UNIVERSITI PUTRA MALAYSIA

PHENOTYPIC VARIABILITY OF BRACHIARIA DECUMBENS AFTER EXPOSURE TO GAMMA IRRADIATION

GHAZALI BIN HUSSIN

FP 2003 1

PHENOTYPIC VARIABILITY OF *BRACHIARIA DECUMBENS* AFTER EXPOSURE TO GAMMA IRRADIATION

GHAZALI BIN HUSSIN

MASTER OF AGRICULTURAL SCIENCE
UNIVERSITI PUTRA MALAYSIA

2003

PHENOTYPIC VARIABILITY OF *BRACHIARIA DECUMBENS* AFTER EXPOSURE TO GAMMA IRRADIATION

By

GHAZALI BIN HUSSIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Agricultural Science

January 2003

Specially dedicated to my parents, family
and friends for their understanding
of my interest

Abstract of thesis presented to the Senate Universiti Putra Malaysia in fullfilment of the requirement for the degree of Master Agricultural Science

PHENOTYPIC VARIABILITY OF *BRACHIARIA DECUMBENS* AFTER EXPOSURE TO GAMMA IRRADIATION

By

GHAZALI BIN HUSSIN

January 2003

Chairman: Associate Professor Dr Mohd Ridzwan Abd. Halim

Faculty: Agriculture

Brachiaria decumbens (Signal grass) has been used for many years as grazing pastures for ruminants. Its toxicity to small ruminants is the main limitation to utilization of this grass. Many researchers have reported several methods to control or alleviate this problem. Since there is a potential to use mutagenesis techniques to obtain variability of the plants, the objective of this study is to observe the phenotypic variability of Signal grass after mutagenic induction with gamma radiation. Further studies can be conducted to make a selection of variants which are free from toxicity.

Brachiaria decumbens seeds were irradiated at doses of 100, 200, 300, 400, 500, 600, 700, 800 and 900 Gy to determine the appropriate doses for mutagenic treatments of the grass. The results showed that, *B. decumbens* seeds were less sensitive to gamma ray than most other species and the LD_{50} was found to be between 800 to 900 Gy.

In order to observe the effects of gamma radiation on phenotypic variability of the grass, the grass seeds were exposed to 900 Gy gamma radiation. Results showed that gamma radiation at 900 Gy increased variability in morphological characteristics and nutrient contents and an obvious phenotypic mutant was detected.

There were significant increases in variabilities of morphological characters (tiller numbers, leaf length, leaf width, leaf weights, stem weights, leaf-to-stem ratio and internode lengths) and nutrient contents (Crude Protein, Ether Extract, Crude Fibre, Acid Detergent Fibre, Neutral Detergent Fibre and Ash) among the treated plants. There was also a positive skew of the frequency distribution curve for the treated plants to the right, indicating that mutagenic radiation can cause increased variability, which allows greater selection potential for desired characteristics.

DNA Polymorphism in the mutant cannot be detected through RAPD. The mutant showed significantly higher leaf width, leaf-to-stem ratio and crude protein compared to control plants.

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains Pertanian

KEPELBAGAIAN FENOTIP *BRACHIARIA DECUMBENS* SELEPAS DIDEDAHKAN KEPADA SINARAN GAMMA

Oleh

GHAZALI BIN HUSSIN

Januari 2003

Pengerusi: Profesor Madya Dr Mohd Ridzwan Abd. Halim

Fakulti: Pertanian

Brachiaria decumbens (Rumput Signal) telah lama digunakan sebagai pastura ragutan kepada ternakan ruminan. Keracunan kepada ruminan kecil merupakan penghalang utama ke atas penggunaan pastura ini. Ramai penyelidik telah melaporkan kaedah-kaedah untuk mengawal atau mengurangkan masalah ini. Oleh kerana terdapat potensi penggunaan teknik mutagenesis untuk menghasilkan kepelbagaian variasi bagi tanaman, objektif kajian ini ialah untuk menghasilkan kepelbagaian variasi fenotip bagi rumput ini selepas diberi rawatan sinaran gamma. Kajian lanjut boleh dibuat untuk membuat pemilihan ke atas varieti yang bebas daripada bahan toksik kepada ruminan kecil.

Bijibenih *Brachiaria decumbens* telah di dedahkan kepada sinaran gamma pada kadar 100, 200, 300, 400, 400, 500, 600, 700, 800 dan 900 Gy untuk mendapatkan kadar (dos) yang sesuai bagi kajian mutagenesis. Keputusan

menunjukkan bahawa rumput ini kurang peka kepada sinaran gamma kerana dos LD_{50} adalah tinggi iaitu antara 800 hingga 900 Gy.

Untuk melihat kesan sinaran gamma ke atas kepelbagaian variasi fenotip, biji benih rumput telah diberi sinaran pada kadar 900 Gy. Keputusan menunjukkan bahawa kesan sinaran gamma telah meningkatkan kepelbagaian variasi dalam sifat-sifat morfologi dan kandungan nutrien dan satu mutan baru telah diperolehi.

Terdapat peningkatan bermakna di dalam kepelbagaian sifat-sifat morfologi (bilangan pokok serumpun, panjang daun, lebar daun, berat daun, berat batang, kadar daun berbanding dengan batang dan panjang ruas) serta kandungan nutrien (Protein Kasar, Ektrak Ether, Serat Kasar, Serat Detergen Asid, Serat Detergen Asid dan Abu) bagi pokok yang dikenakan sinaran gamma. Keluk taburan frekuensi bagi setiap sifat yang dikaji menunjukkan terdapat lebih banyak bilangan pokok disebelah kanan keluk, ini menunjukan sinaran gamma telah menyebabkan peningkatan kepelbagaian dan memberi peluang yang besar untuk membuat pemilihan ke atas sifat yang dikehendaki.

Polymorfisma DNA tidak dapat dikesan pada mutan melalui kajian RAPD. Mutan menunjukkan peningkatan yang bermakna dari segi lebar daun, nisbah daun berbanding dengan batang dan kandungan protein berbanding dengan pokok asal.

ACKNOWLEDGEMENTS

I would like to express my utmost appreciation and gratitude to my supervisor Associate Prof. Dr. Mohd Ridzwan Abd. Halim for his invaluable advice, guidance, discussion and suggestion throughout this project.

I am also indebted to Associate Prof. Dr. Ghizan Salleh, Dr Mihdzar Abdul Kadir, Dr Nazir Basiran and Tn Hj Md Yusof Sudin for their ideas, support and help. Sincere thanks are also due to Mr Abdul Rahim Harun (MINT), Dr Abu Bakar Cik (MARDI), Ms Affrida Abu Hasan (MINT), Mr Azhar Mohamad (MINT), Dr Eusoff Abdul Jamak (MARDI`) for their help, support and guidance throughout the project.

The author also wishes to thank Dato' Dr Hawari Bin Hussein, Director General of Veterinary Services, for his invaluable support and encouragement to pursue this study. Sincere gratitude are also due to Dr Ahmad Suhaimi Omar, Dr Fauziah Bte Embong, Dr Ahmad Salleh and Tn Syed Hussein for their encouragement and support.

To all my staff especially Mr Sharif Saniman, Mrs Normah Miw Abdullah, Mrs Norizan Yahya, Mr Zubir Hasan, Mr Siratulmuni Zakaria, Mr Hamaruddin Pardi and to all involved, I extend my very special thanks for being very cooperative and helpful.

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADF	Acid Detergent Fibre
CF	Crude Fibre
CIAT	International Center For Tropical Agriculture
cm	Centimeter
CP	Crude Protein
CTAB	cetyl-trimethyl ammonium bromide
dATP	deoxyadenosine-5'-triphosphate
dCTP	deoxycytidine-5'-triphosphate
dGTP	deoxyguanosine-5'-triphosphate
DNA	deoxyribonucleic acid
dTTP	deoxythymidine-5'-triphosphate
DVS	Department of Veterinary Services
EDTA	Ethylene-diamine-tetraacetic acid
EE	Ether Extract
g	Gram
Gy	Gray
kR	krad
LD_{50}	Lethal dosage at 50%
LSR	Leaf to Stem Ratio

LWG	Live Weight Gain
MARDI	Malaysian Agriculture Research and Development Institute
M_1V_6	M: Mutant. V_6 : Vegetative at 6^{th} cuttingback
MINT	Malaysian Institute For Nuclear Technology Research
N	Number of Sample
NDF	Neutral Detergent Fibre
NIRS	Near Infra Red Spectroscopy
PCR	Polymerase Chain Reaction
PKC	Palm Kernel Cake
RAPD	Random Amplified Polymorphic DNA
SD	Standard Deviation
SE	Standard Error

TABLE OF CONTENTS

Pages

CHAPTER 1

INTRODUCTION

Brachiaria decumbens (Signal grass) is a stoloniferous, high yielding grass which is well adapted to a wide range of soils in humid tropical areas. It is one of the most important improved grass species, and in recent years increasing interest has been shown on it in various tropical countries, especially Brazil, Colombia, Indonesia and Malaysia. It exhibits high productivity, tolerance towards low soil fertility, drought resistance and is relatively free from pests and diseases (Loch, 1977). This grass has been shown to have an agronomic potential as an excellent pasture species in the local climatic condition (Wong, 1980). It has been extensively planted in most livestock farms in Malaysia as well as by smallholder livestock farmers since it grows well even with substandard management practices.

In Malaysia, sheep and goats grazing on this grass have been observed to have been affected with photosensitization and hepatic jaundice (Suparjo and Wahid, 1980; Abbas Mazni *et al*., 1983a; 1983b; 1985; Shahirudin *et al*., 1983; Zamri Saad *et al*., 1987). Similarly, there were also reports of similar incidence in sheep and cattle in Colombia (Anon, 1978), Brazil (Carmago *et al*., 1976; Dobreiner *et al*., 1976; Nobre and Andrade, 1978; Olievieria *et al*., 1979), Indonesia (Tribudhi *et al*., 1983), West Africa (Opasina, 1985) and Australia (Briton and Paltridge, 1940).

Many researchers have reported the causes of this disorder (Abbas Mazni *et al.*, 1983a; Salam *et al.*, 1988). The association of fungus *Phytomysis chartarum* with the grass is said to be the cause of this disease in Brazil (Carmago *et al.*, 1976; Dobreiner *et al.*, 1976; Nobre and Andrade *et al.*, 1978; Olievieria *et al.*, 1979). The grass it self is not toxic, but it becomes toxic when components within the grass is converted into hepatotoxic product(s) in the rumen of sheep possibly by the ruminal microorganism that is specifically present in this species (Nordin, 1988). Salam (1992) reported that, the cause of this disorder is related to the presence of an alkaloid compound, 3 *Spirostanols* ($C_{27}H_{44}O_3$) in the rumen of sheep intoxicated by *Brachiaria decumbens,* which were tested to be hepatoxic and caused photosensitization. Nordin *et al.* (1993) characterized the compound as *epi-sarsasapogenin* and *epi-smilagenins.*

The symptoms of jaundice in sheep caused by *Brachiaria decumbens* is similar to that of symptoms produced by copper toxicity in sheep when palm kernel cake (PKC) is taken excessively. Some researchers have proposed the use of zinc sulphate to counter the toxicity of PKC. Yusoff *et al.* (1992) proved that sheep can take up to 100% PKC in their diet, but a chelating agent such as sodium molybdate must be incorporated into the feed to ensure its sufficient uptake to protect it against toxicity.

For more extensive use of this grass, effort should be made to reduce or eliminate this toxicity. Mutation breeding techniques through irradiation or chemical mutagens may cause changes in genetic of the plant which may result in mutation into grass which is free of toxicity. Selection of the plant can be made later between the variation derived from the irradiated population.

Since many investigators have reported the usefulness of gamma radiation application in changing the properties of the plant (Charlotte, 1976; Hayward *et al*., 1993; Neal *et al*., 1993), further studies need to be carried out on the effects of gamma radiation in improving morphological characters and nutritive value of this grass.

The objective of this study is to characterise the phenotypic variability of *Brachiaria decumbens* in term of morphological characteristics and nutrient contents as a result of gamma radiation exposure.

CHAPTER 2

LITERATURE REVIEW

2.1 History and Origin of *Brachiaria decumbens*

Signal grass originated from East Africa (Uganda, Kenya, Tanzania, Rwanda, Burundi and Zaire). It was introduced (C.P.1. 1964, C.P.1. 6798) into Australia during the 1930's. Seeds from the populations derived from these two introductions were released for commercial use in 1966. Experimental lines and cultivars have spread very widely to all tropical regions of the world. The main use of signal grass is as a forage in permanent pastures for grazing.

There are six perennial species of the genus *Brachiaria* that are utilized by pastoralists in the tropics with varying degrees of success (Whyte *et al*., 1959). The species are *Brachiaria brizantha, Brachiaria decumbens, Brachiaria dictyoneura, Brachiaria humidicola, Brachiaria mutica* and *Brachiaria ruziziensis* with *Brachiaria decumbens* being most widely used (Loch, 1977). The *Brachiaria* species used in pastures are indistinctive and confusion in their identification often arise (Loch, 1977). However, *Brachiaria brizantha* and *Brachiaria decumbens* represent the two extremities of the range of variation since they integrate completely on all their morphological features (Loch, 1977). The morphological features of *Brachiaria decumbens* have been well described by Mackay (1974) (Figure 1).

Fig. 1: Plants of *Brachiaria decumbens* in the field

2.2 Nutritional Properties

Signal grass provides forage that is palatable to ruminants depending on its regrowth age. It has a moderate to high nutritional quality. Nitrogen concentration decreases from 2.7 to 0.7 % and *in vitro* DM digestibility from 75 to 50% as its age of regrowth increases from 2 to 4 weeks (Mannetje and Jones, 1992).

Chin *et al.* (1979) showed that the leaf to stem ratio, dry matter, crude protein and crude fibre of *Brachiaria decumbens* are 1:1.30, 31%, 6.89% and 31.5 respectively at the age of 6 weeks. Yusuf (1994) reported nutrient contents of *Brachiaria decumbens* at different ages as in Table 1.