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# Chemical Composition and *in vitro* Digestibility of Bahiagrass (*Paspalum notatum* Flügge) Regenerated from Suspension Culture

Kamaruddin SALENG, Mitsuhiro NIIMI[1], Ryo AKASHI[1], Osamu KAWAMURA[1]

The United Graduate School of Agricultural Science, Kagoshima University, [1]Division of Grassland Science, Faculty of Agriculture, University of Miyazaki

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**Summary:** The regenerated plants (RP) from suspension culture originated from a single genotype of bahiagrass (*Paspalum notatum* Flügge cv. Pensacola) were evaluated for cell wall composition and *in vitro* digestibility. Neutral detergent fiber (NDF) contents of RP were generally higher than those of plants germinated from seeds of Pensacola (PEN), but the lignin contents of RP were not statistically different from PEN. The dry matter and NDF of RP were highly digestible by rumen microorganisms compared to PEN. These results suggest that tissue culture techniques could be efficient breeding methods to improve the feed quality of tropical grasses.

**Key words:** Bahiagrass, Cell wall composition, Digestibility, Regenerated plant, Somaclonal variation

## Introduction

The quality of tropical grasses is generally low compared with the temperate grasses. One of the most urgent problems in tropical grasses is low digestibility due to high contents of cell wall components, especially lignin (Akin and Chesson 1989). Recently, genetic engineering strategies such as antisense, sense or ribozyme-mediated suppression of genes encoding enzymes involved in lignification have became powerful approaches for the modification of lignin content (Atanassova *et al.* 1995; Van Doorsselaere *et al.* 1995; Baucher *et al.* 1996). However, it is often difficult to establish appropriate transformation systems in grass species. On the other hand, useful somaclonal variation (Larkin and Scowcroft 1981), which is often heritable (Larkin *et al.* 1984; Breiman *et al.* 1987), may be exhibited in plants regenerated from tissue culture.

In a previous paper, Akashi and Kawamura (1998) found the variation for RAPD markers in the regenerated plants of bahiagrass (*Paspalum notatum* Flügge) which is a valuable tropical grass. The purpose of this study is to evaluate the digestibility and the contents of cell wall components in the plants regenerated from suspension culture of bahiagrass.

Corresponding author: Osamu Kawamura
Division of Grassland Science
Faculty of Agriculture, University of Miyazaki
Miyazaki 889-2192, Japan

責任著者：川村　修
〒889-2192　宮崎市学園木花台西1-1
宮崎大学農学部草地環境科学講座

## Materials and Methods

### Ⅰ. Plant materials

Plants were regenerated from 6 month-old suspension cultures that originated from a single genotype of *Paspalum notatum* cv. "Pensacola" (Akashi *et al.* 1993). After thirty regenerated plants (RP) were randomly chosen, they were planted into individual pots and were placed in a greenhouse. Twenty-eight of these plants survived and were individually transplanted into larger pots (1/2000a Wagner pot) in February 1993 with the basal application of a compound fertilizer (15-15-15) at 60 kg/10 a to grow outdoors. The five control plants, which were germinated from individual seeds of Pensacola (PEN) in April 1993, were grown under the same outdoor conditions. All plants were trimmed in October 1993. The above-ground portions were cut on June 29, 1994 and on June 28 and September 29, 1995 for chemical analyses and digestion trials. After each cutting, all plants were fertilized at the same level of basal application.

### Ⅱ. Chemical analyses and digestion trials

The collected samples were dried for 24 hours at 50 ℃ using an air-forced oven and were ground to pass through a 1-mm mesh for use in the determination of cell wall components and *in vitro* digestibility with rumen microbes, except for 5 strains of RP in 1995 due to inadequately smaller amounts of collected tissue. Neutral detergent fiber (NDF) content, *in vitro* dry matter digestibility (IVDMD) and *in vitro* NDF digestibility (IVNDFD) were determined using the methods of Georing and Van Soest (1970). The acetyl bromide method (Morrison 1972) was used to analyze lignin for the samples collected in 1994. The actual lignin concentration was calculated using the absorbance value calibrated against the acid detergent lignin analysis (Georing and Van Soest 1970; Kawamura and Niijima 1996).

## Results and Discussion

The chemical composition and digestibility are shown in Table 1. The mean values of NDF content were higher in RP than in PEN, indicating a statistically significant difference only in September 1995. The lignin content of RP was not significantly different from that of PEN in June 1994, but IVDMD and IVNDFD of RP were significantly higher than those of PEN in

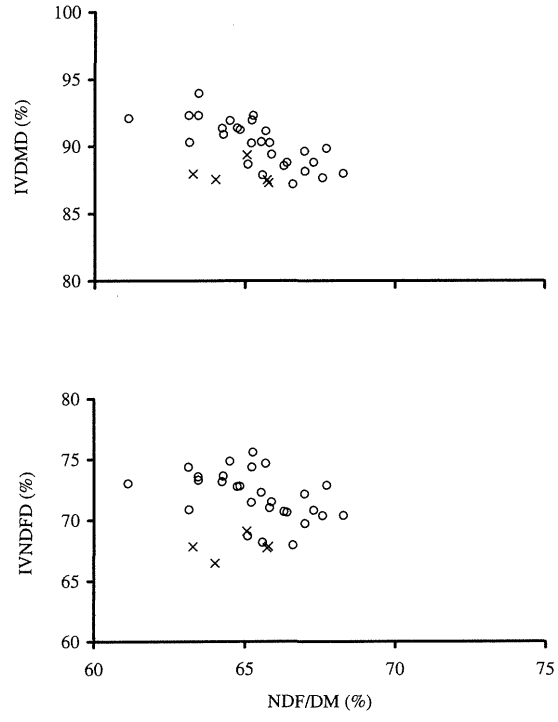
**Table 1.** Chemical composition and *in vitro* digestibility (mean±standard deviation, %) of Pensacola bahiagrass and regenerate plants.

| | | PEN | RP | Difference |
|---|---|---|---|---|
| June 1994 | NDF/DM | 64.77±1.10 | 65.37±1.62 | NS |
| | LIG/DM | 3.30±0.06 | 3.25±0.16 | NS |
| | LIG/NDF | 5.10±0.12 | 4.97±0.19 | NS |
| | IVDMD | 87.95±0.82 | 90.23±1.75 | ** |
| | IVNDFD | 67.81±0.92 | 71.99±2.02 | ** |
| June 1995 | NDF/DM | 66.66±1.60 | 67.47±1.97 | NS |
| | IVDMD | 75.41±1.44 | 79.90±2.51 | ** |
| | IVNDFD | 63.12±1.62 | 70.22±3.55 | ** |
| September 1995 | NDF/DM | 65.75±1.36 | 67.91±1.50 | ** |
| | IVDMD | 74.97±1.84 | 78.36±1.66 | ** |
| | IVNDFD | 61.95±2.17 | 68.16±2.04 | ** |

PEN; pensacola, RP; egenerated plant, NDF; neutral detergent fiber, LIG; lignin, IVDMD; *in vitro* dry matter digestibility, IVNDFD; *in vitro* NDF digestibility, ** : $P < 0.01$, NS; not significant

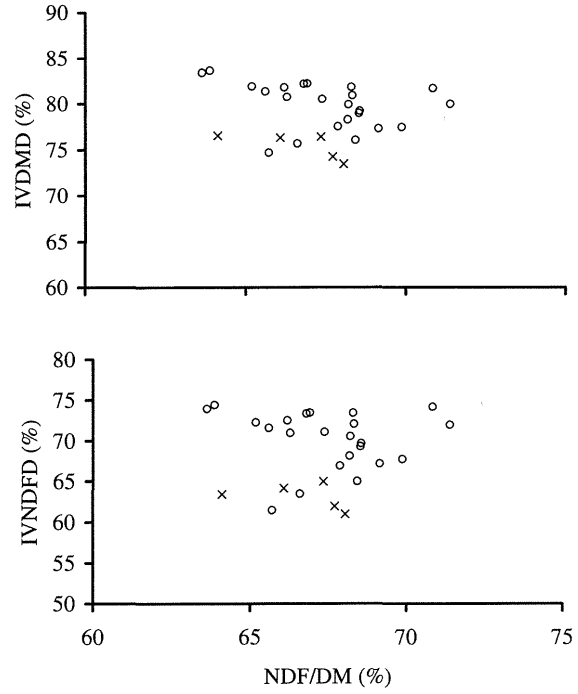
all of the three cuttings. It is well known that the forage having more NDF (cell wall constituents) should be less digestible (Akin and Chesson 1989; Kawamura 1997) because the forages are having relatively less cell contents that are readily digestible and the cell wall is less digestible. Although, in this experiment, NDF content of RP tended to be generally higher than that of PEN, IVDMD and IVNDFD of RP were higher than those of PEN. The digestibility of cell wall constituents is dependent on lignin deposition into the cell wall (Akin and Chesson 1989; Kawamura and Niijima 1996; Kawamura 1997). However, the significant difference of lignin content between PEN and RP was not observed. Therefore, the higher IVNDFD of RP would not be due to the difference in lignin content.

As shown in Figs 1-3, the relations between NDF content and digestibility were common to three cuttings. That is, at the same level of NDF content, IVDMD and IVNDFD were gen-



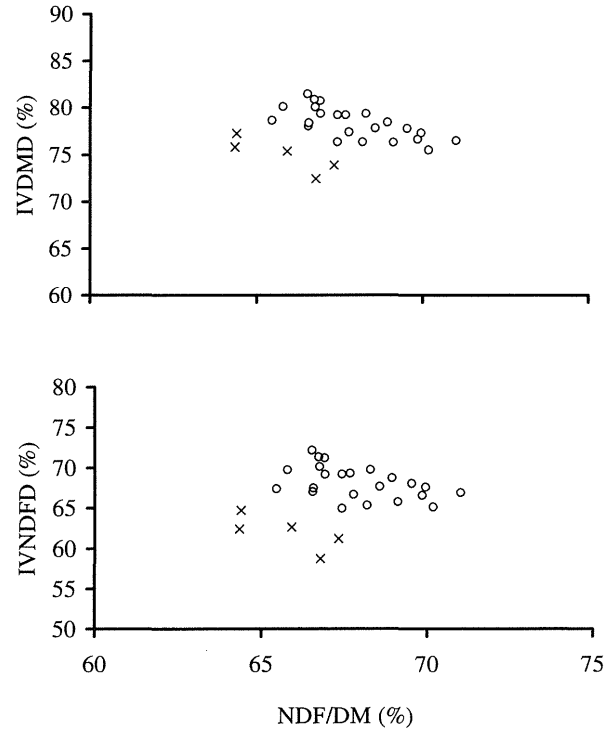

○ RP; regenerated plant
× PEN; Pensacola
As for IVDMD, IVNDFD, NDF; see table 1.

**Fig. 1.** Relationship between *in vitro* DM and NDF digestibilities and NDF content in June, 1994.




○ RP; regenerated plant
× PEN; Pensacola
As for IVDMD, IVNDFD, NDF; see table 1.

**Fig. 2.** Relationship between *in vitro* DM and NDF digestibilities and NDF content in June, 1995.




○ RP; regenerated plant
× PEN; Pensacola
IVDMD, IVNDFD, NDF; see table 1

**Fig. 3.** Relationship between *in vitro* DM and NDF digestibilities and NDF content in September, 1995.

erally higher in RP. It was observed in RP that the relationships between NDF content and IVDMD and between NDF content and IVNDFD were significant in June 1994 and in September 1995. These results indicate that the cell wall of RP reduced the digestibility, but the effect of cell wall on the digestibility may be less in RP than in the control plants. In addition, most RP having more NDF were not so low in IVNDFD. As the digestible NDF contents *in vitro* of PEN and RP are shown in Fig. 4, most RP contain more digestible cell wall than PEN. The significant differences ($p < 0.01$) in digestible NDF content between RP and PEN were observed in all of three cuttings. Recently, the feeding of highly productive cattle, especially the dairy cow, requires that the forage contains a lot of digestible cell wall. Some strains of RP might meet this requirement.

Figures 5 and 6 show the relationship of lignin content to IVDMD and IVNDFD in June 1994, respectively. It was observed that at the same level of lignin content, the digestibility was higher in RP than in PEN, and the relationships in RP between lignin content and IVDMD ($r = -0.7373$, $p < 0.01$) and between lignin content and IVNDFD ($r = -0.6774$, $p < 0.01$) were significant. These results indicate that the lignin deposited into the cell wall of RP reduced the digestibility, but the effect of lignin on the digestibility may be less in RP than in the control plants.

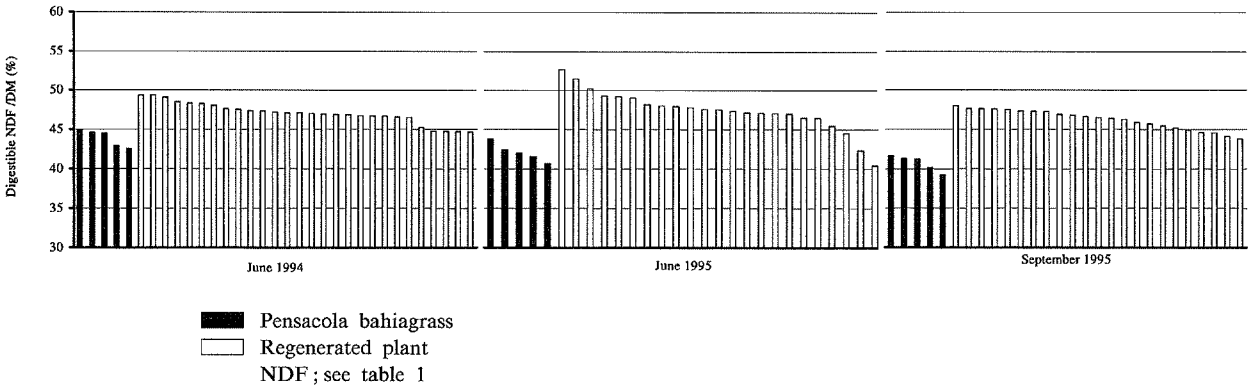



**Fig. 4.** Digestible NDF content (*in vitro*) of Pensacola bahiagrass and regenerated plants.

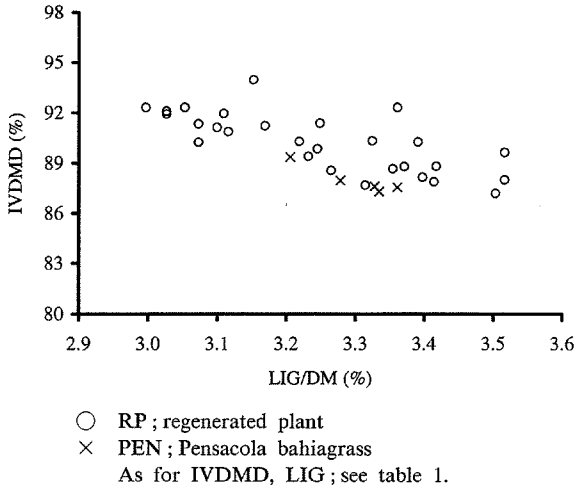



**Fig. 5.** Relationship between *in vitro* DM digestibility and lignin content in June, 1994.

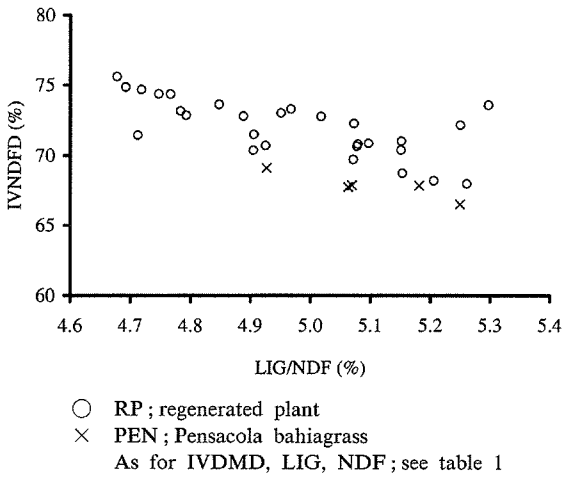



**Fig. 6.** Relationship between *in vitro* NDF digestibility and lignin content in June, 1994.

In conclusion, the tissue culture techniques could be one useful method to improve the digestibility of tropical grasses through a modification of the plant cell wall.

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# バヒアグラス（*Paspalum notatum* Flügge）の懸濁培養細胞由来再分化個体における化学組成および *in vitro* 消化率

カマルディン サレン・新美光弘[1)]・明石　良[1)]・川村　修[1)]

鹿児島大学大学院連合農学研究科，[1)]宮崎大学農学部草地環境科学講座

## 要　約

バヒアグラス（品種Pensacola）の懸濁培養細胞由来再分化個体における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．再分化個体群のNDF含量は，一般に，種子からの植物体（対照個体群）より高かった．リグニン含量には，両個体群の間で，統計的に有意な差がなかった．*In vitro*乾物およびNDF消化率においては，再分化個体群が対照個体群より高かった．これらの結果は，組織培養が暖地型牧草の品質改善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，ソマクローナル変異，バヒアグラス