UNIVERSITI PUTRA MALAYSIA

INDUCED MUTAGENESIS BREEDING THROUGH ACUTE AND CHRONIC GAMMA IRRADIATION FOR YIELD IMPROVEMENT IN TWO BAMBARA GROUNDNUT [Vigna subterranea (L.) Verdc.] VARIETIES

ISMA'ILA MUHAMMAD

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By

ISMA'ILA MUHAMMAD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

July 2021

DEDICATIONS

This thesis is dedicated to my late mother (Khadija Abubakar) for her boundless love, care, and support, who couldn't live to witness this great achievement of my peak academic pursuit. The least I can say Oh ALLAH grant her departed soul eternal rest in Jannatul Firdaus amin. I also dedicated it to the entire members of my family.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

INDUCED MUTAGENESIS BREEDING THROUGH ACUTE AND CHRONIC GAMMA IRRADIATION FOR YIELD IMPROVEMENT IN TWO BAMBARA GROUNDNUT [*Vigna subterranea* (L.) Verdc.] VARIETIES

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July 2021

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Bambara groundnut [*Vigna subterranea* (L.) Verdc.] is a highly nutritious underutilized legume with enormous potentials to sustain global food security. However, limitations due to the crop flower's autogamous and small nature had limited its potential for improvement through conventional breeding with a <2% success rate recorded from the previous studies. Thus, the most viable method of improving this crop is by creating genetic variability through induced mutagenesis. This study was conducted to induce genetic variability in two Bambara groundnut varieties (Ex-Sokoto and Karo) through acute and chronic gamma irradiation to developed high-yielding varieties. Healthy seeds were exposed to acute gamma irradiation using Cesium-137 at the doses of 0 (Control), 25, 50, 75, 100, 125, 150, 175, 200, 250, and 300 Gy. For chronic irradiation, two weeks old healthy seedlings were exposed to the accumulated doses of 0 (Control), 8.52 (Ring 2), 17.04 (Ring 3), 35.56 (Ring 4), 34.09 (Ring 5), 42.61 (Ring 6), 59.65 (Ring 7), 93.74 (Ring 8), 144.87 (Ring 9), 255.64 (Ring 11) and 570.94 (Ring 15) Gy respectively for 852:08 hours in Gamma Green House (GGH) at Nuclear Malaysia until physiological maturity. The experiments were laid down in Randomized complete block design (RCBD) with three replications and four treatments for acute gamma irradiation. However, for chronic gamma irradiation, it was (RCBD) with three replications and eleven treatments at field 15 Faculty of Agriculture, Universiti Putra Malaysia. The results for optimum lethal dose (LD) indicated highly significant differences ($p \leq 0.01$) for all evaluated traits except for internode length, which did not show any significant difference ($p \leq 0.05$). The established lethal doses ($LD_{25\%, 50\%, 75\%}$) for acute gamma irradiation were 73, 160, 248 Gy and 68, 148, 227 Gy for Ex-Sokoto and Karo varieties. Similarly, for chronic gamma irradiation, the established lethal doses ($LD_{25\%, 50\%, 75\%}$) were 47, 250, 444 Gy and 70, 264, 452 Gy for Ex-Sokoto and Karo varieties. The frequency of chlorophyll mutants identified includes; *albina, chlorina, xantha, viridis, maculata*, and *virescent*. The occurrence of a *virescent* type of chlorophyll mutants was the highest between the two varieties. In acute, chlorophyll mutants' overall effectiveness and efficiency were 6.43 and 22.06 among EX-Sokoto, while 4.58 and 17.35 were identified among the Karo variety. Similarly, in the chronic phase, chlorophyll mutants'

overall effectiveness and efficiency were 12.02 and 11.51 among EX-Sokoto and 12.56 and 12.09 among Karo variety. The most noticeable macro mutants identified in this study were linked to the plant height, flowering and maturity period, pods, leaf, and growth habits. The results from both acute and chronic mutagenesis at M_1 to M_4 generations display significant improvements among the different mutagens doses used in this study. It was observed that both acute and chronic gamma irradiation stimulates plant growth at low and moderate gamma irradiation doses compared to the higher gamma irradiation doses. Most of these mutants were identified in the acute phase at the range of 68 to 150 Gy and 73 to 160 Gy for Karo and Ex-Sokoto varieties. Therefore 150 Gy and 160 Gy are regarded as appropriate gamma irradiation dose rates that can be used to induce viable mutants in these varieties. Similarly, among the chronic gamma-irradiated mutants, the most outstanding doses include; 42.61 (ring 7), 25.56 (ring 9), 144.87 (ring 4) and 59.56 (ring 6). Significant improvement was achieved in yield and yield components in both acute and chronic gamma irradiation methods. In the acute phase, the highest mean yield recorded was 10.07 (ESK 250-P11) and 11.60 (KRO 70-P16) t ha^{-1} for EX-Sokoto and Karo varieties. In contrast, 10.37 (ESK R14-P6) and 10.85 (KRO R9-P4) were recorded as the highest mean for EX-Sokoto and Karo variety in the chronic phase. This result is more than two folds of the reported mean yield per hectare in most existing varieties, including the two used in this study. There was high heritability coupled with high genetic advance for most of the studied traits in yield and yield components both in acute and chronic mutagenesis in this study. Therefore, effective selection can be achieved using those traits in subsequent generations. Among acute established mutants, ESK 75-P7 5.11, ESK 75-P15 6.99, ESK 160-P17 7.61, ESK 250-P11 10.07, ESK 250-P7 7.18 and KRO 70-P16 11.60, KRO 70-P5 6.60, KRO 150-P3 11.16, KRO 230-P3 7.68, KRO 230-P3 6.60 were identified as mutants that can be used for further study. For chronic mutants, ESK R6-P9 6.64, ESK R7-P4 6.76, ESK R8-P7 7.47, ESK R11-P5 8.97, ESK R11-P5 6.80, ESK R14-P6 10.37 and KRO R3-P1 6.00, KRO R6-P7 9.44, KRO R7-P3 6.62, KRO R8-P7 7.58, KRO R9-P4 10.85, KRO R11-P9 9.93 can be used. Yield per plant observed a highly significant and positive correlation with most of the rest of the traits studied. In conclusion, this study discovered that induced physical mutagen through acute and chronic gamma radiation effectively induces morphological genetic divergence in Bambara groundnuts and has established the successful approach of induced physical mutagenesis in the two varieties used in this study.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMBIAKBAKAAN MUTAGENESIS INDUKSI MELALUI RADIASI SINARAN GAMMA AKUT DAN KRONIK UNTUK PENAMBAHBAIKAN HASIL DALAM DUA VARIETI KACANG POI BAMBARA [Vigna subterranea (L.) Verdc.]

Oleh

ISMA'ILA MUHAMMAD

Julai 2021

Pengerusi : Muhamad Hazim Nazli, PhD
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Kacang Bambara [*Vigna subterranea* (L.) Verdc.] adalah kekacang yang tinggi khasiat dan berpotensi besar untuk menjamin sekuriti makanan global. Walau bagaimanapun, terdapat keterbatasan kerana bunga tanaman ini yang bersifat autogami dan kecil telah membatasi potensinya, untuk dibiakbaka melalui pembiakbakaan konvensional dengan kadar kejayaan <2% yang dicatat daripada kajian sebelumnya. Oleh itu, kaedah yang paling sesuai untuk membiakbaka tanaman ini adalah dengan mewujudkan kepelbagaian genetik melalui mutagenesis aruhan. Kajian ini dijalankan untuk mengaruhkan kepebagaian genetik ke atas dua varieti kacang Bambara (Ex-Sokoto dan Karo) melalui mutagenesis penyinaran gamma akut dan kronik untuk menghasilkan varieti yang berhasil hasil. Benih yang sihat telah didedahkan kepada sinaran gamma akut menggunakan Cesium-137 pada dos 0 (Kawalan), 25, 50, 75, 100, 125, 150, 175, 200, 250, dan 300 Gy. Manakala, untuk penyinaran kronik, anak benih daripada dua varieti kacang Bambara yang berusia dua minggu telah didedahkan kepada dos terkumpul 0 (Kawalan), 8.52 Gy (Lingkaran 2), 17.04 Gy (Lingkaran 3), 35.56 Gy (Lingkaran 4), 34.09 Gy (Lingkaran 5), 42.61 Gy (Lingkaran 6), 59.65 Gy (Lingkaran 7), 93.74 Gy (Lingkaran 8), 144.87 Gy (Lingkaran 9), 255.64 Gy (Lingkaran 11) dan 570.94 Gy (Lingkaran 15), selama 852:08 jam di dalam Rumah Hijau Gamma (GGH) di Agensi Nuklear Malaysia sehingga matang secara fisiologi. Eksperimen telah dijalankan dalam reka bentuk blok lengkap terawak (RCBD) dengan tiga repikasi dengan empat rawatan bagi penyinaran gamma akut. Walau bagaimanapun untuk penyinaran kronik dengan 11 rawatan, juga dengan reka bentuk RCBD dan tiga replikasi yang dijalankan di Ladang 15, Fakulti Pertanian, Universiti Putra Malaysia. Keputusan penentuan dos maut optimum (LD) menunjukkan perbezaan yang sangat signifikan untuk semua sifat yang dinilai kecuali panjang internod. Dos maut yang ditetapkan ($LD_{25, 50, 75)}$ untuk penyinaran gamma akut masing-masing pada varieti Ex-Sokoto adalah 73, 160 dan 248 Gy sementara 68, 148 dan 227 Gy dicatatkan untuk varieti Karo. Begitu juga untuk penyinaran kronik, dos $LD_{25, 50, 75}$ untuk Ex-Sokoto adalah masing-masing 47, 250, dan 444 Gy, sedangkan 70, 264, dan 452 Gy diperolehi untuk varieti Karo. Frekuensi mutasi

klorofil dikenalpasti termasuk; *albina, chlorina, xantha, viridis, maculata,* dan *virescent*. Kejadian mutan jenis klorofil *virescent* adalah yang paling tinggi antara kedua varieti kacang Bambara. Secara akut, keberkesanan dan kecekapan keseluruhan mutan klorofil masing-masing adalah 6.43 dan 22.06 di antara Ex-Sokoto, sementara itu 4.58 dan 17.35 dikenal pasti di antara varieti Karo. Begitu juga pada fasa kronik, keberkesanan dan kecekapan keseluruhan mutan klorofil adalah maasing-masing 12.02 dan 11.51 di antara Ex-Sokoto dan 12.56 dan 12.09 di antara varieti Karo. Mutan makro yang paling ketara yang dikenal pasti dalam kajian ini adalah berkaitan dengan tinggi pokok, tempoh berbunga dan kematangan, lenggai biji, daun, dan tabiat pertumbuhan. Hasil daripada mutagenesis akut dan kronik pada generasi M_1, hingga M_4 menunjukkan peningkatan yang ketara antara pelbagai dos mutagen yang digunakan dalam kajian ini. Telah diperhatikan bahawa kedua-dua penyinaran gamma, akut dan kronik merangsang pertumbuhan tanaman pada dos penyinaran gamma rendah dan sederhana berbanding dengan dos penyinaran gamma yang lebih tinggi. Sebilangan besar mutan ini dikenal pasti dalam fasa akut pada julat antara 68 hingga 150 Gy, dan 73 hingga 160 Gy masing-masing untuk varieti Karo dan Ex-Sokoto. Oleh itu, 150 Gy dan 160 Gy dianggap sebagai kadar dos penyinaran gamma yang sesuai yang dapat digunakan untuk mendorong mutan yang berdaya hidup dalam varieti ini.

Begitu juga, di antara mutan kronik, dos yang paling berkesan termasuk; 42.61 Gy (Lingkaran 7), 25.56 Gy (Lingkaran 9), 144.87 Gy (Lingkaran 4) dan 59.56 (Lingkaran 6). Peningkatan yang ketara dicapai oleh hasil dan komponen hasil dalam kedua-dua kaedah penyinaran gamma akut dan kronik. Pada fasa akut, hasil min tertinggi dicatatkan ialah 10.07 t ha^{-1} (ESK 250-P11) dan 11.60 t ha^{-1} (KRO 70-P16) untuk varieti Ex-Sokoto dan Karo. Sebaliknya, 10.37 t ha^{-1} (ESK R14-P6) dan 10.85 t ha^{-1} (KRO R9-P4) dicatatkan sebagai min tertinggi untuk varieti Ex-Sokoto dan Karo untuk kaedah kronik. Heritabiliti serta kemajuan genetik yang tinggi juga direkodkan untuk kebanyakan ciri-ciri yang dikaji bagi hasil dan komponen hasil untuk kedua-dua populasi mutagenesis, akut dan kronik. Oleh itu, pemilihan yang berkesan dapat dicapai dengan menggunakan ciri-ciri tersebut pada generasi berikutnya. Hasil setiap pokok memperlihatkan korelasi yang sangat signifikan dan positif dengan kebanyakan sifat yang dikaji. Di kalangan titisan mutan akut maju, ESK 75-P7 5.11, ESK 75-P15 6.99, ESK 160-P17 7.61, ESK 250-P11 10.07, ESK 250-P7 7.18 dan KRO 70-P16 11.60, KRO 70-P5 6.60, KRO 150-P3 11.16, KRO 230-P3 7.68, KRO 230-P3 6.60 dikenal pasti sebagai titisan mutan yang dapat digunakan seterusnya untuk pembangunan varieti. Untuk titisan mutan kronik maju, ESK R6-P9 6.64, ESK R7-P4 6.76, ESK R8-P7 7.47, ESK R11-P5 8.97, ESK R11-P5 6.80, ESK R14-P6 10.37 dan KRO R3-P1 6.00, KRO R6-P7 9.44, KRO R7-P3 6.62, KRO R8-P7 7.58, KRO R9-P4 10.85, KRO R11-P9 9.93 adalah disyorkan untuk pemilihan seterusnya. Kesimpulannya, kajian ini mendapati bahawa mutagen fizikal yang diaruh melalui radiasi gamma akut dan kronik berkesan dalam mendorong dan meningkatkan pencapahan genetik bagi ciri morfologi ke atas kacang Bambara, dan juga telah membuktikan kejayaan pendekatan mutagenesis fizikal teraruh di dalam pembaikan genetik bagi kedua-dua varieti kacang Bambara yang digunakan dalam kajian ini.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

137Cs	Caesium-137
60C	Cobalt-60
GGH	Gamma greenhouse
Gy	Gray
IAEA	International Atomic Energy Agency
kR	Kilo radian
LD_{50}	50% lethal dose
GR_{50}	50% growth reduction
M_1	First generation after mutagenic treatment
M_2	Second generation after mutagenic treatment
M_3	Third generation after mutagenic treatment
M_4	Fourth generation after mutagenic treatment
SA	Sodium azide
SAS	Statistical Analysis System
MVD	Mutant Variety Database
MMS	methyl methanesulfonate
DES	Diethyl sulfate
ENU	N-ethyl-N-nitrosourea

CHAPTER 1

INTRODUCTION

1.1 General introduction

Bambara groundnut [*Vigna subterranea* (L.) Verdc.], is an African indigenous underutilized legume from the Fabaceae family and ranked third most essential food legume next to groundnut (*Arachis hypogaea* L.) and cowpea (*Vigna unguiculata* Walp.), which is predominantly cultivated for subsistence commonly among the low income earning men and women farmers in the semi-arid areas of sub-Sahara Africa (Gbaguidi et al., 2018). It can grow under less fertile soil conditions with low rainfall and widely popular in Africa due to its drought resistance, its quality of producing higher yields when compared to the rest of the more favoured species, like cowpea, common bean, and groundnut (Silué et al., 2016).

It is used both for human and animal consumption as it can improve malnourishment better and improve food availability. The most important and frequently used part of the crop as human food is the seed (Harouna et al., 2018). As part of Africa's diet, the seeds are commonly eaten either by cooking in excess water or roasted as a snack. Therefore, it has a high potential for food and nutritional security among pro-poor households. Furthermore, due to its highly nutritious seeds, which contain 55.5–69.3% carbohydrate, 5.3–7.8% fat, the metabolizable energy value of 362–414 kcal/100g, and 17–24% high-quality protein as well as high amounts of nutritional fibre, Calcium, and Iron including such vitamins as thiamin, riboflavin, niacin, and carotene (Ogundele and Emmambux, 2018).

Researches have revealed that Bambara groundnut production can differ from 375 to 1000 kg ha^{-1} based on the genotype or landrace used (Mohammed et al., 2017). Similarly, in a study conducted by Musa and Singh (2019) to study the influence of nitrogen fixation and the Nbalance of its landraces cultivated on Malaysia's acidic tropical soils, they observed 374-896 kg ha^{-1} in pod yield. The crop produces higher grain yields with a projected world production of 331,000 tons. Valombola et al. (2020) carried out a study to assess the interrelationship among grain yield of Bambara groundnut compared to its numerous yield traits. The result revealed a strong positive correlation between mean pods per plant, 100 seeds weight, and harvest index with grain yield.

Mutation breeding is a form of conventional plant breeding that comprises physical or chemical mutagenesis to assess the genetic variability that will improve varieties with superior traits (Yasmin et al., 2019). Similarly, to select mutants with required agronomic traits, more than 3,222 plant varieties are derived through mutation breeding from more than 170 different plant species worldwide, which have been officially released (Abdulhafiz et al., 2018). Gamma irradiation approach is one of the most efficient and energetic form of electromagnetic radiation, which is more penetrating than other forms of radiation (Kamaruddin et al., 2016). Physical mutagens like gamma rays are safer for health compared to chemical mutagens because they do not require any technique to wash away the mutagen from the planting material (Raina et al., 2017); this is because they are non-toxic. The simplicity of their application plays a significant role in the large acceptability of the technique. About 90% of the developed mutant cultivars were achieved using this technique, with 64% by gamma-rays and 22% by X-rays (Mba, 2013).

1.2 Problem statement

Bambara groundnut is the main source of plant protein in Sub-Saharan Africa and comprises a significant local dish. It has also been identified as a featured crop for Malaysia and positively impacts global food and nutritional security (Belel, 2018). There is increasing support for Bambara groundnut and other underutilized crops from consumers that require higher diversity in their nourishments and from the research communities interested in the potential beneficial crops (Massawe et al., 2016). Despite these significant characteristics, the agro-ecological and genetic potential of Bambara groundnut has not yet been entirely understood due to its autogamous nature. The crop has not received considerable research attention when compared to the other pulses and legumes. It is still cultivated from the local landraces instead of varieties bred precisely for specific agro-ecological environments, thus carrying the underutilized name crop (Muhammad et al., 2020). Several researchers have documented that Bambara groundnut improvement using an artificial conventional breeding approach remains extremely difficult. Many attempts on its hybridization have miserably failed with less than two percent (< 2%) success recorded (Pranesh et al., 2018); this limits its genetic variability for quantitative traits. In Malaysia's tropical humid climate, little is known about the relationship between yield and yield components of Bambara groundnut that may help breeders select promising varieties. Several efforts have been made on induced mutagenesis in many pulses; however, little or no work has been carried out in this underexploited but highly nutritious crop, especially in Malaysia. Therefore, it is imperative to achieve dose-response databases for acute and chronic gamma radiations on Bambara groundnut and develop high-yielding varieties.

1.3 Research hypothesis

The study hypothesised that, different doses of acute and chronic gamma irradiations can induce genetic variability in two Bambara groundnut varieties and can produce viable mutants that will contribute to the performance and yield improvement of the major agronomic traits in this study.

1.4 Main objective

To induce genetic variability in two Bambara groundnut varieties using different doses of acute and chronic gamma irradiations and evaluate their performance on major agronomic traits and identify potential mutants for yield improvement.

Specific objectives:

i. To determine the lethal (LD) and growth reduction (GR) doses of 25%, 50%, and 75% in two Bambara groundnut varieties.

ii. To study the frequency and spectrum of chlorophyll and other observable mutations at M_2 generation of acute and chronic gamma-irradiated mutants.

iii. To assess the mutagenic effectiveness and efficiency of acute and chronic gamma irradiations to increase genetic variability and select economically viable mutants at M_2 generation.

iv. To evaluate yield and agronomic performance of selected M_3 and M_4 mutant generation and compare acute and chronic gamma rays' efficiency in inducing viable mutation for economic traits in Bambara groundnut.

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LIST OF PUBLICATIONS

Journals

Muhammad, I., Rafii, M. Y., Ramlee, S. I., Nazli, M. H., Harun, A. R., Oladosu, Y., … Arolu, I. W. (2020). Exploration of Bambara Groundnut [*Vigna subterranea* (L.) Verdc.] an Underutilized Crop, To Aid Global Food Security : Varietal Improvement, Genetic Diversity and Processing. *Agronomy*, *10*(6), 766; 1–21. https://doi.org/10.3390/agronomy10060766 (Published).

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