UNIVERSITI PUTRA MALAYSIA

# *TURFGRASS VARIETAL IMPROVEMENT FOR SHADE AND DROUGHT TOLERANCE USING GAMMA RAY IRRADIATION*

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# TURFGRASS VARIETAL IMPROVEMENT FOR SHADE AND DROUGHT TOLERANCE USING GAMMA RAY IRRADIATION



**By**

**MOHD ABDUL HALIM BIN BAHARUN AZAHAR**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Master of Science**

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**August 2014**

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Turfgrass breeding aims to improve the characteristics of plants so that they become more desirable agronomically and economically. Alternative methods' using mutagenic treatment is a relatively quick method for improvement of turfgrass. Gamma ray irradiation can be used to improve turfgrass phenotype and enhance tolerance to environmental stress. A series of experiments were conducted to examine the response of turfgrass species to gamma ray irradiation, either in their phenotypic and genotypic characteristics, and to study turfgrass mutant lines under different shade and drought stress conditions. The mutant lines selected for evaluation in these studies were based on desirable characteristics for performance under stress.

Eight gamma ray dosages (0, 20, 40, 60, 80, 100, 150 and 200 Gy) were applied to *Axonopus compressus*, *Zoysia japonica* and *Cynodon dactylon* at the Gamma Cell Laboratory, Malaysian Institute of Nuclear Technology Research (MINT), Bangi, Selangor to identify the optimum dosage for turfgrass mutation. Optimum dosage was needed induce maximum mutation and to increase mutation rate. The optimum dosage was calculated based on 50% radiosensitivity tests on survival rate and plant height. The values 50% of radiosensitivity tests ($LD_{50}$) were determined to be 52, 76 and 90 Gy for *A.compressus, Z.japonica and C. dactylon*, respectively. The turfgrasses were radiated using the optimum dosage of gamma ray to produce numerous mutants. A total of 1500 stolons of each species were radiated and planted in biodegradable seed tray. In order to ensure the inheritance of these characteristics, all mutants were isolated using the cutting back technique.

Most of the mutants had dwarf and semi-dwarf characters. Gamma ray irradiation significantly altered the morphological parameters of turfgrass. The results showed that 2.4%, 2.6% and 1.5% rate of mutation occurred for *A. compressus*, *Z. japonica* and *C. dactylon*, respectively after exposing to the $LD_{50}$ dosages. Thirty six lines from *A. compressus* were recorded as mutants with five (A26-4-1, A61-1-1, A46-2-1, A91-3-5, A13-2-5) showing high potential for further study. Thirty nine lines from *Z. japonica* were recorded as mutant with five (Z131-3-1, Z36-3-1, Z13-1-2, Z12-2-

1, Z2-2-1) of them showing high potential for further study. Twenty two lines from *C. dactylon* were recorded as mutants with five (C43-4-1, C85-1-2, C59-2-2, C41-4-1, C5-3-1) showing high potential for further study, while six (C43-4-1, C42-4-1, C37-5-1, C83-3-2, C95-2-2, C13-3-3) of them were reselected for the shade tolerance study.

In the drought tolerance study, six most tolerant mutant lines (A48-3-5, A64-2-2, A62-3-1, A84-1-1, A26-4-1, A46-2-1) and A0 were subjected to five field capacity treatments of -20, -30, -33 (control), -40 and -50 J/kg and were assessed for visual quality and growth parameters. Shoot and root dry weights were also determined. *A. compressus* showed low quality performance under extreme drought conditions and many had died. A84-1-1 performed the best under drought conditions as it could withstand up to -50 J/kg field capacity, and this was followed by A26-4-1 and A64-2-2. In the shade tolerance study treatments were applied by exposing the grass to three, six, nine or twelve hour's of full sunlight per day. Generally, turfgrass showed slow growth and low quality when exposed to less than 3 hours of sunlight. The quality of *C. dactylon* was much better under long duration of full sunlight. Mutant line C43-4-1 performed the best under shade with its outstanding quality in terms of colour, density and uniformity. Durations with a minimum of at least 6 hours sunlight showed good responses.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PEMBIAKBAKAAN RUMPUT TURF TERHADAP KETAHANAN TEDUHAN DAN KEMARAU DENGAN MENGGUNAKAN SINARAN GAMMA**

Oleh

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Pembiakbakaan rumput bertujuan untuk menambahbaikkan sifat-sifatnya supaya lebih bernilai dari segi agronomi dan ekonomi. Cara alternatif ialah dengan menggunakan kaedah mutagenik yang mana lebih cepat untuk menambahbaikkan rumput. Sinaran gamma digunakan untuk menambahbaikkan fenotip dan ketahanan rumput terhadap tekanan persekitaran. Satu eksperimen bersiri telah dijalankan untuk menguji tindakan rumput terhadap sinaran gamma, samada terhadap sifat-sifat fenotip dan untuk menilai rumput mutan di bawah tekanan naungan dan kemarau. Mutan dipilih berdasarkan sifat-sifat yang diingini dan prestasinya di bawah tekanan.

Lapan dos sinaran gamma (0, 20, 40, 60, 80, 100, 150 dan 200 Gy) telah didedahkan kepada *Axonopus compressus*, *Zoysia japonica* dan *Cynodon dactylon* di Gamma Cell Laboratory, Malaysian Institute of Nuclear Technology Research (MINT), Bangi, Selangor untuk mengenalpasti dos terbaik mutasi rumput. Dos terbaik perlu digunakan supaya memaksimumumkan mutasi dan meningkatkn kadar mutasi. Dos terbaik dikira dengan berdasarkan 50% ujian radiosensitif ke atas kadar hidup dan ketinggian rumput. Nilai dos terbaik iaitu $LD_{50}$ untuk *Axonopus compressus*, *Zoysia japonica* dan *Cynodon dactylon* ialah 52, 76 dan 90 Gy masing-masing. Rumput didedahkan dengan dos sinaran gamma terbaik untuk menghasilkan pelbagai mutan. Sejumlah 1500 stolon untuk setiap spesis diradiasikan dan ditanam dalam bekas semaian. Untuk memastikan sifat-sifat yang diingini, mutan diasingkan dengan menggunakan teknik cutting back.

Kebanyakkan mutan mempunyai sifat-sifat kerdil dan separuh-kerdil. Sinaran gamma secara signifikan telah mengubah sifat-sifat morfologi rumput. Keputusan menunjukkan sebanyak 2.4%, 2.6% and 1.5% kadar mutasi untuk *A. compressus*, *Z. japonica* dan *C. dactylon* masing-masing selepas didedahkan pada dos $LD_{50}$. Sejumlah 36 mutan dari *A. compressus* direkodkan dan lima (A26-4-1, A61-1-1, A46-2-1, A91-3-5, A13-2-5) menunjukan potensi yang besar untuk kajian seterusnya. Sejumlah 39 mutan dari *Z. japonica* telah direkodkan dan lima (Z131-3-1, Z36-3-1, Z13-1-2, Z12-2-1, Z2-2-1) menunjukan potensi yang besar untuk kajian seterusnya. Manakala 22 mutan dari *C. dactylon* telah direkodkan dan lima (C43-4-

1, C85-1-2, C59-2-2, C41-4-1, C5-3-1) daripadanya telah menunjukan potensi yang besar untuk kajian seterusnya, sementara enam (C43-4-1, C42-4-1, C37-5-1, C83-3-2, C95-2-2, C13-3-3) daripadanya dipilih untuk kajian naungan.

Dalam kajian kesan kemarau, enam mutan (A48-3-5, A64-2-2, A62-3-1, A84-1-1, A26-4-1, A46-2-1) yang mempunyai ketahanan paling tinggi dan A0 telah dipilih dan dirawat dengan lima kapasiti lapangan iaitu -20, -30, -33 (sebagai kawalan), -40 and -50 J/kg dan dinilai dari segi kualiti visual dan pertumbuhan. Berat kering pucuk dan akar juga dinilai. *A. compressus* (A0) telah menunjukkan kualiti yang rendah di bawah tahap kemarau yang tinggi dan mengalami kematian. A84-1-1 telah menunjukkan prestasi yang terbaik di bawah keadaan kemarau dan boleh bertahan sehingga -50 J/kg kapasiti lapangan, diikuti oleh A26-4-1 dan A64-2-2. Di samping itu, kajian kesan naungan telah dilakukan dengan mendedahkan rumput kepada tiga, enam, sembilan dan dua belas jam cahaya matahari untuk setiap hari. Umumnya, pertumbuhan rumput menjadi perlahan dan kualiti juga berkurang apabila didedahkan pada 3 jam cahaya matahari. Mutan C43-4-1 mempunyai prestasi terbaik di bawah naungan dengan menunjukkan warna, kepadatan dan kesamaan yang baik. Tempoh masa minimum 6 jam cahaya matahari menunjukkan kesan yang baik.

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I certify that a Thesis Examination Committee has met on August 29, 2014 to conduct the final examination of Mohd Abdul Halim Bin Baharun Azahar on his thesis entitled "**Turfgrass varietal improvement for shade and drought tolerance using gamma ray irradiation**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| AsA | Ascorbic Acid |
| APX | Ascorbate Peroxidase |
| ANOVA | Analysis of Variance |
| CIRP | Christmas Island Rock Phosphate |
| CAT | Catalase |
| CRD | Completely Randomized Design |
| Gy | Gray |
| GSH | Glutathione |
| GR | Glutathione Reductase |
| GMO | Genetically Modified Organism. |
| HSD | Tukey’s Studentized Range |
| LD | Lethality dosage |
| Mm | Milimeter |
| MINT | Malaysian Institute of Nuclear Technology Research |
| $M_1V_1$ | First Cutting Back |
| $M_1V_3$ | Third Cutting Back |
| ROS | Reactive Oxygen Species |
| SAS | Statistical Analyses System |
| SOD | Superoxide Dismutase |

# CHAPTER 1

# INTRODUCTION

Turfgrass is a vegetative ground cover composed of closed cut thickly growing, uniform, inter-twined stems and leaves of plants which form a kind of mat, sward or sod (Beard, 1973). Turfgrass is a monocot plant that belongs to family Poaceae and only 50 grasses have been considered as turfgrasses (Christians, 2007). Each species of turfgrass has different characteristics such as shade tolerance, leaf width and colour, fertility requirements, disease resistance, growth rate, close mowing tolerance, cold hardiness, heat and drought tolerance, uniformity and ability to tolerate traffic and establishment rate (Emmons, 1984). There are about 17 species of turf grasses being used in Malaysia. However, only carpet grass (*Axonopus compressus*), bermudagrass (*Cynodon dactylon*), zoysiagrass (*Zoysia japonica*) and seashore paspalum (*Paspalum vaginatum*) are commercialized in Malaysia. These turfgrasses still have weakness like coarse texture and poor tolerance to environmental stresses.

Issues of enhanced turfgrass improvement depend primarily on increasing new cultivars and their maintenance. In Malaysia, little research has been done so far on turfgrasses. Turfgrass requires genetic variation in useful traits for turfgrass improvement and breeding. However, the desired variation is lacking especially for warm season grasses. There are several potential turfgrasses in Malaysia but due to lack of certain characteristics, these turfgrasses are not top performers. Given this challenge, existing and appropriate new technologies need to be integrated into turfgrass research, in order to focus on problems related to improving turfgrass breeding and development of new cultivars.

Turfgrass industry is still in the process of development in Malaysia. Indeed, there is a lack of turfgrass varieties and cultivars. Currently, there are several ways to obtain genetic variation. One of them is hybridization. However, hybridization is difficult in turfgrass since the florets are very small for hand emasculation and pollination, which is a limiting factor of many research institutes. Besides that, another way to obtain genetic variation is through mutation breeding. Mutations can occur either spontaneously or can be induced.

Induced mutation is a relatively quicker method for improvement of turfgrass and has been used in recent years as a valuable supplement to breeding and development of better crop cultivars (Awan, 1991). Induced mutation plays a vital role in creating additional genetic variations and gamma rays irradiation is one of the mutagenic agents available. Normally large plant populations are required to raise a segregating population. A better way would involve efficient management of first and second generations that could give the greatest possibility for selection of different mutants. For the improvement of a crop, the extent of genetic variability is more important than the total variability. The inheritance of important economic traits such as yield, quality, adaptation, pest and stress resistance, upon which much of the future of plant improvement depends, can be understood through the analysis of a wide range of induced mutations.

There are also important lessons to be learned from the attempts of hybridists and mutation breeders to introduce abiotic and biotic stress resistance into plants (Casler and Van Santen, 2010). The effects of physical and chemical mutagens are well characterized and are very similar to the spontaneous mutation arising *in vitro* or somaclonal variation. Somaclonal variation has contributed to the development of abiotic and biotic stress resistant varieties in major crops. Biotic factor due to the environment are one of the limitations to turfgrass potential. For examples *A. compressus* cannot tolerate drought conditions while *C. dactylon* can poorly tolerate shade conditions. Thus, these situations became limiting factors for turfgrass and reduces turfgrass potential.

In view of the above limitations, several studies were conducted to achieve the following objectives:

I. Determine the optimum dose of gamma rays irradiation for the development of morphological variations in common *C. dactylon, Z. japonica* and *A. compressus*.
II. Asses the morphological variations obtained through gamma rays irradiation.
III. Screen the *C. dactylon* and *A. compressus* mutants for shade and drought tolerance.

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