.A. Webster & W.S. Fargo. 1987.

Morphometric variations in the alates of greenbug (Homoptera: Aphididae) biotypes. Ann. Entomol. Soc. Am. 80: 306-311.

Lowe, H.J.B. 1974. Intraespecific variation of *Myzus persicae* on sugar beet (*Beta vulgaris*). Ann. Appl. Biol. 78: 15-26.

Mayo, Z.B. & K.J. Starks. 1972. Sexuality of the greenbug *Schizaphis graminum* in Oklahoma. Ann. Entomol. Soc. Am. 65: 671-675.

Miller, M.P. 1998. AMOVA-PREP freeware version 1.01 in mpm@nauvaux.ucc.nau.edu

Porter, D.R., J.D. Burd, K.A. Shufran, J.A. Webester & G.L. Teetes. 1997. Greenbug (Homoptera: Aphididae) biotypes: selected by resistant cultivars or preadapted opportunists. J. Econ. Entomol. 90: 1055-1065.

Puterka, G.J. & D.C. Peters. 1988. Rapid technique for determining greenbug (Homoptera: Aphididae) biotypes B, C, E and F. J. Econ. Entomol. 81: 396-399.

Puterka, G.J. & D.C. Peters. 1990. Sexual reproduction and inheritance of virulence in the greenbug *Schizaphis graminum* (Rondani), p.289-317. In R.K. Campbell & R.D. Eikenbary (eds.), Aphid-plant genotype interactions, Amsterdam, Elsevier, 378p.

Salvadori, J. & G.E.L. Tonet. 2001. Manejo integrado dos pulgões do trigo. EMBRAPA, Passo Fundo, 52p.

Saxena, R.C. & A.A. Barrion. 1987. Bioypes of insect pests of agricultural crops. Insect Sci. Appl. 8: 454-458.

Shufran, K.A., D.C. Margolies & W.C. Black. 1992. Variation between biotype E clones of *Schizaphis graminum* (Homoptera:Aphididae) . Bull. Entomol. Res. 82: 407-416.

Shufran, K.A., J.D. Burd, J.A. Anstead & G. Lushai. 2000. Mitochondrial DNA sequences divergence among greenbug (Homoptera: Aphididae) biotypes: evidence for host adapted races. Insect Mol. Biol. 9: 179-184.

Sneath P.H.A & R.R. Sokal. 1973. Numerical taxonomy; the principle and practice of numerical classification. V. H. Freeman, San Francisco, 537p.

Tonet, G.E.L. 1999. Identificação de biótipos de *Schizaphis graminum* que ocorrem em cereais de inverno no Brasil, p.590-595. Anais da XVIII Reunião Nacional da Pesquisa de Trigo, Passo Fundo.

Wilhoit, L.R. & T.E. Mittler. 1991. Biotypes and clonal variation in greenbug (Homoptera: Aphididae) populations from a locality in California. Environ. Entomol. 20: 757-767.

Received 28/03/03. Accepted 12/10/03.