Analysis of DNA size, content and cell cycle in leaves of Napier grass (*Pennisetum purpureum* Schum.)

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Summary. Mesophyll cell nuclei isolated from leaves of *Pennisetum purpureum* were analysed by flow cytometry to determine the nuclear DNA content and the percentage of cells in different phases of the cell cycle. Samples taken from base, middle and tip regions of leaves 2 to 8 (leaf 1, which was adjacent to the meristem, was too small to sample) showed no significant differences in the amount of DNA per G1 nucleus due to either age or position. The average amount of DNA per G1 nucleus was 5.78 pg. Although the majority of cells for each sample were in G1, samples taken from older leaves had higher percentages of cells in G2 and S phases. More specifically, base and middle regions of older leaves had a higher percentage of cells in G2 than all three positions in younger leaves. Electrophoretic analysis of nuclear DNA from leaves 2 to 7 showed no evidence of degradation or difference in fragment size for any sample or position. This study was compared to previous work on the relationship between leaf age and embryogenic competence in *Pennisetum purpureum*. The results suggest that changes in the cell cycle, and/or a loss or fragmentation of the nuclear DNA, are not responsible for loss of embryogenic competence in mature leaf tissue.

Key words: Cell cycle – Embryogenic competence – Nuclear DNA – *Pennisetum purpureum* – Tissue culture

Introduction

The loss of embryogenic (or morphogenic) competence in mature and differentiated tissues of the Gramineae is widely recognized (Vasil and Vasil 1986; Vasil 1987).

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This has limited the choice of explant material to tissues that are meristematic, such as immature embryos, young inflorescences and the basal region of young leaves. Several studies using leaf explants have shown that temporal and spatial gradients exist within leaves of the Gramineae, such that the youngest (basal) portion of the leaf is the most likely to form embryogenic callus (Wernicke and Brettell 1980; Haydu and Vasil 1981; Lu and Vasil 1981; Wernicke et al. 1981; Hanning and Conger 1982; Ho and Vasil 1983; Wernicke and Milkovits 1984; Linacero and Vazquez 1986; Joarder et al. 1986). Such gradients form the basis of a report by Hesemann and Schröder (1982) which found that the content of DNA per nucleus decreased with increasing leaf age in *Secale cereale* (rye). Furthermore, Beaulieu et al. (1985) have reported that the DNA obtained from mature parts of *Triticum aestivum* (wheat) leaves appears to be fragmented when compared to DNA obtained from immature tissues. They proposed that higher levels of nuclease activity, either spontaneous or induced, were responsible for reduced fragment size. It has been suggested that both fragmentation and/or loss of DNA may be related to the loss of cell division and/or morphogenic competence in mature leaf tissues (Hesemann and Schröder 1982; Beaulieu et al. 1985).

Another factor to be considered regarding the loss of embryogenic competence in mature tissue explants is the cell cycle. Fukuda and Komamine (1981a, b), in their studies of tracheary elements, have shown that the cell cycle is an important factor controlling differentiation. It is also known that the G1 phase is a major control point for both division and differentiation in cultured cells (Fukuda and Komamine 1981a; Gould 1983). In his review, Gould (1983) has suggested that the cell cycle might play a key role in the regeneration of plants from tissue cultures.
In an effort to further understand the factors responsible for the loss of embryogenic competence in mature, differentiated tissues of the Gramineae, we have examined the DNA from leaves of *Pennisetum purpureum* (Napier grass) and the percentage of cells in the various phases of the cell cycle. This was done to determine if loss or fragmentation of the DNA and/or cell cycle factors were related to the loss of embryogenic competence in this species.

**Materials and methods**

Shoots of *Pennisetum purpureum* Schum. (accession number PP-9, obtained from Dr. S.C. Schank) were collected from field grown plants. Two of each of the eight youngest leaves (except leaf 1, which was too small) were separated and used for the analysis. Samples weighing approximately 60 mg were taken from the base, middle and tip regions of each leaf, except for leaf 2 which was too small to yield a sample from the middle region. Samples were labelled both according to their developmental age and position relative to the meristem (leaf 1 being the youngest and closest to the meristem) and according to the location of the sample along the leaf blade (base = B, middle = M, tip = T). For example, sample 3B would represent the base region of the third leaf out from the meristem. Nuclei were isolated from the leaf tissue and stained for flow cytometry according to Galbraith et al. (1983) and Galbraith (1984). Flow cytometry was used to determine both the average amount of DNA per G1 nucleus and the percentages of cells in the G1, S and G2 phases of the cell cycle. In all, 5,000 nuclei were sampled from every position on each of 14 leaves. Seven leaves from one shoot (ages 2 to 8) were analysed in one experiment. Six leaves from a second shoot (ages 2 to 7) and the eighth leaf from a third shoot were used to form a second data set (see Table 1 for leaf lengths). The cell cycle values were recorded as the percentages of cells in G1, S and G2 phases from samples of 5,000 nuclei per leaf position.

Statistical analyses were performed on the data from both experiments, using the percentages as observations. Cochran's test for homogeneity of variance was followed by an analysis of variance (ANOVA) to examine the effect of leaf position and age on the amount of DNA and the percentages of cells in the G1, S and G2 phases of the cell cycle. Since no middle region sample could be obtained from leaf 2, no leaf 2 data were included in the statistical analyses. Factors shown to be significant by the F-test (\(\alpha = 0.05\)) were further examined using Duncan's multiple range test. Four separate analyses were conducted; one for DNA amount and one for each of the three cell cycle phases (G1, S and G2).

The size of the DNA was determined by isolating DNA from leaves 2 to 7 according to the methods of Dellaporta et al. (1983) and Rogers and Bendich (1985). Five to six leaves per age class and 1--4 g of leaf tissue per sample position were used with the Dellaporta method. One leaf per age class and 100 mg per sample position were examined using the methods of Rogers and Bendich. The DNA was run out on 0.8% to 1.0% agarose gels with ethidium bromide using a BRL lambda HindIII marker for size determination.

**Results**

The analysis of variance showed no significant difference in DNA amount per G1 nucleus for either sample position (\(\alpha = 0.05, v = 12, v = 18\)) or age (\(\alpha = 0.05, v = 5, v = 18\); Fig. 1). For all samples, the average amount of DNA per G1 nucleus was 5.78 pg. No correlation was seen between DNA amount and leaf length (Fig. 1). Cell cycle data indicated that the majority of cells in all samples were in G1 (58.2% to 85.2%, Table 2). However, older leaves contained a significantly higher percentage of cells in G2 and a lower percentage in G1 than younger leaves (Fig. 2). The data obtained for leaf 2 samples, which were not used in the statistical analyses, fell within the range of the trends based on leaves 3 to 8 (Fig. 2, Table 2). The base and middle regions of older leaves possessed a higher percentage of cells in G2 when compared to all positions in younger leaves, as well as the tip regions of older leaves. The opposite was true for G1 phase (Table 2, Fig. 3). The F-test demonstrated that leaf age has a significant effect on the percentage of cells in S phase. This is in contrast to Duncan's multiple range test which failed to detect individual means significantly different from the others. A slight trend showing an increase in the percentage of cells in S phase with increasing leaf age can be seen in Fig. 2. The position of the sample, however, did not appear to have a significant effect on the percentage of

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**Table 1. Leaf length data for cell cycle analysis grouped by leaf age**

<table>
<thead>
<tr>
<th>Leaf Age</th>
<th>Replicate 1 Leaf Length</th>
<th>Replicate 2 Leaf Length</th>
<th>Average Length</th>
</tr>
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<td>2</td>
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<td>68</td>
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<td>100</td>
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</tr>
<tr>
<td>8</td>
<td>99</td>
<td>110</td>
<td>104.5</td>
</tr>
</tbody>
</table>

**Fig. 1.** Leaf length and DNA content grouped by leaf age. For DNA content (given in picograms) each sample point is the mean DNA content taken from the percentage of G1 nuclei in a sample of 30,000 (15,000 from each of 2 experiments). Leaf age numbers refer to the position of the leaf with respect to the meristem. Note that no samples were significantly different with respect to DNA amount.