

Genetic Variability of *Brachiaria ruziziensis* Clones to *Collaria oleosa* (Hemiptera: Miridae) Based on Leaf Injuries

Daniela Maria da Silva¹, Jair Campos de Moraes¹, Alexander Machado Auad^{2*},
Marcy das Graças Fonseca², Fausto Souza Sobrinho²

¹Department of Entomology, Universidade Federal de Lavras, Lavras, Brazil; ²Entomology Laboratory, Embrapa Dairy Cattle Research Station, Juiz de Fora, Brazil.

Email: *amauad@cnpgl.embrapa.br

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ABSTRACT

Collaria oleosa causes leaf injuries to the main forage grasses used for cattle feeding in Brazil. The aim of this work was to determine resistance of *B. ruziziensis* clones against *C. oleosa*. Eighty *B. ruziziensis* clones were maintained in greenhouse, in which *C. oleosa* natural infestations have been occurring in previous years. After 40 days, damage score and chlorophyll content reduction were assessed for all clones. By using these parameters, genetic gain was estimated based on REML/BLUP mixed models. We observed significant differences for damage scores and chlorophyll content reduction among *B. ruziziensis* clones, evidencing genetic variability in this forage specie in regard to resistance against *C. oleosa*. Gain derived from selection of the 10 best clones was 18.2% and 5.80% when considering the damage score and chlorophyll content reduction, respectively. The clones CNPGL BR 10, CNPGL BR 64, CNPGL BR 97 and CNPGL BR 40 presented the highest genetic gain for both damage score and chlorophyll content reduction, and then they will be selected to continue the *B. ruziziensis* breeding program with the possibility of maximizing genetic gain for the next generations.

Keywords: Signal Grass; Bug; Damage Score; Chlorophyll Content

1. Introduction

Grasses of *Brachiaria* genus are agronomically well-adapted to several types of soils [1], being characterized by their higher flexibility in use, management and tolerance to several limitations and/or adverse conditions when compared to other species of forage grasses [2].

Among *Brachiaria* species cultivated in Brazil, *B. ruziziensis* is diploid and it reproduces sexually, allowing selection and recombination of superior genotypes [3]. Additionally, this species is of high palatability and quality. On the other hand, this species is susceptible to insect pests, such as the grass bug *Collaria oleosa* (Distant, 1863) (Hemiptera: Miridae), which is widely distributed within South America including several Brazilian States [4].

This grass bug has been causing serious damage to the

shoot of *B. ruziziensis*. Grass bug adults and nymphs cause partial or total leaf lamina desiccation, compromising the yield and plant nutritional value [5]. Under favorable conditions, grass bug populations can reach high levels within a short period of time [6]. Yet *C. oleosa* injuries often occur in *Brachiaria* and elephant grass pastures, producers and technicians are not able to associate the injury to the causing agent which leads to gradual increase in *C. oleosa* population every year, due to the use of wrong strategies to solve the problem.

Recently, Auad *et al.* [7] reported that injuries related to grass bugs were caused by the stylet insertion in leaf epidermis through stomata which leads to pigmentation loss. Plant defensive response against insect attack is an important tool to select resistant genotypes that will be less damaged by insect pests. The selection is possible given the high genetic variability in *B. ruziziensis* available by the breeding program from Embrapa Gado de

*Corresponding author.

Leite, Brazil [8]. Furthermore, we believe that the use of resistant varieties is an important component of integrated management of the grass bug by helping to keep its populations below economic threshold levels.

Therefore, the aim of this work was to assess the genetic variability and the resistance of *B. ruziziensis* clones to *C. oleosa*. The selection of resistant parents will contribute to the advance of breeding program of this grass.

2. Material and Methods

It was assessed 80 clones originated from *B. ruziziensis* breeding program of Embrapa Gado de Leite (Brazil), along with the controls *B. brizantha* cv. Marandu (resistant), *B. decumbens* cv. Basilisk and *B. ruziziensis* cv. Kennedy (susceptibles). These grasses were selected as controls in the experiment because they are resistant and susceptible to spittlebug [9,10]; and there is no previous knowledge about the resistance against *C. oleosa* damage.

To obtain seedlings, plants were reproduced by cloning (cuttings) and cultivated in greenhouse. After 30 days, the plants were transplanted to pots of 1 kg containing a mix of soil, sand and manure (3:1:1) and kept in the greenhouse (4.0 m high × 4.5 m wide × 6.5 m length) of Embrapa Gado de Leite/Brazil, which had presented issues in keeping *C. oleosa* natural infestations in previous years. Before starting the experiment, the plant height was standardized by trimming clones to reduce to 10 cm high.

After 40 days that clones were in contact with *C. oleosa*, percentage of damaged leaf area was assessed by two independent evaluators using a score scale of 1 - 5 based on visual pattern, being 1 = no damage, 2 = 25% of injury in leaf area, 3 = 50% of injury in leaf area, 4 = 75% of injury in leaf area and, 5 = leaf area totally desiccated [10]. To assess chlorophyll content reduction (CCR), a portable chlorophyll meter SPAD (Soil Plant Analysis Development) was used to measure injured leaves (IL) and non-injured leaves (NIL) picked at random. The measurement was done in the mid-third of a full-expanded leaf, in a total of ten samples per plant. The percentage of chlorophyll content reduction was given by the formula $CCR (\%) = (NIL \times IL / NIL) \times 100$.

The experiment consisted of three replicates in a completely randomized design. The data in chlorophyll content reduction and damage score were tested by analysis of variance and means were compared by Scott Knott test at 5% of probability using the software SISVAR 5.1 (Universidade Federal de Lavras-Minas Gerais, Brazil). Pearson's correlation for the chlorophyll content reduction and damage score was estimated using the software BioEstat 3.0 (Universidade Federal de Belém, Brasil).

In order to estimate the genetic gain using the collect-

ed data (damage score and chlorophyll content reduction), the statistical analysis was based on REMUBLUP mixed models, carried out in the software *Selegen-RemlBlup*, in accordance with Resende [11]; Resende and Dias [12]. The statistical model was given by: $y = Xb + Zg + e$; in which y , b and e are data vectors of fixed effects (blocks), random effects (genotype accesses) and random errors, respectively; X and Z are incidence matrices for b and g , respectively.

3. Results and Discussion

We observed significant differences for damage scores ($F = 1.490$, $P = 0.013$) and chlorophyll content reduction ($F = 1.288$, $P = 0.049$) among *B. ruziziensis* clones (**Table 1**), evidencing genetic variability in this forage species for resistance against *C. oleosa*. This variability will be used in breeding program of *B. ruziziensis* grass aiming at selecting genotypes that have both favorable agro-nomical traits and resistance against the grass bug.

Damage inflicted by *C. oleosa* in the score scale ranged from 1.50 to 4.66, being clone CNPGL BR 10 the lowest rating and CNPGL BR 50 the highest rating. Thirty-six clones had the damage score significantly low (1.50 to 2.87), including the three control cultivars (*B. brizantha*, *B. decumbens* and *B. ruziziensis*). Forty-four clones were suitable hosts to grass bug as they had a high damage score (3.00 to 4.66) (**Table 1**). Surprisingly, the spittlebugs-susceptible controls were resistant to the mirid *C. oleosa*. This might have occurred because spittlebugs feed on the host plant xylem and phloem, while grass bugs feed on leaf parenchyma.

From the clones studied, CNPGL BR 19; CNPGL BR 4; CNPGL BR 26; CNPGL BR 58 were reported by Souza Sobrinho *et al.* [3] as being resistant to spittlebugs based on damage scores. This method therefore seems to be promising to identify and select resistant clones to both pests and these clones might be used in future breeding cycles.

From all the tested genotypes, 62.5% obtained the lowest chlorophyll content reduction, with means lower than 25.21% in comparison with other tested accesses (**Table 1**). As these clones have not lost much chlorophyll content in injured leaves, they were less affected by herbivory and we predict that losses would be consequently lower in terms of yield and forage quality. Within this group, we can observe that the genotypes CNPGL BR 3; CNPGL BR 30; CNPGL BR 64 showed chlorophyll content reduction of 0.78%; 7.06% and 8.75%, respectively. On the other hand, in 37.5% of *B. ruziziensis* clones, chlorophyll content reduction was significantly high in a way that clones CNPGL BR 91; CNPGL BR 68 and CNPGL BR 74 had chlorophyll content reduction of 41.64%; 42.59% and 44.80%, respectively. There was a significant lower chlorophyll content

Table 1. Score damage (1 to 5) and chlorophyll content reduction (%) of *B. ruziziensis* clones and controls.

Clones	Damage Score	Chlorophyll Content Reduction	Clones	Damage Score	Chlorophyll Content Reduction
CNPGL BR 2	2.66 a	2.57 a	CNPGL BR 49	2.25 a	27.14 b
CNPGL BR 3	2.50 a	0.78 a	CNPGL BR 50	4.66 b	34.35 b
CNPGL BR 4	2.66 a	15.82 a	CNPGL BR 52	3.50 b	26.88 b
CNPGL BR 5	3.25 b	28.28 b	CNPGL BR 53	2.50 a	18.15 a
CNPGL BR 6	3.60 b	25.21 a	CNPGL BR 54	3.20 b	26.40 b
CNPGL BR 7	3.00 b	18.19 a	CNPGL BR 55	3.00 b	19.40 a
CNPGL BR 8	2.50 a	24.19 a	CNPGL BR 56	3.08 b	22.31 a
CNPGL BR 9	2.50 a	15.30 a	CNPGL BR 57	3.33 b	26.57 b
CNPGL BR 10	1.50 a	10.21 a	CNPGL BR 58	2.80 a	16.40 a
CNPGL BR 11	3.00 b	18.40 a	CNPGL BR 60	3.50 b	27.87 b
CNPGL BR 15	3.33 b	14.05 a	CNPGL BR 61	3.50 b	25.16 b
CNPGL BR 13	3.50 b	29.90 b	CNPGL BR 62	4.16 b	26.52 b
CNPGL BR 14	3.25 b	22.77 a	CNPGL BR 63	3.25 b	36.62 b
CNPGL BR 17	3.25 b	19.03 a	CNPGL BR 64	2.33 a	8.75 a
CNPGL BR 18	3.00 b	22.02 a	CNPGL BR 66	3.33 b	23.33 a
CNPGL BR 19	2.83 a	33.38 b	CNPGL BR 68	3.33 b	42.59 b
CNPGL BR 20	2.50 a	22.18 a	CNPGL BR 69	3.50 b	29.73 b
CNPGL BR 21	2.75 a	30.80 b	CNPGL BR 70	2.70 a	27.36 b
CNPGL BR 23	3.00 b	21.24 a	CNPGL BR 72	3.00 b	17.04 a
CNPGL BR 24	2.66 a	27.27 b	CNPGL BR 73	2.50 a	19.15 a
CNPGL BR 25	1.75 a	24.09 a	CNPGL BR 74	3.50 b	44.80 b
CNPGL BR 26	2.16 a	21.43 a	CNPGL BR 75	2.70 a	12.90 a
CNPGL BR 27	2.50 a	15.67 a	CNPGL BR 76	2.87 a	25.56 b
CNPGL BR 29	3.00 b	11.98 a	CNPGL BR 77	3.33 b	31.07 b
CNPGL BR 30	3.25 b	7.06 a	CNPGL BR 79	4.00 b	30.96 b
CNPGL BR 32	3.25 b	20.23 a	CNPGL BR 80	2.66 a	13.40 a
CNPGL BR 33	2.41 a	19.00 a	CNPGL BR 82	2.83 a	18.91 a
CNPGL BR 34	2.83 a	27.86 b	CNPGL BR 83	3.10 b	19.43 a
CNPGL BR 35	3.40 b	16.59 a	CNPGL BR 84	3.41 b	23.80 a
CNPGL BR 36	3.50 b	35.53 b	CNPGL BR 89	3.83 b	33.54 b
CNPGL BR 37	3.66 b	13.05 a	CNPGL BR 90	3.08 b	21.13 a
CNPGL BR 38	2.83 a	34.73 b	CNPGL BR 91	3.00 b	41.64 b
CNPGL BR 39	3.30 b	16.95 a	CNPGL BR 93	3.08 b	17.96 a
CNPGL BR 40	2.50 a	13.67 a	CNPGL BR 96	2.20 a	19.40 a
CNPGL BR 41	3.25 b	22.23 a	CNPGL BR 97	2.40 a	10.17 a
CNPGL BR 42	3.25 b	37.15 b	CNPGL BR 99	2.83 a	36.16 b

Continued

CNPGL BR 43	2.80 a	24.41 a	CNPGL BR 100	3.20 b	18.40 a
CNPGL BR 44	3.00 b	20.59 a	<i>B. brizantha</i>	1.62 a	21.14 a
CNPGL BR 46	2.75 a	38.89 b	<i>B. decumbens</i>	2.60 a	18.18 a
CNPGL BR 48	2.80 a	18.15 a	<i>B. ruziziensis</i>	2.70 a	27.77 b

reduction in the controls *B. decumbens* and cultivar Marandu (*B. brizantha*), but not in *B. ruziziensis* (Table 1).

From the 36 genotypes classified in the group of lowest damage score, 66.7% showed the lowest chlorophyll content reduction of insect-injured leaves. Therefore, it was clear that there is a positive significant correlation ($t = 3.6639$; $P = 0.0004$) between the visual damage assessment and chlorophyll content measures of *C. oleosa* injured leaves. The positive correlation between insect leaf damage and chlorophyll content reduction allows a direct selection method. Thus, it is possible to estimate *C. oleosa* damage only by means of plant chlorophyll content readings without relying on visual and subjective damage score assessment. Furthermore, chlorophyll content readings are easy and fast and therefore it allows assessment of a high number of plant materials with low costs and no detriment in the data quality.

By analyzing the parameters damage score and chlorophyll content reduction in the current study, we found that there is a great genetic variability among *B. ruziziensis* clones in regard to resistance against *C. oleosa*. The same parameters were also used for selection of soybean [13,14] and millet [15] that were resistant to stinkbugs. Diaz-Montano *et al.* [16] used the same parameter and equipment to measure the chlorophyll content in order to separate resistant wheat plants against the aphids *Aphis glycines* Matsumura. They showed that susceptible wheat accesses had low chlorophyll content and, consequently decreased photosynthetic capacity.

Given the genetic variability of *B. ruziziensis* clones, it was possible to estimate genetic parameters and genetic gain from selection using damage score and chlorophyll content reduction (Tables 2 and 3). Juhász *et al.* [17]; Abreu *et al.* [18]; Faria Neto *et al.* [19]; Missio *et al.* [20]; Costa *et al.* [21]; Costa *et al.* [22] also used the genetic parameters and genetic gain with from selection to select the best genotypes of several crop plant based on plant height and diameter.

The individual heritability (h^2g) was estimated between 0.16 and 0.14 for both assessed traits (Table 2), and it is considered moderate according to the classification proposed by Resende [11]. Heritability indicates the degree by which individuals pass their traits over the generations and it can also infer about the genetic control of traits. In this sense, a high heritability value indicates that there is a good chance of genetic gain from selection

Table 2. Values of genetic parameters for chlorophyll content reduction (CCR) and damage score (DS) for *B. ruziziensis* clones.

Variable	DS	CCR
Vg	0.0027	22.34
Ve	0.013	133.92
Vp	0.016	156.26
h2g	0.16 ± 0.07	0.14 ± 0.06
h2mc	0.54	0.50
Acclon	0.73	0.70
CVgi%	14.28	6.24
CVe%	32.07	15.28
CVr%	0.44	0.41
Average	36.83	75.70

Vg: genotypic variance; Ve: environmental variance; Vp: phenotypic variance for individuals; $h^2g = h^2$: genotypic heritability for individuals; h^2mc mean genotypic heritability for individuals; Acclon: accuracy; CVgi%: coefficient of genotypic variation; CVe%: coefficient of environmental variation; CVr = CVg/CVe = coefficient of relative variance.

[23].

The mean heritability for clones was higher than 50% for damage score and chlorophyll content reduction (Table 2) showing that the variability of plant resistance trait against *C. oleosa* can be transferred to next generations. Also, these results indicate the efficiency of selection within *B. ruziziensis* for plant resistance trait. Similarly, Sharma *et al.* [24] also reported a high heritability for the first (50%) and the second generations (82%) of a sorghum resistance trait against the fly *Atherigona soccata* (Rondani).

According to Juhász *et al.* [17], accuracy (Acclon) refers to the correlation between the expected genetic values and the true genetic value of an individual. The higher the accuracy to a given individual, the higher is the reliability of the assessment and the expected genetic value, as well as the higher is the gain from selection. In the current work, we estimated in 73% for the damage score and 70% for the chlorophyll content reduction of insect-injured leaves (Table 2), reinforcing the reliability of the results.

It was found higher values of environmental variance (Ve) in comparison to the genotypic variance (Vg) for

Table 3. Values of expected genetic gain for damage score (DS) and chlorophyll content reduction (CCR) of *B. ruziziensis* clones and controls.

Chlorophyll content Reduction				Damage Score			
Clones	Gain	clones	Gain	Clones	Gain	clones	Gain
3	9.05	20	3.05	<i>B. brizantha</i>	41.8	55	-5.67
97	8.19	41	-2.97	10	38.63	82	-5.48
29	7.53	14	-2.9	64	32.14	19	-5.32
75	7.07	18	-2.83	26	28.34	90	-5.16
64	6.77	25	-2.75	25	26.03	68	-4.97
40	6.51	8	-2.67	96	23.7	93	-4.8
30	6.29	2	-2.6	33	21.93	7	-4.64
10	6.11	66	-2.53	97	20.52	63	-4.48
72	5.97	76	-2.45	73	19.35	17	-4.32
27	5.8	43	-2.38	40	18.16	23	-4.15
93	5.65	52	-2.3	49	17.1	11	-4.02
53	5.52	61	-2.23	53	16.21	60	-3.88
37	5.37	49	-2.15	9	15.39	100	-3.75
58	5.25	6	-2.08	<i>B. decumbens</i>	14.69	83	-3.58
33	5.13	60	-2.01	24	14.01	72	-3.45
80	5.03	62	-1.93	3	13.38	5	-3.31
35	4.94	57	-1.86	20	12.81	52	-3.18
39	4.84	34	-1.79	8	12.32	56	-3.07
15	4.76	54	-1.72	27	11.86	42	-2.93
9	4.65	24	-1.64	2	11.43	30	-2.82
48	4.55	5	-1.57	48	11.02	29	-2.69
<i>B. decumbens</i>	4.46	21	-1.5	75	10.64	77	-2.55
7	4.37	79	-1.43	46	10.26	84	-2.42
4	4.29	70	-1.36	<i>B. ruziziensis</i>	9.91	36	-2.31
100	4.22	<i>B. ruziziensis</i>	-1.29	38	9.55	32	-2.17
90	4.14	69	-1.21	91	9.23	54	-2.04
73	4.05	13	-1.14	80	8.9	57	-1.9
26	3.98	77	-1.06	4	8.6	15	-1.79
32	3.9	36	-0.98	70	8.31	69	-1.66
96	3.83	63	-0.9	99	8.03	66	-1.52
83	3.77	42	-0.81	21	-7.79	35	-1.38
<i>B. brizantha</i>	3.69	19	-0.73	41	-7.52	13	-1.25
56	3.62	89	-0.65	14	-7.3	61	-1.11
11	3.55	46	-0.56	44	-7.06	79	-0.98

Continued

44	3.48	50	-0.45	34	-6.84	39	-0.84
82	3.41	37	-0.39	76	-6.62	37	-0.71
17	3.35	89	-0.3	43	-6.43	89	-0.54
55	3.28	6	-0.22	18	-6.24	6	-0.38
84	3.21	62	-0.12	74	-6.03	62	-0.19
23	3.13	50	0.00	58	-5.86	50	0.00

both characteristics. None of the characteristics showed coefficient values of relative variation above one (**Table 2**). According to Vencovsky [25], for experiments with two or three replicates, values for the ratio CV_{gi}/CV_e closer to one mean higher chances of gain from progeny selection.

Numbers in **Table 3** demonstrate the improvement of population average for the assessed trait. In regard to DS (damage score), gain was substantially higher compared to those assessed for CCR (chlorophyll content reduction). The material assessed in this work showed, on average, that gain varied between 41.80% and 0% for damage score and 9.05% and 0% for chlorophyll content reduction (**Table 3**). It is expected that desirable alleles will build up in the population over the selection cycles and then there will be a higher number of resistant plants to *C. oleosa*.

The cultivar Marandu (*B. brizantha*) and the clones CNPGL BR 10, CNPGL BR 64, CNPGL BR 26, CNPGL BR 25, CNPGL BR 96, CNPGL BR 33, CNPGL BR 97, CNPGL BR 73, CNPGL BR 40 were classified as the best ten given their high genotypic values for score damage. Values of gain from selection were for some of these clones higher than 18.2% (**Table 3**). Regarding chlorophyll content reduction, the gain from selection of the best genotypes (CNPGL BR 3, CNPGL BR 97, CNPGL BR 29, CNPGL BR 75, CNPGL BR 64, CNPGL BR 40, CNPGL BR 30, CNPGL BR 10, CNPGL BR 72, CNPGL BR 27) were higher than 5.80% (**Table 3**).

For damage score, the extension between averages was 0.77 (**Table 3**). The cultivar Marandu (*B. brizantha*) presented, on average, 2.61 for damage score and the gain from selection was 41.80% based on the genotypic values. The extension between chlorophyll content reduction averages was 0.35 and the genotype CNPGL 3 showed the highest average (4.13) and gain from selection of 9.05% (**Table 3**), considering the genotypic values. All controls presented genotypic values higher than the overall average for damage score, what was not true only for chlorophyll content reduction of *B. ruziziensis* cv. Kennedy (**Tables 3**).

We reported considerable genetic variability in *B. ruziziensis* resistance against *C. oleosa* herbivory. The

genotypes CNPGL BR 10, CNPGL BR 64, CNPGL BR 97, CNPGL BR 40 showed the highest values of genetic gain from selection for damage score and chlorophyll content reduction.

4. Conclusion

The genotypes CNPGL BR 10, CNPGL BR 64, CNPGL BR 97, CNPGL BR 40 therefore will be selected for continuing *B. ruziziensis* breeding program with a possible increase in gain over the next generations.

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