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NOTES

Genetic variability in *Brachiaria ruziziensis* for resistance to spittlebugs

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ABSTRACT - *The aim of this study was to evaluate the resistance of* B. ruziziensis *clones to the spittlebug species* Deois schach *and* Mahanarva spectabilis. *Together with the cultivar Marandu (*B. brizantha*) and the progenies 77s, 85s and 106s as controls, 71* B. ruziziensis *clones were evaluated in a randomized block design. Six spittlebug eggs about to hatch were artificially infested and 40 days later evaluated for the percentage of surviving nymphs and plant damage based on a rating scale. Analysis of variance identified significant differences for nymph survival and plant damage of both spittlebug species. Since nymph survival and damage scores of the clones 100, 95, 19, 42, 93, 4, 92, 47, 26, 63, and 58 were similar to Marandu, these can be considered resistant to insect pests. These results, besides demonstrating the existence of genetic variability for a possible selection, offer hope for an identification of* B. ruziziensis *spittlebug-resistant genotypes.*

Key words: Forage breeding, *Mahanarva spectabilis, Deois schach,* plant resistance.

INTRODUCTION

Forage grasses of the genus *Brachiaria* were introduced in Brazil in the mid-1960´s and rapidly expanded throughout the country due to their great adaptability to various soil types, aside from the favorable agronomic characteristics that make their cultivation attractive (Wenzl et al. 2001 and Wenzl et al. 2003, Rao et al. 2006).

Among the Brachiaria species grown in Brazil, *B. ruziziensis* is the only diploid species and has sexual reproduction, allowing selection and recombination of superior genotypes (Souza Sobrinho 2005). However, despite the good palatability and forage quality, it is susceptible to spittlebugs (Lascano and Euclides 1996, Keller-Grein et al. 1996, Souza Sobrinho 2005, Sotelo et al. 2008).

Spittlebugs are considered the main pests of forage crops, particularly of the genus *Brachiaria* (Miles et al. 2006, Sotelo et al. 2008). This strong concern is due to the severe damage caused by the sucking of nymph and adult insects from the host plant, among other factors. At high population densities, adult bugs can induce the death of the above-ground part of plants, reducing dry matter and forage quality. Among the most frequent species in southeastern Brazil are those of the genera *Mahanarva* (Auad et al. 2007) and *Deois*. Since the chemical control of insect pests is economically unfeasible, the search for spittlebug-resistant genotypes is the most indicated control method.

After years of research on the interaction of insect pest species with *B. decumbens*, *B ruziziensis* and *B. brizantha*, the first two were determined as standards of susceptibility and the latter of resistance (Valério et al. 1997, Cardona et al. 2004). However, considering the genetic variability within *B. ruziziensis* for yield-related and forage quality traits (Souza Sobrinho et al. 2009)

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the search for genotypes less susceptible to spittlebug infestation should be prioritized. The purpose of this study was therefore to evaluate the resistance of *B.ruziziensis* clones to the spittlebug species *Deois schach* and *Mahanarva spectabilis*.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse of Embrapa Gado de Leite, in Juiz de Fora (MG), where 71 *B. ruziziensis* clones were evaluated, together with the resistant control cultivar Marandu (*B. brizantha*) (Miles et al. 2006) and the progenies 77s, 85s and 106S, selected as resistant in previous trials. The experiment was arranged in a completely randomized block design, with five replications. Each unit (PVC tube, diameter 5 cm, height 8 cm) contained one plant.

Seedlings were produced by cloning hydroponic plants grown in a greenhouse. Upon removal, the tillers were transplanted into plastic tubes (35 cm^3), containing commercial pine bark substrate. After 30 days these plants were planted in rearing units of spittlebugs, containing a substrate mixture of soil, sand and manure (1: 1:1).

Adult insects of the two species *D. schach* and *M. spectabilis* were collected in the field and the greenhouse and placed in cages in the Entomology Laboratory of Embrapa Gado de Leite (Juiz de Fora, MG) to produce eggs, in a climatic chamber (28 °C, RH 70 ± 10% and 14h photoperiod) until near hatching. Plants were infested by applying six spittlebugs eggs to each rearing unit.

Forty days after hatching the experiment was evaluated by counting the nymph survivors and assessing plant damage, based on a pattern of a visual 1 - 5 grade scale: 1 = no damage, 2 = 25% affected leaf area, 3 = 50% affected leaf area, 4 = 75% affected leaf area and, 5 = entire leaf area affected and dry (Cardona et al. 1999).

Based on the results of this first evaluation, clone plants with similar nymph survival and plant damage rate as the resistant standard (Marandu - *B. brizantha*) were selected. These genotypes were evaluated again with the two insect species, by the methodology described above.

In both experiments, the data collected were transformed into $(x+1)^{0.5}$, subjected to analysis of variance and the means compared by the Scott and Knott test ($P < 0.05$).

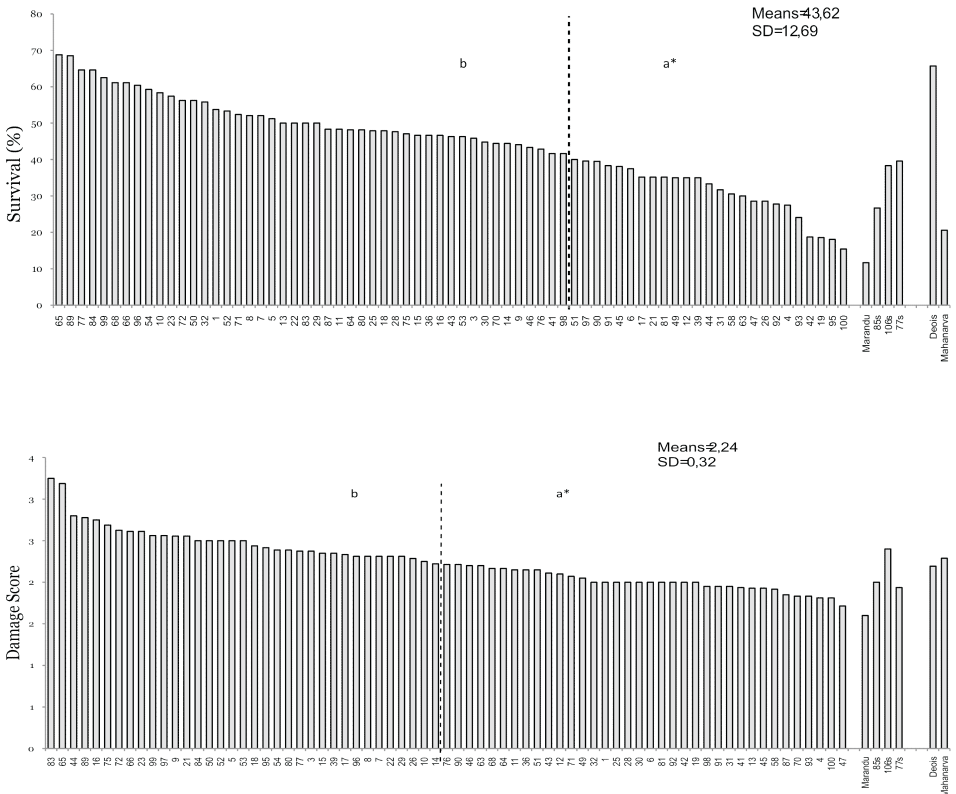
RESULTS AND DISCUSSION

The analysis of variance identified significant differences of the clones, for nymph survival as well as damage level, showing the existence of genetic variability among *B. ruziziensis* clones for resistance to *Mahanarva spectabilis* and *Deois schach*. The effect of spittlebugs was also significant indicating that these influenced the clones differently. The interaction between clones and species was however not significant for both traits, showing that the performance of the *B. ruziziensis* clones under the attack of the insect pests studied is consistent. For this reason, and due to the need for identification and selection of genetic resistance to the different species, the results are presented and discussed based on the means of the two spittlebug species evaluated.

The Scott-Knott test separated the genotypes into two distinct groups of mean spittlebug survival. The group with lower survival means contained 25 clones (100, 95, 19, 42, 93, 4, 92, 47, 26, 63, 58, 31, 44, 39, 49, 12, 81, 17, 21, 6, 45, 91, 90, 97, and 51), along with the resistance standard cv. Marandu (*B. brizantha*) (Miles et al. 2006). Nymph survival of these genotypes did not reach 40% and they were in the mean 63.7% superior to the 46 clones classified in the worst group and 6.8% better than the control mean (Figure 1).

According to Cardona et al. (1999), only genotypes with nymph survival below 30% are considered resistant to insect pests. According to this standard, the clones 100, 95, 19, 42, 93, 4, 92, 47, 26, and 63 may also be considered resistant to insect pests, aside from cv. Marandu (*B. brizantha*). The clone with the lowest nymph survival had a very similar mean (15.42%) to Marandu (11.67%).

In the visual assessments of plant damage the amplitude of variation of the means was 1.38, corresponding to 61.6% of the overall experimental mean, separated into two distinct groups by the Scott-Knott test (Figure 1). The group with least damage consisted of 37 clones, plus cv. Marandu, and the control progenies 85 and 77. Low damage symptoms observed in these progenies confirmed the previous results. The mean of the group of best clones was very similar to that of the controls, indicating the potential of the genotypes tested for spittlebug resistance (Figure 1).



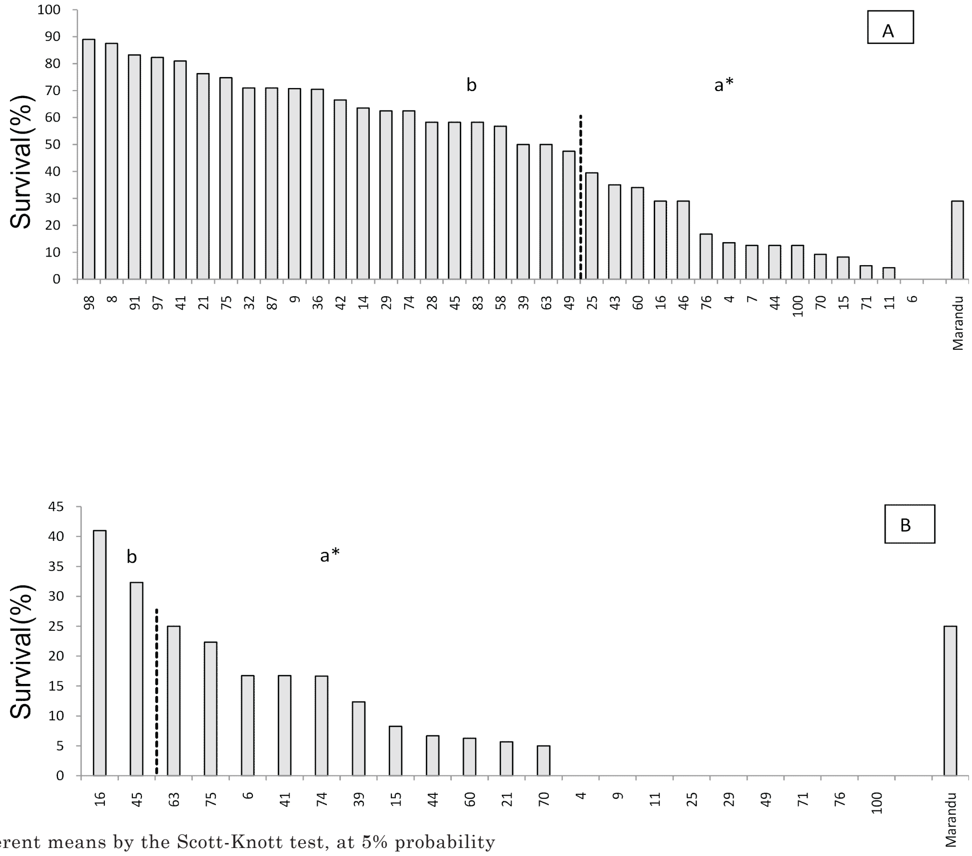
* a and b - different means by the Scott-Knott test, at 5% probability
** SD = standard deviation.

Figure 1. Means of nymph survival (%) and plant damage scores of *M. spectabilis* and *D. schach* on different *B.ruziziensis* clones

In terms of nymph survival rate and also the plant damage caused by these insects, the clones 100, 95, 19, 42, 93, 4, 92, 47, 26, 63, and 58, along with the controls Marandu and progeny 85s performed best (Figure 1). These genotypes did not only belong to the best group identified by Scott-Knott, but the values for each trait were below the mean and the respective standard deviation. According to the criterion of nymph survival mentioned by Cardona et al. (1999), these genotypes can be considered spittlebug- resistant. In the practice, the association between low nymph survival and little damage observed in plants is highly favorable, since plant drying, which is the main visual symptom of the insect attack, and the consequent reduction of forage available to animals are avoided.

The results of the second experiment, in which the best clones of the first were evaluated, confirmed the resistance of most genotypes (Figure 2). The insects caused no damage to the plants that would have allowed a visual detection, so only nymph survival was evaluated. In most cases, low percentages of nymph survival of both spittlebug species (*D. schach* and *M. spectabilis*) were observed. For *D. schach*, 91% of the clones were classified in the same group as the resistant control, with an average percentage of nymph survival always below 25%, equal to the value observed for cv. Marandu. Of these genotypes, the nymph survival rate of nine clones was 0 (zero) (Figure 2). The mean nymph survival of *M. spectabilis* clones on *B. ruziziensis* was 47.4%, against 29% on Marandu. Of the 37 clones tested, 15 were statistically similar to that cultivar, with a mean of 17.4% nymph survival (Figure 2). For both spittlebug species, mean nymph survival of the clones classified in the best group by the Scott-Knott test (Figure 2) was lower than of cv Marandu and can therefore be considered resistant, according to the criteria of Cardona et al. (1999).

By recurrent selection in an interspecific population of *Brachiaria* from the crossing of *B. ruziziensis* (tetraploidized), *B. decumbens* and *B.*



* a and b - different means by the Scott-Knott test, at 5% probability

Figure 2. Means of nymph survival (%) of *M. spectabilis* (A) and *D. schach* (B) on different *B. ruziziensis* clones in the second experiment

brizantha, Miles et al. (2006) was able to identify genotypes that were more resistant to spittlebug (*Aeneolamia varies*) than Marandu; though only in the fifth selection cycle. In the sixth cycle, clones with lower average damage and nymph survival than the control were identified for the three tested spittlebug species (*A. varies*, *A. reducta*, and *Zulia carbonaria*). The results of the present study provide evidence of the existence of genetic variability, allowing selection, but also offer hope for the identification of *B. ruziziensis* genotypes resistant to spittlebugs, the main forage pest, in the short term. This would correct the main limiting factor to an expanded use of this crop by producers, the spittlebug susceptibility (Pereira et al. 2001, Souza Sobrinho 2005).

The results obtained in these experiments involving *M. spectabilis* and *D. schach* must be confirmed in field trials, possibly involving other spittlebug species. The existence of *B. ruziziensis* clones with great potential for genetic improvement for spittlebug resistance was however confirmed. In addition, efforts should be made to link this resistance to other important traits such as yield and forage quality, for which genetic diversity has already been proven (Souza Sobrinho et al. 2009).

The results involving *B. ruziziensis* breeding reveal great possibilities for the selection of genotypes with greater forage potential, which could surpass even the cultivars of most widespread use in Brazil (Souza Sobrinho et al. 2009). Nevertheless, the problem of spittlebug susceptibility, the main limiting characteristic against a broader use of this species as forage, had not been solved so far (Souza Sobrinho 2005). The results of this study show that there is also variability for this trait and indicate the possibility of selection of resistant genotypes in the short term.

These findings are at odds with Miles et al. (2006) who mentioned that this grass species has a narrow genetic base. In the cited study, the authors point out the existence of alleles that negatively affect nymph development of spittlebugs on grasses of African origin (among them *Brachiaria*), although the physical and biochemical bases of this resistance are not known. The identification of clones with low rates of nymph development and plant damage, similar to cv. Marandu, indicate the presence of these same alleles in *B. ruziziensis*. It is noteworthy that this species is the only of its kind produced in Brazil that is diploid and sexual; after the identification of resistant genotypes, intercrossing and the breeding of improved populations are therefore possible (Souza Sobrinho 2005). Through successive cycles of evaluation and selection in recurrent selection, the frequency of favorable alleles in the population can be increased (Ramalho et al. 2000), raising the chances of identification of spittlebug-resistant genotypes. The combination of this trait with higher yields and the acknowledged palatability and forage quality (Souza Sobrinho 2005, Miles et al. 2006) will make this species more attractive for farmers, with potential gains in animal performance (meat and milk) due to the quality of this forage.

CONCLUSIONS

There is great genetic variability among *B. ruziziensis* clones for spittlebug-resistance. The clones 100, 95, 19, 42, 93, 4, 92, 47, 26, 63, and 58 are resistant to *M. spectabilis* and *D.schach*.

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Variabilidade genética em *Brachiaria ruziziensis* para a resistência às cigarrinhas-das-pastagens

RESUMO - *Objetivou-se avaliar a resistência de clones de* B. ruziziensis *em relação às espécies de cigarrinhas* Deois schach *e* Mahanarva spectabilis*. Foram avaliados 94 clones de* B. ruziziensis*, juntamente com a cultivar Marandu (*B. brizantha*) e as progênies 77s, 85s e 106s, utilizadas como testemunhas, em DBC. Efetuou-se a infestação artificial com seis ovos de cigarrinhas próximos à eclosão e foram avaliadas, 40 dias após, a porcentagem das ninfas sobreviventes e o dano causado às plantas. Foram detectadas diferenças significativas para a sobrevivência ninfal e os danos às plantas para as duas espécies de cigarrinhas. Os clones 100, 95, 19, 42, 93, 4, 92, 47, 26, 63 e 58 mostraram sobrevivência de ninfas e notas de dano semelhantes à cultivar Marandu, e podem ser considerados resistentes aos insetos-praga. Esses resultados, além de evidenciarem a existência de variabilidade genética, permitem vislumbrar a identificação de materiais de* B. ruziziensis *resistentes às cigarrinhas-das-pastagens.*

Palavras chave: melhoramento de forrageiras, *Mahanarva spectabilis*, *Deois schach*, resistência de plantas.

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