Variations in Morphological and Feeding Characteristics in Plants Regenerated from Embryogenic Suspension Cultures Derived from a Single Genotype of Bahiagrass (*Paspalum notatum* Flügge)

Kamaruddin Saleng*, Mikio Nasu, Mitsuhiro Nimi, Ryō Akashi and Osamu Kawamura**

Faculty of Agriculture, Miyazaki University, Miyazaki 889-2192, Japan
* United Graduate School of Agricultural Sciences, Kagoshima University, 890-0015, Japan
(Present address: Faculty of Agriculture, “45” University, Makassar Jl. Urip Sumoharjo Km 4, Makassar 90232, South Sulawesi, Indonesia)
** Corresponding author

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**Synopsis**


We have investigated the extent of variation in morphological and feeding characteristics in plants regenerated from six-month-old embryogenic suspension cultures originated from a single genotype of bahiagrass (*Paspalum notatum* Flügge). Twenty-eight regenerated plants were randomly selected and planted individually in pots and grown outdoors. Five control plants, which were obtained from individual seeds, were grown under the same condition. Substantial phenotypic variation was observed among regenerants, whereas the control plants were reasonably stable. Large variation was observed in the number of tillers, ranging from 10 to 84. Regenerants were compared in six morphological traits, cell wall constituents, lignin and *in vitro*-digestibility. All regenerants had higher NDF contents than the control plants although there was only a small difference in dry matter and NDF digestibility between regenerants and control plants. In this study, regenerated plants showed different morphological and feeding characteristics, and *in vitro*-digestibilities were negatively correlated with plant length (−0.56, p<0.01).

Key words: Bahiagrass, Cell wall constituents, Digestibility, Morphological characteristics, Plant regeneration, Somaclonal variation.

**Introduction**

Introduction of genetic variation into breeding stocks is of significant importance in plant breeding. Many *in vitro*-techniques are being used to complement conventional plant breeding methods for different crop species. Somaclonal variation is one of these *in vitro*-approaches. This method selects for naturally occurring genetic variation in plants regenerated from somatic cells in culture and thus provides an increased genetic base for breeding new value-added varieties. The technique has been successfully used to generate agriculturally useful variation in many different crop species.

Bahiagrass (*Paspalum notatum* Flügge) has a tremendous forage value, particularly in the tropical and subtropical regions, and its normal reproductive mode is by obligate apomixis. However, improvement of this species through conventional breeding has been extremely difficult because of apomixis which is an obstacle to hybridization. Akashi et al. described the successful regeneration of bahiagrass from embryogenic callus and embryogenic suspension cultures. Because bahiagrass has a low dry matter digestibility, we were interested in determining if variability in morphological characteristics, dry matter characters related to cell wall constituents (CWC) and digestibility could be generated through tissue culture. Changes in these traits would indicate that somaclonal variation could be used to modify the forage qualities of the species. In a general sense, this approach could be a model for improving the quality in other grasses using tissue culture technology through the generation of somaclonal variation. The objectives of this study were (i) to determine whether qualitative traits were affected by somaclonal variation in bahiagrass plants regenerated from embryogenic suspension cultures; and (ii) to examine if plant length, leaf blade length,
leaf blade width, number of tillers, internode length and dry matter yield are the most consistent variables to use when screening for somaclonal variation for feeding character.

**Materials and Methods**

1. **Plant materials**

Plants were regenerated from 6-month-old suspension cultures obtained from a single seed of bahiagrass (*Paspalum notatum* Flügge) cv. "Pensacola". The procedure for the regeneration of the plants from bahiagrass suspension cultures is described elsewhere.

Thirty regenerated plants (RP) were randomly selected, transferred into individual small pots (1/5,000 a size) and placed in a greenhouse. Twenty-eight of these plants survived and were transplanted into larger size pots (1/2,000 a) in February 1993 with the basal application of the compound fertilizer (15-15-15) at 60 kg/10 a and were grown outdoors. The five control plants, which were obtained from individual seeds of ‘Pensacola’ (PEN) in April 1993, were grown under the same conditions. The above-ground portions were collected as the sample for chemical analyses and digestion trials in October 1993.

2. **Morphological comparison**

Regenerants were compared to the control plants for six morphological traits. Measurements were taken for a) plant length; b) leaf blade length and c) leaf blade width, taken from the trifoliate leaf of the longest stem; d) number of tillers; e) internode length of the longest stem at the first ear emergence; f) dry matter yield. Each measurement except the yield was repeated ten times. The mean of the ten measurements was used to define the six morphological traits.

3. **Chemical analyses and digestion trials**

The collected samples were dried for 24 hours at 50°C using an air-forced oven and were then ground and passed through a 1-mm mesh for the determination of cell wall components and *in vitro* digestibility with rumen microbes. Neutral detergent fiber content as CWC, *in vitro*-dry matter digestibility (IVDMD) and NDF digestibility (IVNDFD) were determined by the method of Georghiou and van Soest. The acetyl bromide method was used to estimate the lignin content.

**Results and Discussion**

Often the inducement of somaclonal variation occurs whenever plant cells and tissues are cultured *in vitro*. Regeneration of grass species via somatic embryogenesis is believed to be less subject to somaclonal variation than regeneration via organogenesis, since embryo ontogeny is sensitive to disruption by any induced abnormality. However, even though bahiagrass regenerates via embryogenic callus.

We were able to identify variations for random amplified polymorphic DNA (RAPD) markers in regenerated plants from 6 month-old suspension cultures obtained from a single seed of bahiagrass (*Paspalum notatum* Flügge) cv. "Pensacola".

Analyses of morphological characteristics and chemical composition to assess the somaclonal variation in plants regenerated from embryogenic suspension cultures originated from a single genotype of bahiagrass are provided as histogram in Fig. 1. Substantial phenotypic variations were observed in the regenerated plants, but only small variations in the controls. Plant length of the regenerated plants ranged from 7 to 24 cm, compared with 7 to 14 cm for the control plants. Leaf blade length of the regenerated plants varied from 5 to 29 cm, compared with 19 to 30 cm for the control plants. Leaf blade width of the regenerated plants ranged from 0.3 to 0.7 cm,
compared with 0.3 to 0.5 cm for the control plants. Number of tillers of the regenerated plants varied from 10 to 83, compared with 4 to 18 for the control plants. Internode length of the regenerated plants ranged from 0.1 to 0.6 cm, compared with 0.2 to 0.5 cm for the control plants. Dry matter of the regenerated plants varied from 50 to 250 g/m², compared with 100 to 200 g/m² for the control plants. An outstanding variant was identified and valued 84 for the number of tillers, which is about nine times the number found in control.

Histograms of the chemical composition and digestibility are shown in Fig. 2. NDF content of the regenerated plants ranged from 58 to 65%, compared with 55 to 58% for control plants. Dry matter digestibility in vitro of the regenerated plants varied from 85 to 93%, compared with 92 to 95% for the control plants. NDF digestibility in vitro of the regenerated plants ranged from 73 to 75%, compared with 72 to 74% for control plants. Digestible NDF content of the regenerated plants varied from 41 to 47%, compared with 39 to 42% for control plants. The lignin content (absorbance) per DM of the regenerated plants ranged from 7.2 to 8.0, compared with 7.4 to 7.6 for the control plants. The lignin content (absorbance) per NDF of the regenerated plants varied from 12.0 to 13.2, compared with 13.1 to 13.8 for the control plants. All regenerants had higher NDF content than control plants although there were small differences in IVDMD and IVNDFD between the two groups. Accordingly, most of the regenerants were higher in percentage of digestible NDF than control plants. This indicates that a proportion of high quality fiber might have been contained in the regenerants.

Correlation coefficients of morphological characteristics with digestibility and chemical composition in regenerated plants of bahiagrass are shown in Table 1. Dry matter digestibility was negatively correlated with plant length, but positively with leaf blade and internode length, showing the highest correlation with plant length (−0.56, p<0.01). Neutral detergent fiber digestibility was negatively correlated with plant length, and positively correlated with leaf blade, number of tillers and internode length, showing the highest correlation with leaf blade width (0.53, p<0.01). Lignin/DM was positively correlated

![Fig. 2. Histograms of chemical composition and digestibility in vitro.](image)

<table>
<thead>
<tr>
<th>Plant length</th>
<th>Leaf blade length</th>
<th>Leaf blade width</th>
<th>Number of tiller</th>
<th>Internode length</th>
<th>DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVDMD</td>
<td>−0.56**</td>
<td>−0.24 NS</td>
<td>0.39*</td>
<td>0.18 NS</td>
<td>0.37*</td>
</tr>
<tr>
<td>IVNDFD</td>
<td>−0.41*</td>
<td>−0.10 NS</td>
<td>0.53**</td>
<td>0.40*</td>
<td>0.39*</td>
</tr>
<tr>
<td>NDF/DM</td>
<td>−0.06 NS</td>
<td>0.04 NS</td>
<td>−0.14 NS</td>
<td>−0.10 NS</td>
<td>0.03 NS</td>
</tr>
<tr>
<td>Lignin/DM</td>
<td>−0.17 NS</td>
<td>−0.22 NS</td>
<td>−0.24 NS</td>
<td>−0.35 NS</td>
<td>0.09 NS</td>
</tr>
<tr>
<td>Lignin/NDF</td>
<td>−0.11 NS</td>
<td>−0.14 NS</td>
<td>0.12 NS</td>
<td>0.01 NS</td>
<td>0.11 NS</td>
</tr>
<tr>
<td>Digestible NDF/DM</td>
<td>0.22 NS</td>
<td>0.09 NS</td>
<td>−0.39*</td>
<td>−0.28 NS</td>
<td>−0.27 NS</td>
</tr>
</tbody>
</table>

NS: not significant; *: p<0.05; **: p<0.01.
with DM, but NDF/DM and lignin/NDF were not found to be correlated with any of the morphological characteristics recorded.

In conclusion, although regenerated plants used in this study originated from embryogenic suspension cultures derived from a single genotype of bahiagrass, they showed differences in morphological and feeding characteristics. Digestibility of regenerated plants was negatively correlated with plant length. This finding suggests that tissue culture techniques are potential breeding methods for the improvement of feed quality in warm-season grasses. Investigations of variations in the progenies of the regenerated plants are now in progress.

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References


要旨


バヒアグラス（品種ペンサコラ）の単一変種由来懸濁培養細胞から再分化した個体における外部形態、細胞壁構成物質含量およびルーメン微生物によるin vitro消化率を調査した結果、これらの性状に変異が認められた。全ての再分化個体のNDF含量は、種子からの植物体（対照個体）のそれより高かった。再分化個体の消化率は単月と有意な負の相関があった。これらの結果は、組織培養が暖地型牧草の品質改良のための育種操作として有用であることを示唆している。

キーワード：再分化個体、細胞壁構成物質、消化率、ソマクラーノル変異、バヒアグラス。