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Article

Genetic Dissimilarity in *Conyza sumatrensis* Revealed by Simple Sequence Repeat (SSR) Markers

*Dissimilaridade Genética entre Biótipos de **Conyza sumatrensis** Revelada por Marcadores Moleculares Microssatélites*

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ABSTRACT - In view of the rapid evolution of *Conyza sumatrensis* populations resistant to glyphosate, it is necessary to understand the genetic diversity aimed to improve strategies for managing this weed. We investigated the genetic dissimilarity among 15 biotypes of *C. sumatrensis* from different geographic regions using microsatellite loci. The biotypes, were cultivated in a greenhouse to obtain vegetal material for DNA extraction. Nineteen microsatellite markers (SSR), were developed for *C. sumatrensis* biotypes. The genetic dissimilarity was estimated by the Jaccard coefficient (JC) and the biotypes grouped by the UPGMA method. The results demonstrated a high dissimilarity (JC = 7.14 to 82.62) of the analyzed material, with the biotypes forming five groups, being one group formed just by the susceptible biotype and in the others grouped by biotypes from distinct locations in the same group The high genetic diversity of *C. sumatrensis* indicates that the biotypes may show different responses to different management strategies, and that the mechanisms of resistance to herbicides and characteristics of evolution of populations due to adaptability may be some of the factors involved in the genetic variability of the species.

Keywords: polymorphism, SSR, tall fleabane, genetic variability.

*RESUMO - Tendo em vista a rápida evolução das populações de **Conyza sumatrensis** resistentes ao glifosato, é necessário entender a diversidade genética com vistas a melhorar as estratégias de manejo dessa planta daninha. Diante do exposto, objetivou-se com este trabalho investigar a dissimilaridade genética entre 15 biótipos de **C. sumatrensis** de diferentes regiões geográficas usando marcadores moleculares microssatélites. Os biótipos foram cultivados em casa de vegetação, para obtenção de material vegetal para extração de DNA. Dezenove marcadores microssatélites (SSR) foram desenvolvidos para os biótipos de **C. sumatrensis**. A dissimilaridade genética foi estimada pelo coeficiente de Jaccard (JC), e os biótipos, agrupados pelo método UPGMA. Os resultados demonstraram alta dissimilaridade (JC = 7,14 a 82,62) do material analisado, com os biótipos formando cinco grupos. A alta diversidade genética de **C. sumatrensis** indica que os biótipos podem apresentar distintas respostas a diferentes estratégias de manejo e que os mecanismos de resistência a herbicidas podem ser um dos fatores envolvidos na variabilidade genética das espécies.*

Palavras-chave: polimorfismo, SSR, buva, variabilidade genética.

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INTRODUCTION

Weeds regularly adapt to the most varied selection pressures to which they are exposed, including climate, soil, management practices and anthropic disturbances (Powles and Yu, 2010; Begg et al., 2012). The ability to adapt quickly imposes constant challenges to producers and researchers around the world due to the resulting reduction of agricultural production capacity. The best example of adaptation of these plants to management practices is the evolution of resistance to herbicides (Jasieniuk et al., 1996; Powles and Yu, 2010). Herbicide resistance of weeds involves numerous weed species and herbicides, mainly glyphosate.

Of the many species belonging to *Conyza* Less genus, some of them are important weeds that cause economic losses in agriculture due to its worldwide occurrence, resistance to herbicides ans other traids which turn these species into first grade weeds (Bossdorf et al., 2005; Concenço and Concenço, 2016). Among these species, *Conyza sumatrensis* (Retz.) E. Walker (popularly called tall fleabane) stands out. This species is native to South America and has spread to warmer regions of all continents, has an annual or biannual cycle, and reproduces only via seed, mainly by self-pollination (Thebaud et al., 1996; Case and Crawley, 2000; Hao et al., 2009). The economic relevance of this weed is related to the efficient seed dispersal and their prolific seed production; and the presence of resistance to several herbicide mechanism of action (Steckel and Gwathmey, 2009; Heap, 2019).

The occurrence of *C. sumatrensis* biotypes resistant to glyphosate in several regions of Brazil has led to the assumption that there is high genetic dissimilarity between these populations, since the main method of dispersal of *C. sumatrensis* seeds is by the wind, in addition to contamination of machinery and seed lots. To *C. canadensis*, the seeds can regularly disperses at least 500 m from source populations, while a relatively small number of seeds long distances, 99% of the seed was found within 100 m of de source (Dauer et al., 2007). *C. sumatrensis* biotypes resistant to glyphosate have been reported in Brazil, France, Paraguay, Greece and Spain countries. In Brazil, this species was identified with simple resistance to glyphosate in 2010 (Heap, 2019).

Although most populations of *Conyza* spp. are resistant to glyphosate, there are still biotypes that are susceptible to this herbicide. Thus, according to the basic precepts of population genetics, the existence of genetic variability in individuals within a population increases the chance of some individuals responding differently to changes in the environment and, therefore, guarantees preservation of the species (Allendorf and Luikart, 2007). Weed populations with greater genetic dissimilarity may be an obstacle to control because the plants exhibit variable responses to herbicides and/or variable responses to different concentrations of herbicides. Moreover, the occurrence of an unfavorable event can eliminate all susceptible individuals and allow survival of the resistant weeds (Kissmann, 2003). *C. sumatrensis* showed high molecular diversity in resistant biotypes, indicating that these biotypes have a high potential to colonize new areas, which increases its potential as a weed (Marochio et al., 2017).

To study this genetic dissimilarity among weed populations, the use of molecular markers has been employed, since these reveal the variability at the genomic level in a fast, simple and effective way (Ferreira and Grattapaglia, 1995). Microsatellite markers, also called simple sequence repeats (SSR), are molecular markers consisting of one to six base pairs repeated in tandem. These regions often occur in plant genomes and exhibit great variation, and such markers also allow genetic discrimination of closely related individuals, as well as the distinction between populations (Varshney et al., 2005).

Research on *Conyza* spp. was carried out with the objective of obtaining information on the evolution, origin and dispersion of the resistance of the species to glyphosate herbicide (Ren et al., 2010; Okada et al., 2013; Okada et al., 2014; Marochio et al., 2017). In these studies, analysis of the microsatellite markers was based on the presence of multiple alleles per locus favoring the genetic diversity among populations and subpopulations, as is the case of most weeds (Zhang et al., 2009), thereby contributing to management strategies for these plants. The high and low values of genetic similarity between the *C. sumatrensis* biotypes may be used to investigate the efficiency of similar and different strategies, respectively, to control infestations in different areas (Marochio et al., 2017).Therefore, the objective of this study was to investigate the genetic dissimilarity in *C. sumatrensis* biotypes from different regions of Brazil, analyzing polymorphism of microsatellite loci.

MATERIAL AND METHODS

Plant material

The biotypes used in this study are described in Table 1. The seeds were collected from different sites, areas with a history of glyphosate application in burndown and Roundup Ready (RR) soybean crops, where farmers had observed no evidence of weed control after glyphosate application. Fifteen biotypes of *C. sumatrensis* were selected, 14 resistant and one susceptible to glyphosate herbicide (Figure 1). Each plant was placed in a separate paper bag to avoid mixing the seeds from the different collection sites.

The biotypes were sown in April 2016, in a greenhouse at the Universidade de Passo Fundo, in Passo Fundo, State of Rio Grande do Sul, in pots with a volume capacity of 500 mL, at a depth of 0,5 cm. The seeds were irrigated daily and, when the seedlings emerged, these were transferred to other pots with the same characteristics as the previous ones and kept until leaves were collected for DNA extraction. In this plants the herbicide was not applied.

To confirm resistance, glyphosate at a dose of 1440 g a.e. ha^{-1} was applied in a parallel experiment when the plants had reached the stage of 4 to 6 true leaves and were classified according to the response to the herbicide (Rizzardi et al., in press).

Table 1 - Biotypes of *Conyza sumatrensis* used in the study of genetic dissimilarity, collection sites and responses to glyphosate. Passo Fundo - 2017

Byotipe	City	State	Response to glyphosate[(1)]
DIO 01	Diamante do Oeste	PR	R
MIS 01	Missal	PR	R
ITA 01	Itaporã	MS	R
BOM 05	Bonito	MS	R
MAR 02	Maracaju	MS	R
POP 06	Ponta Porã	MS	R
IND 01	Indápolis	MS	R
TUP 02	Tupanciretã	RS	R
SAB 01	Santa Bárbara do Sul	RS	R
JAR 04	Jari	RS	R
CAR 01	Carazinho	RS	R
QUEV 04	Quevedos	RS	R
JUC 05	Júlio de Castilhos	RS	R
BAG 04	Bagé	RS	R
PON 03	Pontão	RS	S

Figure 1 - Geographic location of the biotypes in their respective locations in the states of Mato Grosso do Sul (MS), Rio Grande do Sul (RS) and Paraná (PR). Passo Fundo - 2017.

DNA extraction

DNA extraction was performed at the Embrapa Trigo Biotechnology Laboratory, located in Passo Fundo, Rio Grande do Sul, Brazil, according to the methodology described by Doyle and Doyle (1990). The collection of *C. sumatrensis* leaves took place in September 2016. DNA was extracted from young leaves. Leaves were collected and then conditioned in aluminum foil, duly identified, and packed in a styrofoam box with liquid nitrogen and transported to the laboratory. The DNA was extracted from a total of 120 plants, comprising 15 biotypes with 8 replicates (plants) of each biotype. DNA extracted from several individual plants of the same biotype was combined to produce a single sample (Michelmore et al., 1991).

For DNA extraction, 300 mg of leaf tissue was macerated in liquid nitrogen and conditioned in microtubes, duly identified. Extraction buffer containing 2% CTAB [20 mM EDTA, 100 mM Tris-HCl pH 8.0, 1.4 M NaCl, plus 0.2% 2-mercaptoethanol (1 mL) was added to each microtube. The microtubes were vortexed and incubated at 65 °C in a water bath for 60 min, gently inverting the microtubes every 10 min. Subsequently, the microtubes were removed from the water bath and cooled to room temperature for 5 min. After this procedure, 700 µl of chloroform-isoamyl alcohol prepared in the ratio of 24:1 was added, and the contents were gently stirred for 10 min. After this time, the samples were centrifuged for 10 min at 5,000 rpm and the aqueous phase (supernatant) then transferred to new labeled microtubes. Ice-cold isopropanol (500 µL) was added to the supernatant and incubated at -20 °C for 2 h. The tubes were then centrifuged for 5 min at 10,000 rpm and the supernatant was discarded. The precipitate was first washed with 70% and 95% ethanol and, after discarding these solutions, was left at room temperature for complete drying.

After drying, the precipitate was resuspended in 100 µl of TE buffer, containing 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA (pH 8.0), and gently shaken for complete solubilization of the DNA. Finally, in order to digest the RNA present in the samples, 0.3 µL of RNAse (10 mg mL^{-1}) was added to the samples and left to stand at 37 °C for 30 min and were subsequently stored in an ultra-freezer at -80 °C for subsequent use.

DNA quantification

In order to quantify and verify the integrity of the extracted DNA, the samples were submitted to 0.8% agarose gel electrophoresis, prepared with TBE buffer in 1× concentration, pH 8, with a voltage of 80 V. The samples were compared with phage marcador markers of 50 and 100 ng. For visualization, the gel was stained with 0.5 µg mL^{-1} ethidium bromide. The gel was then photographed under UV light using Gel Doc XR+ digital gel documentation system (Bio-Rad). The samples were also quantified in a Nanodrop UV-visible spectrophotometer to determine the DNA concentration of each sample and, consequently, the dilution required for the polymerase chain reaction (PCR).

Molecular markers and genetic dissimilarity

For the analysis of genetic dissimilarity, 19 microsatellite markers (SSR) developed for the genus *Conyza* were selected from the literature, aiming to obtain the largest number of tags in the genome of *C. sumatrensis* (Table 2). The SSR markers IUB-1, IUB-2, IUB-3, IUB-4, IUB-5, IUB-6, IUB-8 and HNCA-2 (Whitton et al., 1997), were used, but the amplification was not successful and these were removed from the analysis. The PCR amplifications were performed in isolation for each marker in 10 µL of solution containing 0.2 µM of each primer or primer oligonucleotide (F = forward and R = reverse), 0.2 mM dNTP, 2.5 mM $MgCl_2$, 0.75 U Taq DNA polymerase, 1× buffer and 100 ng DNA from each study biotype.

PCR reactions were conducted in a GeneAmp Thermal Cycler 9700 (Applied Biosystems-ABI) using the following program: one cycle at 94 °C for 3 min; 5 cycles of 94 °C for 1 min, 60 °C for 1 min (decreasing at 1 °C per cycle down to 55 °C), 72 °C for 1 min; 30 cycles of 94 °C for 1 min, 55 °C for 1 min; and a cycle of 72 °C for 10 min. The amplification products were separated on 2% agarose gel for approximately 2 h at 110 V and visualized using a Gel Doc XR+ (Bio-Rad) digital gel documentation system. The ladder marker used was 100bp.

Table 2 - Relationship between microsatellite markers (primers), nucleotide sequence (F = forward and R = reverse) and concentrations used in the amplification reaction of DNA samples from *Conyza sumatrensis*. Passo Fundo – 2017

Primer	Nucleotide sequence	Volume per 10 µL of reaction
HW02[(1)]	F: AGTATTTGGCAATCAAAATTCG R: TCACAATCACAAACAACACAAA	0.18 µL
HW04[(1)]	F: GCCACCCTATTGTTTTGGTTAT R: AACTTGCATGGTAGTCAACGTC	0.18 µL
HW06[(1)]	F: CTTGCATGGTAGTCAACGTCAT R: CAGAGGTGGTCATGTGATGTG	0.18 µL
HW07[(1)]	F: GTGTGGCGCTACTCATTTCC R: TGATCACACCTGCGATTTGT	0.38 µL
HW14[(1)]	F: AAACTAAGGGTGATTGGGGAAT R: TGGTAGCCAAAAAGCTACAAA	0.25 µL
HW21[(1)]	F: ATAGTCGAATTGGTCACGATTTG R: GCAGTTTTCACTCTTCTCTCGAA	0.18 µL
HW27[(1)]	F: TTTCATAGTCGAATTGGTCACG R: CCGGTAGCAGTTTTCACTCTTC	0.20 µL
HW29[(1)]	F: CTACTTGTTCAATTTATCCATAC R: AAACTGGTTACTTCTCTTCC	0.38 µL
HWSSR01[(2)]	F: TATGTTGTACGACTGACTGAGTC R: CCATTGACTGTAGACCAGTGTG	0.25 µL
HWSSR03[(2)]	F: TTGACTCCAACTCGTAGTGTATG R: ACGTTAAATCTCTCGTGTCCTTC	0.20 µL
HWSSR04[(2)]	F: GGAAAACTCCTGTCATAGTATTAGC R: ATTAAAATCTAGCAAGGCCGAAC	0.20 µL
HWSSR09[(2)]	F: CATGAGTTTGAGTTATCCCAGAT R: CGAATACTTTCAATGCTTACGAC	0.18 µL
HWSSR11[(2)]	F: ATCGTTGACATCTGACTCTGC R: GATTCTTGCTCTGGTTCCTTG	0.20 µL
HWSSR12[(2)]	F: CATAACAAACGGATTAGTGGCG R: ATTATTGACGACCAACAACACC	0.25 µL
IUB 7[(3)]	F: TATAAACTGCCCCTTCTCTCC R: GAGGTTGAAGCATGCAGTTC	0.35 µL
OSU 1[(3)]	F: ACAAGTCGGCTGGTGAGC R: ACATGAAACACGAGCTAAACCA	0.18 µL
OSU 2[(3)]	F: CACTTCTCACACTTTGGGCA R: CCAAATAATTACCATCATGCCA	0.18 µL
OSU 4[(3)]	F: AGAACTGGCAGCTTGGAAAA R: GTCCAAATGGTGGAAAACTACC	0.18 µL
HNCA 1[(3)]	F: TTGGAGATGTGTTTGTGTTCTAGAG R: ACCTACACCTTAGTTAAACCTTGCC	0.18 µL

Source: [(1)] Wang et al. (2008). [(2)] Okada et al. (2014). [(3)] Whitton, Rieseberg and Ungerer (1997).

The genetic dissimilarity between the biotypes was estimated by the Jaccard coefficient (Jaccard, 1991). The biotypes were grouped using the UPGMA clustering method (Unweighted Pair-Group Mean Average), and the biotypes were considered as operative taxonomic units (OTUs) and as binary characters the bands obtained by the markers. The program used for data analysis was Genes Package (Cruz, 1998). The value of the polymorphism information content (PIC) was determined using the following formula:

$$PIC = 1 - \Sigma Pij^2$$

where Pij^2 is the allele j^{th} frequency, encompassing all alleles per locus (Nei, 1973).

RESULTS AND DISCUSSION

The methodology used in this study, described by Doyle and Doyle (1990) and using leaves as the biological material, was efficient in producing quality DNA for amplification using microsatellite primers and the results of these amplifications were reproducible.

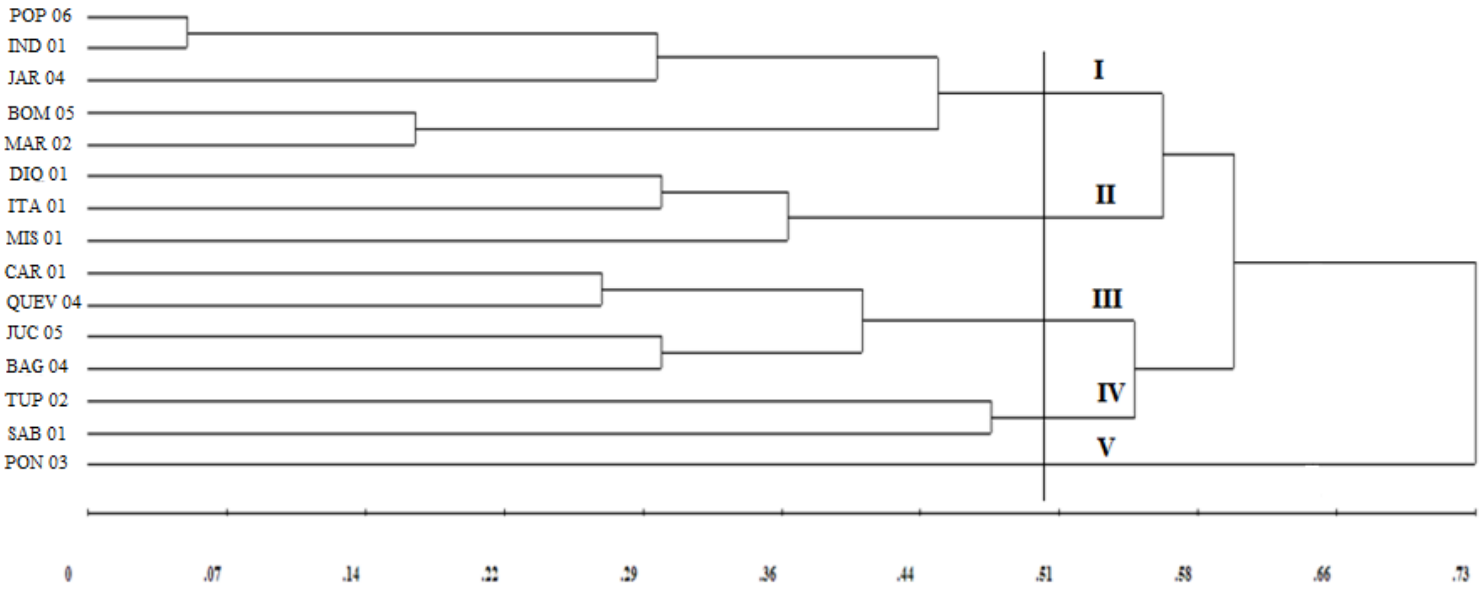
For the analysis of genetic dissimilarity, 19 molecular markers of the microsatellite type were used, of which 16 (84.2%) presented polymorphism (Table 2). Sixteen markers were as follows: HW02, HW04, HW06, HW07, HW14, HW21, HW27, HW29, HWSSR01, HWSSR03, HWSSR09, HWSSR11, HWSSR12, OSU 2, OSU 4 and HNCA 1. Thus, the 16 primers were selected for having defined bands and reproducibility of the fragments between the amplified samples.

Results were obtained from analysis of the gels regarding the presence or absence of each allele for each marker, followed by allele frequency. These were compiled by the UPGMA clustering method (which deals with the average link between groups) and were used to obtain the Jaccard matrix (Figure 2) and the dendrogram (Figure 3) in order to identify the relationship of genetic dissimilarity between all the biotypes.

Biotype	POP 06	IND 01	JAR 04	BOM 05	MAR 02	DIO 01	ITA 01	MIS 01	CAR 01	QUEV 04	JUC 05	BAG 04	TUP 02	SAB 01	PON 03
POP 06	0														
IND 01	0.4231	0													
JAR 04	0.3044	0.32	0												
BOM 05	0.5185	0.5172	0.36	0											
MAR 02	0.6897	0.5172	0.4231	0.1739	0										
DIO 01	0.6786	0.6333	0.5556	0.4	0.3333	0									
ITA 01	0.6071	0.6667	0.5926	0.44	0.375	0.0526	0								
MIS 01	0.5769	0.6667	0.5926	0.6552	0.6552	0.52	0.5	0							
CAR 01	0.6071	0.7273	0.6667	0.7188	0.7188	0.5556	0.5385	0.48	0						
QUEV 04	0.5769	0.6207	0.6429	0.6071	0.5556	0.3182	0.2857	0.5	0.48	0					
JUC 05	0.6071	0.6452	0.6667	0.5862	0.5862	0.5	0.48	0.5926	0.5185	0.4167	0				
BAG 04	0.5769	0.7097	0.7333	0.6552	0.6552	0.6296	0.6154	0.6667	0.5385	0.56	0.2727	0			
TUP 02	0.6552	0.6452	0.5714	0.5862	0.5862	0.5	0.5385	0.5926	0.4615	0.6429	0.3333	0.4167	0		
SAB 01	0.6429	0.7188	0.6071	0.6667	0.6667	0.5926	0.5769	0.5769	0.5	0.6296	0.375	0.52	0.3044	0	
PON 03	0.8214	0.875	0.7857	0.75	0.7931	0.7308	0.72	0.6667	0.7857	0.8148	0.6923	0.8148	0.64	0.4286	0

The numbers in the first row and first column correspond to DIO 01, MIS 01, ITA 01, BOM 05, MAR 02, POP 06, IND 01, TUP 02, SAB 01, JAR 04, CAR 01, QUEV 04, JUC 05, BAG 04 and PON 03.

Figure 2 - Jaccard matrix demonstrating the genetic distances between the 15 biotypes evaluated. Passo Fundo – 2017.



Biotypes: POP 06, IND 01, JAR 04, BOM 05, MAR 02, DIO 01, ITA 01, MIS 01, CAR 01, QUEV 04, JUC 05, BAG 04, TUP 02, SAB 01 and PON 03. The cut-off point around 0.50 indicates the formation of the different groups.

Figure 3 - Dendrogram of genetic distance obtained from the Jaccard coefficient for the 15 evaluated biotypes. Passo Fundo – 2017.

The cophenetic correlation coefficient obtained was 0.84, indicating that the results obtained were efficient, since this analysis verifies the measure of distortion between the values of the initial matrix of dissimilarity and those derived from the dendrogram. It should be emphasized that the estimate of cophenetic correlation ranges from zero to one; the higher the value of this coefficient, the more representative is the dendrogram in relation to the genetic distances matrix (Cruz et al., 2011).

The dendrogram demonstrated the dissimilarity of the analyzed material, in which the highest dissimilarity value was observed between DIO 01 and TUP 02 (82.62%) and the lowest between POP 06 and IND 01 (7.14%). Five groups were formed using the formula proposed by Mojena (1977), obtaining a cut-off value of 0.50. Of the five groups formed, group I contained the biotypes POP 06 (MS), IND 01 (MS), JAR 04 (RS), BON 05 (MS) and MAR 02 (MS). Group II was formed by the biotypes DIO 01 (PR), ITA 01 (MS) and MIS 01 (PR). Group II was composed of the biotypes CAR 01 (RS), QUEV 04 (RS), JUC 05 (RS) and BAG 04 (RS).

The group IV was formed by the biotypes TUP 02 (RS) and SAB 01 (RS). Group V only contained the biotype PON 03 (RS), which was notable as this was the only susceptible biotype studied. Formation of the distinct groups indicates the genetic dissimilarity of the studied materials, because in group I there were four biotypes from MS and one from RS, in group II two biotypes from PR and one from MS. In groups II and IV, all the biotypes came from RS. However, the genetic dissimilarity values were high (Figure 3), confirming the high genetic dissimilarity of the biotypes. The average coefficient of similarity obtained was 54.24%, confirming the high diversity among the analyzed biotypes from the microsatellite markers used. The number of alleles varied from one to four, with the mean number of alleles being 2.68 (Table 3).

The results obtained in the dendrogram show some relationship between the genetic dissimilarity obtained as a function of the geographical distribution of the biotypes. However, it is noted that there is genetic distance between individuals that were geographically closer, and in the same way there is genetic proximity between geographically distant individuals. For example, biotypes from Quevedos, Tupanciretã and Júlio de Castilhos have a genetic distance index of 55 and 65% (Figure 3). These results are closely related to the high genetic variability in the species. In comparison to the species of *C. canadensis*, *C. bonariensis* and *C. sumatrensis*, the highest number of alleles was found in *C. sumatrensis*, which is possibly the species with the greatest genetic diversity among the three studied (Marochio et al., 2017).

The PIC values ranged from 0.0 to 0.68 and the mean value was 0.44. Microsatellite markers used to study genetic variability in tomato populations found a mean PIC of 0.63, with a maximum value of 0.81 and a minimum of 0.17 (Aguirre et al., 2017). Moreover, the lower mean value reported in the present study is due to the greater amount of polymorphic markers used in comparison to the others studies, which explains the mean value of lower PIC. Also, the value of 0.44 corroborates the results obtained by Jaccard's coefficient. This is because PIC is an indicator of a marker's informative ability in genetic studies (segregation, population identification, and paternity control). Calculation of PIC depends on the number of alleles, their frequencies and the sample size. According to the classification of Botstein et al. (1980), markers with PIC values greater than 0.5 are considered to be very informative, values between 0.25 and 0.50 informative, and values lower than 0.25 not very informative. The numbers of amplified alleles in each of the 16 primers analyzed are presented in Table 3. The highest numbers of alleles observed were for the primers HW21, HWSSR12 and OSU 2, presenting four alleles in the samples from *C. sumatrensis*.

Table 3 - Polymorphic markers used, number of alleles, approximate fragment size, and polymorphism information content (PIC). Passo Fundo – 2017

Primer	Number of alleles	Approximate fragment size (pb)	PIC
HW02	3	188	0.55
HW04	3	211	0.45
HW06	3	230	0.56
HW07	1	229	0
HW14	1	209	0
HW21	4	174	0.53
HW27	3	183	0.67
HW29	1	216	0
HWSSR01	2	176	0.45
HWSSR03	2	190	0.48
HWSSR09	3	202	0.59
HWSSR11	3	190	0.48
HWSSR12	4	273	0.68
OSU 2	4	17	0.75
OSU 4	3	225	0.26
HNCA 1	3	102	0.65

The reproduction system of the species also exerts influence on the genetic differentiation between biotypes. For self-pollinating species, higher genetic variability is expected, mainly due to the low crossing rate between the individuals (Hamrick and Godt, 1996). However, as the studied specie is self-pollinating, the high genetic dissimilarity found in the evaluated populations indicates that there is a greater chance of finding individuals that respond differently to the selection pressure exerted by the environment, such as the application of herbicides, so that this guarantees species preservation (Allendorf and Luikart, 2007). It is also worth mentioning that, in weeds populations with high genetic dissimilarity, there is a greater chance of selecting individuals that will not suffer an adaptive cost due to deleterious effects, guaranteeing the preservation and perpetuation of these individuals (Marochio et al., 2017). The genetic structuring of *C. sumatrensis* populations may indicate that different populations require different management strategies for successful weed control.

The formation of four groups of resistant biotypes with a genetic distance of 50% may indicate that the action of the herbicide on selection of resistant *C. sumatrensis* biotypes is not selecting the same alleles or the same frequency of these alleles in the loci microsatellites analyzed in a uniform manner in resistant populations. Glyphosate-resistant biotypes of *C. sumatrensis* from Paraná (Brazil) also revealed high genetic variability (Marochio et al., 2017), but the susceptible biotypes remained together with herbicide-resistant biotypes, contrary to what we have shown.

With regard to *C. canadensis*, which has been studied with the objective of investigating the evolution and dissemination of glyphosate resistance, Okada et al. (2013) confirmed the existing relationship with high diversity and the herbicide resistance selection, and the genetic variability founded in the resistant biotypes of *C. sumatrensis* in our study corroborates with this results. Thus, it is possible that the pre-existing genetic diversity in the studied populations, together with different management practices adopted, may influence a heterogeneous selection process in the resistant biotypes.

The mechanisms of resistance to glyphosate in *Conyza* spp. may be related to the genetic variability found in the species. This factor could be related to or unrelated to the site of action of the herbicide (Sammons and Gaines, 2014). Thus, for *Conyza* spp., the mechanisms of resistance reported in the literature involve the over-expression of EPSPs, sequestration in the vacuole, differential translocation and metabolism of the herbicide (Feng et al., 2004; Ge et al., 2010; Gonzáles-Torralva et al., 2012). In this way, it becomes evident that there are different mechanisms of resistance associated with glyphosate which may influence the process of differentiated selection of resistant populations.

There is genetic variability among *C. sumatrensis* populations from different geographic regions in Brazil, including biotypes which are susceptible or resistant to glyphosate. The mechanisms of resistance to herbicides and characteristics of evolution of populations due to adaptability may be some of the factors involved in the genetic variability of the species.

Thus, based on the results of this study, it is clear that different biotypes from different locations may respond differently to the imposed selection pressure. Thereby, each situation must be addressed with specific strategies, including the elaboration of management strategies and the choice of herbicides.

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