Male and female sporogenesis and gametogenesis in apomictic Brachiaria brizantha, Brachiaria decumbens and F1 hybrids with sexual colchicine induced tetraploid Brachiaria ruizienis

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Summary

A calendar of female sporogenesis and gametogenesis was made for both apomictic tetraploid (2n = 4x = 36) Brachiaria brizantha and Brachiaria decumbens and their apomictic F1 hybrids with sexual tetraploid (2n = 4x = 36) Brachiaria ruizienis. Microgametogenesis was used as a reference. Apospory was facultative in both species and hybrids. Environmental conditions had variable effects on the level of apomixis according to each genotype. Ratios of segregation into sexuals and apomicts in the interspecific hybrids suggest an oligogenic determinism with dominant apomixis in the genus Brachiaria. Highly apomictic and partially male fertile hybrids were identified and will be used in an improvement program to transfer genes for apomixis into the sexual species B. ruizienis.

Introduction

This research is part of an improvement program dealing with the transfer of gene(s) for apomictic reproduction from the two apomictic species Brachiaria brizantha (Hochst) Stapf and Brachiaria decumbens Stapf to Brachiaria ruizienis Germain & Evrard, an important tropical forage grass in the tribe Paniceae (Heering, 1989).

Induced tetraploid (2n = 4x = 36) B. ruizienis, used as the female parent and crossed with these two tetraploid (2n = 4x = 36) apomictic species resulted in interspecific F1 hybrids among which some were partially male and female-fertile (Lutts et al., 1991). Identification of fertile apomictic F1 hybrids would allow us to envisage their utilization as pollen parent in future backcrosses to B. ruizienis. Some should be superior plants morphologically close to the female progenitor and express both hybrid vigour and apomictic reproduction.

The potential for apomictic reproduction in Brachiaria needs a thorough study of megasporogenesis and embryo sac development. Gobbe et al. (1982) previously made such a study on diploid and induced tetraploid sexual B. ruizienis. In our study of apomictic species B. decumbens and B. brizantha, the different steps of megagametogenesis have been observed chronologically in relation to microgametogenesis. A calendar was established and should help in identifying early apomictic and sexual development.

The second aim of this research was to analyse cytologically and identify the mode of reproduction of F1 hybrids. The ratio of segregation into apomicts and sexuals among the interspecific hybrids may provide valuable information on the genetical determinism of apomixis within the genus Brachiaria. Most studies (Harlan et al., 1964; Taliaferro & Bashaw, 1966; Bashaw, 1980; Savidan, 1982; Dujardin & Hanna, 1989) suggested a simple mono or oligogenic determinism for apomixis in many genera of Paniceae and Andropogoneae.

Finally, the cytological analysis of the apomictic F1 hybrids should allow us to assess the effects of hybridization on the expression of apomictic reproduction.
Table 1. Quantitative characteristics of megagametogenesis of apomictic Brachiaria brizantha, B. decumbens and F1 hybrids with B. ruziziensis

<table>
<thead>
<tr>
<th>Species or hybrid</th>
<th>Number (per ovule)</th>
<th>Percentages ovule with multiple ES**</th>
<th>sterility</th>
<th>min. sex.</th>
<th>max. pot. sex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aposporic initials</td>
<td>gametophyte</td>
<td>mature gametophyte</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. brizantha (g*)</td>
<td>1-4</td>
<td>3.0</td>
<td>1.4</td>
<td>70</td>
<td>20</td>
</tr>
<tr>
<td>B. brizantha (nc*)</td>
<td>1-12</td>
<td>3.9</td>
<td>2.3</td>
<td>93.9</td>
<td>3</td>
</tr>
<tr>
<td>B. decumbens (g)</td>
<td>3-11</td>
<td>3.1</td>
<td>1.75</td>
<td>69.1</td>
<td>13.2</td>
</tr>
<tr>
<td>B. decumbens (nc)</td>
<td>3-9</td>
<td>2.7</td>
<td>1.8</td>
<td>67.4</td>
<td>6</td>
</tr>
<tr>
<td>F1(B) (g)</td>
<td>5-15</td>
<td>3.2</td>
<td>2.3</td>
<td>88.4</td>
<td>11.4</td>
</tr>
<tr>
<td>F1(D) (g)</td>
<td>4-10</td>
<td>2</td>
<td>1.5</td>
<td>63.5</td>
<td>4.2</td>
</tr>
</tbody>
</table>

* g: greenhouse.
* nc: natural conditions.
** ES: embryo sac.

Material and methods

Our material consisted of two ecotypes of Brachiaria brizantha native to the Mosso Plain (Burundi) and to Rubona (Rwanda) and two ecotypes of B. decumbens from the Mosso Plain and from Mwanza (Tanzania). These collections were introduced into our greenhouse at Louvain-la-Neuve (Belgium) and grown at 25°C day 20°C night temperature, 60% relative humidity and submitted to 12 hour photoperiod.

The F1 population consisted of 27 F1 hybrids (F1(D)) from the cross B. ruziziensis × B. decumbens and 6 F1 hybrids (F1(B)) from the cross B. ruziziensis × B. brizantha, where sexual B. ruziziensis plants used as female progenitors were colchicine induced tetraploids (2n = 4x = 36) (Lutts et al., 1991). Pollen grain fertility was estimated on 500 pollen grains per plant according to the procedure of Bronckers (1963).

Apomictic and sexual plants in the F1 population were identified by the clearing procedure of Young et al. (1979). One hundred ovules collected from spikelets at anthesis were observed on each hybrid. The criteria for distinguishing apomictic and sexual embryo sacs were those previously used by several researchers (Knox & Heslop-Harrison, 1963; Pritchard, 1967; Savidan, 1982) and consisted of 1) the structure of embryo sac (8 nucleate for the sexual and 4-nucleate for the apomictics), 2) the number of embryo sacs per ovule (sexual plants have generally only one sac per ovule while apomictic plants may have several), 3) the position of nuclei in relation to the central vacuole (all nuclei are concentrated at the same end in an apomictic embryo sac and are distributed at both ends in a sexual embryo sac).

As multiple embryo sacs after the heading stage were not precisely delimited and were not easy to observe in spikelets, ovules were fixed, sectioned and stained with hematoxylin-fast green.

The material used in this study was collected 1) in the greenhouse (126 and 86 flowers for B. brizantha and B. decumbens, respectively, and 2) in natural conditions (65 and 58 flowers for B. brizantha at Rubona and for B. decumbens at Mwanza respectively) in order to observe the environmental effects on gametogenesis of the apomictic species. For two F1(B) and four F1(D) apomictic interspecific hybrids obtained from distinct female progenitors, 75 and 71 flowers, respectively, were analyzed. Two levels of sexuality were considered: on the one hand, the minimum sexuality, defined as the percentage of ovules containing exclusively a single embryo sac, and, on the other hand, the maximum potential sexuality defined as the percentage of ovules having a sexual embryo sac in presence or absence of aposporic embryo sacs.

Results

Apospory in B. decumbens and B. brizantha was facultative: the two species possessed ovules with only a sexual gametophyte or with both sexual and aposporic embryo sacs (Table 1). Regarding the reproductive behaviour, B. decumbens seemed much more uniform than B. brizantha, which behaved differently in the greenhouse than in natural conditions. In nature, sex-