UNIVERSITI PUTRA MALAYSIA

PHENOTYPIC AND MOLECULAR VARIATION AMONG SELECTED Curcuma alismatifolia GAGNEP. MUTANTS DERIVED FROM ACUTE AND CHRONIC GAMMA IRRADIATION

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By

SIMA TAHERI

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

November 2014

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Dedication

This thesis is dedicated to

My beloved father and mother

Whose love and blessings have been sources

Of Inspiration to me

And my

Beloved sisters Sara and Sepideh

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

PHENOTYPIC AND MOLECULAR VARIATION AMONG SELECTED *Curcuma alismatifolia* GAGNEP. MUTANTS DERIVED FROM ACUTE AND CHRONIC GAMMA IRRADIATION

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November 2014

Chairman: Thohirah Lee Abdullah, PhD
Faculty: Agriculture

Studies were conducted to study phenotypic and molecular variation among selected *Curcuma alismatifolia* mutants through acute and chronic gamma irradiation and to develop new variants of *C. alismatifolia*. In acute gamma irradiation, rhizomes in the sprouting bud stage were irradiated at eight different doses of 0, 10, 20, 25, 35, 40, 60, and 100 Gy. Radiation sensitivity tests revealed that the LD_{50} of the varieties were achieved at 21 Gy for 'Chiang Mai Red', 23 Gy for 'Sweet Pink', 25 Gy for 'Kimono Pink', and 28 Gy for 'Doi Tung 554'.

From the analysis of variance (ANOVA), significant variations were observed for vegetative traits, flowering development, and rhizome characteristics among the four cultivars of *C. alismatifolia* and dose levels as well as the dose × variety interaction. In first generation ($M_1V_{1)}$, as many as 52 chlorophyll mutants were observed, resulting in a mean mutation frequency of 38.5 % in which 13.1 and 19.3% were produced by 10 and 20 Gy doses, respectively. Individual mutant DT20-1 with two-tone purple bract color was the most attractive mutant generated.

In SSR analysis,the obtained results indicated the high efficiency of polymorphic SSR loci for genetic studies among treated and non-treated *C. alismatifolia* individual plants. 20 Gy acutely irradiated individuals showed a higher mean percentage of polymorphic loci (62.5%) than the 10 Gy (59.38%) and non-treated (22%) ones. By HRM analysis, all studied irradiated individual plants produced melting curves with different melting temperature ($T_{m)}$ from their control individual plants.

In chronic gamma irradiation, plants that were exposed to radiation at doses of 14.6, 33, and 87.4 Gy showed significant decreases in the vegetative traits as compared to the controls. Interestingly, low doses of gamma irradiation stimulated the vegetative growth and promoted earlier flowering of mutants compared to controls. However, higher dose rates decreased the flowering capacity and reduced the quality of the rhizomes of *C. alismatifolia* mutants. In SSR analysis, the overall genetic variability for the varieties studied showed that chronic gamma irradiation particularly at higher dose rates (14.6, 33, and 87.4 Gy) were able to induce more genetic variations to genome of studied *C. alismatifolia* individual plants.

In order to study the morphological and genetic stability of mutants, for both acute and chronic gamma irradiations, studies were continued into the second generation (M_1V_2). In acute gamma irradiationchlorophyll mutation frequency decreased significantlyat 10 and 20 Gy irradiated plants in M_1V_2. Individual DT20-1 with two tone purple color at first generation produced solid mutant with deeper purple bracts in M_1V_2. In order to gain distinctness, uniformity and stability (DUS) of mutants, the rhizomes of all individual plants were grown in M_1V_3 generation and observations were continued. Nine individual plants with bract color variations and dwarfism were selected for further studies in M_1V_4 generation.DT20-1 mutant that maintained the deep purple color in M_1V_3 was considered a desirable potential mutant. SSR analysis in the second generation showed that the percentage of polymorphic loci decreased from 59.3% to 40.6% for 10 Gy individual plants. Similarly, at 20 Gy, percentage of polymorphic loci decreased from 62.5% to 50.0 %.

In chronic gamma irradiation, none of the flower shape and color variations observed in first generation were transferred to the next generation. The effects induced by chronic gamma radiation on these plants are more likely to be physiological changes at sublethal dosages rather than irreversible genetic effects. In SSR analysis the average number of alleles, mean Shannon's information index, and the mean PIC values decreased in second generation as compared to first generation. The percentage of polymorphic loci decreased from 59.38% in M_1V_1 to 37.5% in M_1V_2.

In conclusion, the above studies demonstrated that the mutation frequencies induced by acute gamma irradiation were higher than that of chronic gamma irradiation. In general, new variants of C. *alismatifolia* varieties developed in this study have the potential to be introduced to the Malaysian flower industry.

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

VARIASI FENOTIP DAN MOLEKUL ANTARA MUTAN *Curcuma alismatifolia* GAGNEP. TERPILIH HASIL DARIPADA SINARAN GAMMA AKUT DAN KRONIK

Oleh

SIMA TAHERI

November 2014

Pengerusi: Thohirah Lee Abdullah, PhD
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Kajian telah dijalankan untuk menilai variasi fenotip dan molekul antara mutan *Curcuma alismatifolia* terpilih melalui sinaran gamma akut dan kronik serta memperkenalkan varian *C. alismatifolia* yang baru. Dalam sinaran gamma akut, rizom dalam peringkat mata tunas bercambah telah diiradiasi dengan lapan dos berlainan (0, 10, 20, 25, 35, 40, 60, dan 100 Gy). Ujian kesensitifan radiasi telah menunjukkan bahawa LD_{50} untuk varieti-varieti adalah seperti berikut: 'Chiang Mai Red': 21 Gy, 'Sweet Pink': 23 Gy, 'Kimono Pink': 25 Gy, 'Doi Tung 554': 28 Gy.

Dari analisis varians, variasi yang nyata telah diperhatikan untuk ciri-ciri vegetatif, perkembangan bunga dan ciri-ciri rizom antara keempat-empat kultivar *C. alismatifolia*, paras dos dan juga interaksi di antara dos × varieti. Dalam generasi pertama (M_1V_1), sebanyak 52 mutan klorofil telah diperhatikan, mencatatkan kekerapan min mutasi sebanyak 38.5% di mana 13.1 dan 19.3% adalah dihasilkan oleh dos 10 dan 20 Gy masing-masing. Mutan DT20-1 dengan daun pelindung yang mempunyai dua ton warna, adalah mutan yang paling menarik.

Dalam analisis SSR, keputusan yang diperoleh menunjukkan bahaw alokus SSR yang polimorfik adalah sangat berkesan dalam kajian genetic antara individu *C. alismatifolia* yang dirawat dan tidak dirawat. Individu yang diiradiasi dengan 20 Gy secara akut mencatatkan peratus min lokus polimorfik yang lebih tinggi (62.5%) berbanding dengan 10 Gy (59.38%) dan yang tidak dirawat (22%). Kesemua individu yang dirawat menghasilkan lengkung peleburan dengan suhu peleburan (Tm) yang berlainan berbanding dengan individu kawalan melalui analisis HRM.

Dalam sinaran gamma kronik, tumbuhan yang didedahkan kepada sinaran pada dos 14.6, 33, dan 87.4 Gy menunjukkan pengurangan yang nyata dalam ciri-ciri vegetative berbanding dengan kawalan. Yang menariknya, sinaran gamma padaparas dos yang rendah mampu merangsang pertumbuhan vegetative dan mengalakkan pembungaan awal tumbuhan mutan berbanding dengan tumbuhan kawalan. Namun begitu, kadar dos yang tinggi mengurangkan kapasiti berbunga dan kualiti rizom mutan *C. alismatifolia*. Dalam analisis SSR, variasi genetic keseluruhan untuk varieti-varieti yang dikaji menunjukkan bahawa sinaran gamma kronik, terutamanya

pada kadar dos yang tinggi (14.6, 33, dan 87.4 Gy) berkeupayaan untuk mengaruhkan lebih banyak variasi genetic pada genom individu *C. alismatifolia* yang dikaji.

Untuk mengkaji kestabilan morfologi dan genetic mutan-mutan yang dihasilkan daripada sinaran gamma akut dan kronik, pemerhatian serta kajian telah diteruskan sehingga generasi kedua (M_1V_2). Dalam sinaran gamma kronik, kekerapan mutasi klorofil telah berkurang dengan nyata pada individu M_1V_2 yang disinar dengan 10 dan 20 Gy. Individu DT20-1 dengan dua ton warna ungu pada generasi pertama menghasilkan mutan yang stabil dengan warna ungu yang lebih gelap pada daun pelindungnya dalam M_1V_2. Untuk memperoleh mutan yang menpunyai kelainanan, keseragaman dan kestabilan (DUS), rizom-rizom dari kesemua individu telah ditanam dalm generasi M_1V_3 dan permerhatian telah diteruskan. Sembilan individu dengan variasi warna daun pelindung dan kekerdilan telah dipilih untuk kajian selanjutnya dalam generasi M_1V_4. Mutan DT20-1 yang mengekalkan warna ungu tua dalm M_1V_3 telah dianggap sebagai mutan yang berpotensi. Analisis SSR dalam generasi kedua menunjukkan bahawa peratusan lokus polimorfik telah berkurangan daripada 59.3 % kepada 40.6 % untuk individu yang diiradiasi dengan 10 Gy. Trend yang sama telah ditunjukkan untuk individu yang diiradiasi dengan 20 Gy, di mana peratusan lokus polimorfik berkurangan daripada 62.5 % kepada 50.0 %.

Dalam sinaran gamma kronik, tidakada variasi bentuk dan warna bunga yang diperhatikan dalam generasi pertama dipindahkan ke generasi seterusnya. Kesan yang diaruhkan oleh sinaran gamma kronik pada tumbuhan ini berkemungkinan besar merupakan perubahan fisiologi pada dos sublethal berbanding dengan kesan genetic tidak berbalik. Dalam analisis SSR purata nombor alel, min indeks maklumat Shannon dan min nilai PIC berkurangan pada generasi kedua berbanding dengan generasi pertama. Peratus lokus polimorfik berkurangan daripada 59.38% dalam M_1V_1 kepada 37.5% dalam M_1V_2.

Kesimpulannya, kajian ini telah menunjukkan bahawa kekerapan mutasi yang diaruhkan oleh sinaran gamma akut adalah lebih tinggi daripada sinaran gamma kronik. Secara umumnya, varian baru *C. alismatifolia* yang dihasilkan daripada kajian ini mempunyai potensi untuk diperkenalkan ke dalam industry bungaan di Malaysia.

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I certify that a Thesis Examination Committee has met on 3 November 2014 to conduct the final examination of Sima Taheri on her thesis entitled "Phenotypic and Molecular Variation among Selected *Curcuma alismatifolia* Gagnep. Mutants Derived from Acute and Chronic Gamma 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 Doctor of Philosophy.

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This thesis was submitted to the Senate of 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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°C	degree Celsius
µl	microliter
µM	micromole
^{137}Cs	caesium-137
^{60}Co	Cobalt-60
AFLP	amplified fragment length polymorphism
ANOVA	analysis of variance
C.V.	coefficient of variation
CE	capillary electrophoresis
CMR	Chiang Mai Red
CRD	Complete randomized design
CTAB	Cetyltrimethylammonium bromide
DNA	deoxyribonucleic acid
DT	Doi Tung 554
DUS	distinctness, uniformity and stability
dNTP	2'-deoxynucleotide 5'-triphosphate
EDTA	Ethylenediaminetetraacetic acid
EMS	ethyl-methane-sulphonate
ENH	ethyl-nitrosourea
FAO	Food and agriculture organization
G1	Gap 1
G2	Gap 2
GGH	Gamma greenhouse
Gy	Gray
H	Nei's (1973) gene diversity
h	hours
He	Expected heterozigosity
H_O	observed heterozygosity
HRM	high resolution melting
I	Shannon's information index
IAEA	International Atomic Energy Agency
IR	ionizing radiations
ISSR	inter-simple sequence repeat
kR	Kilo radian
KP	Kimono Pink
LD50	50 % lethal dose
LSD	Least significant difference
M	Mitosis
M_1V_1	First vegetative generation of irradiated populations
M_1V_2	Second vegetative generation of irradiated populations
M_1V_3	Third vegetative generation of irradiated populations
MAB	Mutation-assisted breeding
MNH	methyl-nitroso-urea
mg	miligram
min	Minutes
mM	milimolar
n_a	Observed number of alleles
n_e	Effective number of alleles

NTSYS	numerical taxonomy multivariate analysis system
PCA	principal component analysis
PCR	polymerase chaine reaction
PIC	polymorphic information content
PVP	polyvinyl pyrrolidone
RAPD	random amplified polymorphic DNA
RCBD	randomized complete block design
RFLP	restriction fragment length polymorphism
ROS	reactive oxygen species
S	Synthesis
SA	sodium azide
SAS	statistical analysis system
SNP	single nucleotide polymorphism
SSR	simple sequence repeats
SP	Sweet Pink
TBE	Tris/Borate/EDTA
TE	Tris EDTA buffer
UPGMA	Unweighted pair group method using arithmetic averages
UV	ultraviolet

CHAPTER 1

INTRODUCTION

1.1 Floriculture industry in Malaysia

The Malaysian cut flower is a relatively new industry as compared to other agricultural business but have grown from it's rather low position into a commercial yield in the mid-eighties. Efficient increase in the flower production and export in response to local and foreign demands are witnesses of such tremendous growth in the last decade. It is expected this developing trend will continue in the future along with growing affluence of the local population and that of the developed countries as well as improved market opportunities. In the Tenth Malaysia Plan (2011-2015), cut flowers with qualified potential to meet the growing local and foreign demands and to generate higher income for producers are preferred. In 2006, exports from Malaysia for temperate flowers, orchids, foliages and dried flowers were RM100 million a figure that only represented about 33 % of the world market (Ahmad *et al.*, 2007). This scenario showed that the Malaysian floriculture industry still has the potential for expansion to bring more revenue to the country as well as the industry.

In recent years, the Malaysian floriculture industry has shown a remarkable growth promoted by urbanization and landscaping activities and has become a dynamic industry in the agricultural export sector. Thus, as a contribution of the Tenth Malaysian Plan, which emphasized more on the agriculture sector, the introduction of a new flowering crop is vital as it has vast potential in contributing to Malaysia's economic development. In the quest for new variety of ornamental plants to further enhance the lucrative floriculture industry, ornamental Zingibers, which have long been known of their showy inflorescences, present the highest potential (Lee, 2007).

1.2 Overview of ornamental gingers

The Zingiberaceae or ginger family is a family of flowering plants comprising about 90 genera and approximately 1400 species which continues to grow as more new species are being identified (Chapman, 1995). The genera of flowering gingers include *Alpinia, Curcuma, Etlingera, Globba, Hedychium, Kaempferia and Zingiber*. Ornamental gingers with spectacular and brightly colored flowers are amazingly versatile group of plants with a wide range of shapes, size, and colors. These gingers are cultivated in many parts of the tropics and subtropics as landscape plants, pot flowers or cut flowers. The genus *Curcuma* is considered to have originated in the Indo-Malayan Region (Purseglove, 1972), has a widespread occurrence in the tropics of Asia to Africa and Australia. *Curcuma is* the genera in the Zingiberaceae family that have recently gained much attraction.

1.3 Status of *C. alismatifolia*

C. alismatifolia, native to northern Thailand was introduced to the world flower market in the early 1990s. The basic physiology of flowering for *C. alismatifolia* has been intensively studied (Fukai and Udomdee, 2005; Azuma and Takano, 1994; Hajiladi*et al.*, 1997a, Kuehny *et al*., 2002) and year-round cut flower production has been established. Presently, the species is grown predominantly in Thailand and China where they are the native (indigenous) to the South East Asia (Paz, 2003). Although successful breeding programs of *Curcuma* such as production of inter-specific hybrids have been achieved in Thailand (Wongpiyasatid *et al.*, 2009) only a few have been introduced to the world flower market because of the lack of commercial bodies to lead these flowers onto the world market. Similar growing conditions here in Malaysia offer a great potential and this opportunity is presently unexploited.

This plant has a high potential to enhance the floriculture industry in Malaysia as it is becoming a favorite in the international market – as cut flower production, potted plant as well as for exterior landscapes. It is also becoming a highly demanded plant in Japan, Holland, USA, Taiwan and Israel where it is grown commercially as a cut flower.

Curcuma alismatifolia have colorful, long-lasting inflorescence, with a 180-200 days production cycle and with few pest problems. They are herbaceous perennials with short fleshy rhizomes and tuberous roots, often with a dormancy period (Khuankaew *et al*., 2010). In Thailand, rhizomes of *Curcuma* in commercial production are harvested during the three months dormant period of storage and distribution (Lee, 2007). Other characteristics that make them attractive to the floricultural industry are ease of production, unique foliage, and numerous flowering stems per pot. In this study, three cultivars of *C. alismatifolia*, 'Chiang Mai Red', 'Sweet Pink', 'Kimono Pink', and one hybrid namely 'Doi Tung 554' were chosen due to their free flowering habits and attractive flower colour and shape.

1.4 The role of induced mutation in producing new cultivars of ornamental plants

Mutation breeding is one of the methods for generating genetic variation and obtaining new cultivars of ornamental plants during the past decades (Ahloowalia and Maluszynski, 2001; Broertjes *et al*., 1976; Zalewska and Jerzy, 1997). Induced mutations have been utilized for the past 70 years to produce mutant cultivars by changing the plant characteristic for a significant increase in plant production among both seed and vegetatively propagated crops. The most common mutagens used are: chemical (alkylating agents)-ethyl-nitrosourea (ENH), methyl-nitroso-urea (MNH), sodium azide (SA), ethyl-methane-sulphonate (EMS), and physical agents–X-rays, gamma rays, fast neutrons, ultra violet, and laser (Jain, 2002). Among these mutagens, gamma rays and EMS are widely used for mutation induction. The advantages of physical mutagens are accurate dosimetry and reasonable reproducibility, as well as high and uniform penetration of multicellular system particularly by gamma rays. Extensive work has been carried out for the last 30 years for improvement of different ornamental crops. Gamma-rays have been most successfully used and 76 new mutant varieties with

changed flower color/shape, and chlorophyll variation in leaves have been developed and released in different ornamentals (Datta, 2009).

1.5 The application of biotechnology in mutation programs

The estimation of genetic variation on the basis of morphological traits alone, which are the product of gene and environmental interactions, does not determine the actual level of genetic variation among studied individuals (Sigrist *et al*., 2011). In order to reveal the induced variation in genomic DNA of mutants, appropriate molecular markers are required. Among different classes of molecular markers, Simple sequence repeats (SSR) are useful for a variety of applications in plant genetics and breeding. SSR also known as microsatellites are a class of molecular markers based on tandem repeats of short (1-6 bp) DNA sequences which are ubiquitously distributed throughout eukaryotic genomes. These repeat sequences with codominant inheritance are found to be abundant in plant genomes and are frequently highly polymorphic, even among closely related cultivars. Variation could be due to insertion or deletion of nucleotides. In addition, SSR markers have also been shown to have transferability across different species and plants, a fact that increase their value in plant genetic studies. Inter-simple sequence repeat (ISSR) involves amplification of DNA segments present at an amplification distance in between two identical SSR repeat regions oriented in opposite directions (Masumbuko and Bryngelsson, 2006). Based on the presence of the SSR throughout genome, ISSR analysis involves polymerase chain reaction (PCR) amplification of genomic DNA using a single primer that targets the repeat, which is anchored at 3' or 5' end. Unlike SSR markers, ISSR does not require sequence information or prior genetic studies for primer synthesis (Joshi *et al*., 2000). Besides ISSRs have been demonstrated to provide highly reproducible results and generate abundant polymorphism in a number of crop species. Therefore it provides an efficient tool for assessment of DNA variation induced by gamma irradiation.

SSRs and ISSRs have been highly popular genetic markers for last two decades because of the above mentioned benefits. However, the traditional protocols used for SSR, employ loci-specific primers to PCR amplify the DNA fragment containing nucleotide repeats, and separate the PCR products using laborious electrophoresis. Nevertheless, high resolution melt (HRM) analysis is a technique that measures the disassociation of double-stranded DNA at high temperature resolution, and permits the analysis of genetic variations (SNPs, mutations, methylation) in PCR amplicons (Gundry *et al*., 2003). This technique allows mutation scanning without the need for costly labeled probes, as it uses high fidelity heteroduplex- detecting double-stranded DNA binding dyes, such as EvaGreen which exhibit equal binding affinities for GC-rich and AT-rich regions and no sequence preference (Lochlainn *et al*., 2011).

However, due to the lack of new local varieties, most of the varieties planted in Malaysia were imported from Thailand. In addition, unlike other well-established bulbous floral crops, studies on *C. alismatifolia* for induced mutation have not been fully investigated. The development of new varieties of *Curcuma* is expected to reduce the grower's dependence on Thailand's cultivars in the long term.

The present study is aimed to use acute and chronic gamma rays to induce morphological and DNA variations in *C. alismatifolia* and to develop new variants of *C. alismatifolia*, as well as to compare the effectiveness of acute and chronic gamma irradiation in inducing desired morphological and DNA variation.

1.6 Research objectives

This study was designed to achieve the following general objectives:

1. To induce morphological and DNA variations in *C. alismatifolia*cultivars through acute and chronic gamma irradiation.
2. To develop new variants of *C. alismatifolia*using acute and chronic gamma irradiation.
3. To compare the effectiveness of acute and chronic gamma irradiation in induction of desired morphological and DNA variations in *C. alismatifolia*.

REFERENCES

Abdullah, T. L., Endan, J., & Nazir, B. M. (2009). Changes in Flower Development, Chlorophyll Mutation and Alteration in Plant Morphology of *Curcuma alismatifolia* by Gamma Irradiation. *American Journal of Applied Sciences*, 6(7), 1436.

Adamu, A. K. & Aliyu, H. (2007). Morphological effects of sodium azide on tomato (*Lycopersicon esculentum* Mill).*Scientific World Journal*, 2(4), 9-12.

Agarwal, M., Shrivastava, N. & Padh, H. (2008). Advances in molecular marker techniques and their applications in plant sciences.*Plant Cell Reports*, 27(4), 617–631.

Ahloowalia, B. S., & Maluszynski, M. (2001). Induced mutations - A new paradigm in plant breeding.*Euphytica*, 118(2), 167-173.

Ahloowalia, B. S., Maluszynski, M. & Nichterlein, K. (2004). Global impact of mutation-derived varieties.*Euphytica*, 135(2), 187-204.

Ahmad, Z., Abu Hassan, A., Salleh, S., Ariffin, S., Shamsudin, S. & Basiran, M.N. (2012). Improvement of Malaysian Ornamental Plants through Induced Mutation.*Pertanika Journal of Tropical Agricultural Science*, 35 (3), 631-636.

Ahmad, S. H., Mohamed, M. T. M., Abdullah, T. L., Malik, A. A. & Rashid, R. *Malaysia horticulture perspectives: education, research and extension*, paper presented at the International Symposium on Prospects of Horticulture Industry in Pakistan, Faisalabad. March 2007.

Al-bashir, M. (2004). Effect of gamma irradiation on fungalload, chemical and sensory characteristics of walnuts *(Juglans regia* L.).*Journal of Stored Products Research*, 40 (4), 355–362.

Al-Safadi, B. & Simon, P. W. (1996). Gamma irradiation-induced variation in Carrots (*Daucus carota* L.).*Journal* of the *American Society* for *Horticultural Science*, 121 (4), 599 –603.

Ansell, S. W., Schneider, H., Pedersen, N., Grundmann, M., Russell, S. J. & Vogel, J. C. (2007). Recombination diversifies chloroplast trnF pseudogenes in *Arabidopsis lyrata*. *Journal of Evolutinary Biology*, 20(6), 2400–2411.

Aparajita, S., Senapati, S. K. & Rout, G. R. (2008). Identification and genetic relationships among nine Albizzia species based on morphologicaland molecular markers.*Plant Biosystems*, 142(1), 30–9.

Apavatjrut, P., Sirisawad, T., Sirirugsa, P., Voraurai, P. & Suwanthada, C. *Studies on chromosome number of seventeen Thai Curcuma species*. Paper presented at the meeting of the 2nd Thailand National Conference on Flower and Ornamental Plant, Chiang Mai, 14-17 Feb 1996.

Apavatjrut, P., Somboon, A., Puangpen, S. & Chiara, A. (1999). Molecular markers in the identification of some early flowering *Curcuma* L. (Zingiberaceae) species.*Annals of Botany*, 84 (4), 529–534.

Arlett, C. F. & Potter, J. (1971). Mutation to 8-azaguanine resistance induced by v-radiation in a Chinese hamster cell line.*Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 13 (1), 59-65.

Arnold, N. P., Barthakur, N. N. & Tanguai, M. (1998). Mutagenic effects of acute gamma irradiation on miniature roses: Target theory approach.*Hortscience*, 33(1), 127-139.

Arthofer, W., Steiner, F. M. & Schlick-Steiner, B. C. (2011). Rapid and cost-effectivescreening of newly identified micro satellite loci by high resolution meltinganalysis. *Molecular Genetics and Genomics*, 286(3), 225–235.

Atienzar, F. & Jha, A. N. (2006). The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: A critical review. *Mutation Research/Reviews in Mutation Research,*613(2), 76–102.

Atienzar, F. A., Venier, P., Jha, A. N. & Depledge, M. H. (2002). Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations.*Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 521(1), 151–163.

Atienzar, F. A., Cordi, B., Donkin, M. E., Evenden, A. J., Jha, A. N., & Depledge, M. H. (2000). Comparison of ultraviolet-induced genotoxicity detected by random amplified polymorphic DNA with chlorophyll fluorescence and growth in a marine macroalgae, *Palmariapalmata*. *Aquatic Toxicology*, 50(1), 1-12.

Atienzar, F. A., Conradi, M., Evenden, A. J., Jha, A. N. & Depledge, M. H. (1999). Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in Daphnia magna exposed to benzo[a]pyrene. *Environmental Toxicology* and *Chemistry*, 18(10), 2275–2282.

Azhar, M., Rusli, I. & Sobri H. (2009). *Gamma greenhouse for chronic irradiation in plant mutation breeding*. Paper presented at International Nuclear Conference. PWTC, Kuala Lumpur. June 2009.

Azuma, A., & Takano, K. (1994). Studies on the flowering control of *Curcuma alismatifolia*. II. On rest of corm, and its breaking of rest. The storage condition of corm.*Bulletin of Kochi Agriculture Research Center*, 3, 37-45.

Babaei, A. (2010). Geneic diversity analysis of Nemat rice mutant (*Oriza sativa* L.) via RAPD markers.*American-Eurasian Journal of Agriculture and Environmental Science*, 8(4), 452-456.

Babaei, N., Abdullah, N. A. P., Saleh, G. & Abdullah, T. L. (2012). Isolation and characterization of microsatellite markers and analysis of genetic variability in *Curculigo latifolia* Dryand.*Molecular Biology Reports*, 39(11), 9869–9877.

Bari, G. (1971). Effect of chronic and acute gamma irradiation on morphological characters and seed yield in Flax.*Radiation Botany*, 11 (4), 293-302.

Bhatia, C. & Swaminathan, M. S. (1963). Frequency and spectrum of mutations induced by radiations in some varieties of bread wheat.*Euphytica*, 12(1), 97-112.

Bory, S., Silva, D., Risterucci, A. M., Grisoni, M., Besse, P. & Duval, M.F. (2008). Development of microsatellite markers in cultivated vanilla: Polymorphism and transferability to other vanilla species. *Scientia Horticulture*, 115(4), 420–425.

Botstein, D., White, R. L., Skolnick, M. & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American journal of human genetics*,32(3), 314-331.

Boyer, V., Vichot, L., Fromm, M., Losset, Y., Tatin-Froux, F., Guétat, P. & Badot, P. M. (2009). Tritium in plants: a recent of current knowledge. *Environmental and Experimental Botany*, 67(1), 34–51.

Briscoe, D. A., Malpica, J. M., Robertson, A., Smith, G. J., Frankham, R., Banks, R. G. & Barker, J.S.F. (1992). Rapid loss of genetic variation in large captive populations of *Drosophila* files: implications for the genetic management of captive populations. *Conservation Biology*,6 (1), 416-425.

Broertjes, C., Roest, S., & Bokelmann, G. S. (1976). Mutation breeding of *Chrysanthemum morifolium* Ram.using*in vivo* and *in vitro* adventitious bud techniques. *Euphytica*, 25(1), 11-19.

Broertjes, C. & van Harten, A. M. (1988). Applied Mutation Breeding for Vegetatively Propagated Crops. Amsterdam: Elsevier.

Bruggemann, E., Handwerger, K., Essex, C. & Storz, G. (1996). Analysis of fast neutrongenerated mutants at the *Arabidopsis thaliana* HY4 locus.*The Plant Journal*, 10(1), 755–760.

Brunborg, G. & Williamson, D.H. (1978). The relevance of the nuclear division cycle to radiosensitivity in yeast.*Molecular and General Genetics MGG*,162 (3), 277-286.

Bunya-atichart, K., Ketsa, S. & van Doorn, W. G. (2004). Postharvest physiology of *Curcuma alismatifolia* flowers.*Postharvest Biology and Technology*, 34(2), 219–226.

Burki, H. J., Lam, C. K. & Wood, R. D. (1980). UV-light-induced mutations in synchronous CHO cells.*Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 69(2), 347-356.

Chagne, D., Gasic, K., Crowhurst, R. N., Han, Y., Bassett, H. C., Bowatte, D. R., Lawrence, T. J., Rikkerink, E. H. A., Gardiner, S. E. & Korban, S. S. (2008). Development of a set of SNP markers present in expressed genes of the apple. *Genomics*, 92 (5), 353–358.

Chahal, G. S. & Gosal, S. S. (2002). *Principles and Procedures of Plant Breeding*. Oxford: Alpha Science International Ltd. Narosa Press.

Chapman, T. S. (1995). *Ornamental Gingers: A guide to selection & cultivation.*Published by Timothy Sean Chapman, St. Gabriel, Louisiana.

Chopra, V. L. (2005). Mutagenesis: Investigating the process and processing the outcome for crop improvement. *Current Science*, 89, 353–359.

Chun, E. H. L.,Vaughn, M. H. & Rich, A. (1963). The isolation and characterization of DNA associated with chloroplast preparations. *Journal of molecular biology*, 7(2), 130-141.

Chuantang, N. & Yazhi, L. (1998). The radiation induced mutation of canna (*Canna* L.). *Acta Agricultute Nucleatae Sinica*, 2, 33-39.

D'Amato, F., Scarascia, G. T., Monti, L. M. & Bozzini, A. (1962). Types and frequencies of chlorophyll mutations in Durum wheat induced by radiations and chemicals.*Radiation Botany*, 2(3), 217-239.

Das, A., Kesari, V., Satyanarayana, V. M., Parida, A. & Rangan, L. (2011). Genetic Relationship of *Curcuma* Species from Northeast India Using PCR-Based Markers.*Molecular Biotechnology*, 49(1), 65-76.

Datta, S. K., Chakrabarty, D. & Mandal, A. K. A. (2001). Gamma ray-induced genetic manipulations in flower colour and shape in *Dendranthemum grandiflorum* and their management through tissue culture.*Plant Breeding*, 120(1), 91–92.

Datta, S. K. (2009). A report on 36 years of practical work on crop improvement through induced mutagenesis.In Q. Y. Shu.*Induced Plant Mutations in the Genomics Era* (pp. 253-256). Rome.Food and Agriculture Organization of the United Nations.

De Hertogh, A. A. & Le Nard, M. (1993). Botanical aspects of flower bulbs. In *The Physiology of Flower Bulbs*, ed. A.A. De Hertogh, and M. Le Nard, pp. 7–20. Amsterdam: Elsevier.

Devarumath, R. M., Kalwade, S. B., Kawar, P. G. & Sushir, K. V. (2012). Assessment of Genetic Diversity in Sugarcane Germplasm Using ISSR and SSR Markers.*Sugar Tech*, 14(4), 334–344.

Dhakshanamoorthy, D.,Selvaraj, R. & Chidambaram, A. L. A. (2011). Induced mutagenesis in *Jatropha curcas* L. using gamma rays and detection of DNA polymorphism through RAPD marker. *C. R. Biologies*, 334(1), 24–30.

Distefano, G., Caruso, M., Malfa, S. L., Gentile, A. & Wu, S. B. (2012). High resolution melting analysis is a more sensitive and effective alternative to gel-Based platforms in analysis of SSR. An example in Citrus. *PloS one*.**7**, e44202.

Dogbevi, M. K., Vachon, C. & Lacroix, M. (2000). Physicochemical properties of dry red kidney bean proteins and natural micro-flora as affected by gamma irradiation. *Journal of Food Science*, 64(3), 540–542.

Donini, P. & Sanino, A. Induced mutation in plant breeding: Current status and future outlook, In *Somaclonal Variation and Induced Mutations in Crop Improvement*, Kluwer Academic Publishers, London. Mohan Jain, S Brar, DS Ahloowalia, BS Ed. 1998.

Doyle, J. J. & Doyle, J. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytocheical Bulletin*, 19, 11-15.

Duangent, J. (2006). *Mutational breeding of Curcuma longa for medicinal purposes using colchicine and gamma rays.*Unpublished master's dissertation. Mahidol University. Thailand.

Eralia, M. &Wittwer, C. T. (2010). High Resolution Melting Analysis for Gene Scanning.*Methods*, 50(4), 250–261.

Erickson, D. L. & Fenster. C. B. (2006). Intraspecific hybridization and the recovery of fitness in the native legume *Chamaecrista fasciculata*. *Evolution*, 60(2), 225–233.

Eroglu, Y., Eroglu, H. E. & Ilbas, A. I. (2007). Gamma ray reduces mitotic index in embryonic roots of *hordeum vulgare* L. *Advances in Biological Research*, 1(1-2), 26-28.

Fang, D. Q., Roose, M. L., Krueger, R. R. & Federici, C. T. (1997). Fingerprinting trifoliate orange germ plasm accessions with isozymes, RFLPs, and inter-simple sequences repeat markers. *Theoretical and Applied Genetics*, 95 (1), 211–219.

Fu, H. W., Li, Y. F. & Shu, Q. Y. (2008). A revisit of mutation induction by gamma rays in rice (*Oryza sativa* L.): implications of microsatellite markers for quality control. *Molecular breeding*, 22 (2), 281–288.

Fukai, S., & Udomdee, W. (2005). Inflorescence and flower initiation and development in *Curcuma alismatifolia* Gagnep (Zingiberaceae). *Japanese Journal of Tropical Agriculture*, 49(1), 14-20.

Gady, A. L. F., Hermans, F. W. K., Van de Wal, H. B. J. M., van Loo, E. N., Visser, G. F. R. & Bachem, C. W. B. (2009). Implementation of two high through-put techniques in a novel application: detecting point mutations in large EMS mutated plant populations. *Plant Methods*, 5(1), 13.

Ganopoulos, I., Argiriou, A. & Tsaftaris, A. (2011). Microsatellite high resolutionmelting (SSR-HRM) analysis for authenticity testing of protected designation oforigin (PDO) sweet cherry products. *Food Control*, 22(3), 532–541.

Ghariani, S., Trifi-Farah, N., Chakroun, M., Marghali, S. & Marrakchi, M. (2003). Genetic diversity in Tunisian perennial ryegrass revealed by ISSR markers. *Genetic Resources and Crop Evolution*, 50(8), 809-15.

Gianfranceschi, L., Seglias, N., Tarchini, R., Komjanc, M. & Gessler, C. (1998). Simple sequence repeats for genetic analysis of apple. *Theoretical and Applied Genetics*, 96(8), 1069–1076.

Ginger: Postharvest Care and Market Preparation; National Agricultural Research Institute, Technical Bulletin No. 23: East Coast Demerara, Guyana, 2004.

Golkar , P., Arzani, A.& Rezaei, A. M. (2011). Genetic Variation in Safflower (*Carthamus tinctorious* L.) for Seed Quality-Related Traits and Inter-Simple Sequence Repeat (ISSR) Markers. *International Journal of Molecular Sciences*,12(4), 2664-2677.

Goulao, L. & Oliveira, C. M. (2001). Molecular characterisation of cultivars of apple (Malus x domestica Borkh.) using microsatellite (SSR and ISSR) markers. *Euphytica*, 122(1), 81-89.

Giridharan, M. P. & Balakrishnan, S. (1992). Gamma ray induced variability in vegetative and floral characters of ginger. Indian Cocoa, *Arecanut and Spices Journal*, 15(3), 68–672.

Guasmi, F., Touil, L., Feres, K., Elfelah, W., Triki, T. & Ferchichi, A. (2008). Genetic diversity of Tunisian barley accessions based on microsatellite markers. *Biotechnology*, 7(4), 781-786.

Gudcove, I. N. & Grodzinsky, D. M. (1982). Cell radiosensitivity variation in synchronouly-dividing root meristems of *Pisum sativum* L. during the mitotic cycle. *International Journal of Radiation Biology*, 41(4), 401-409.

Gunckel, J. E. (1957). The effect of ionizing radiation on plants: morphological effects. *The Quarterly Review of Biology*, 32(1), 46-56.

Gundry, C. N., Vandersteen, J. G., Reed, G. H, Pryor, R. J, Chen, J.& Wittwer, C. T. (2003). Amplicon melting analysis with labeled primers: A closed-tube method for differentiating homozygotes and heterozygotes. *Clinical Chemistry*, 49 (3), 396-406.

Gupta, M. N., Laxmi, V., Dixit, B. S., & Srivastava, S. N. (1982). Gamma ray induced variability in *Costus speciosus. Progressive Horticulture*, 14**,** 193-197.

Gupta, M., Chyi, Y. S., Romero-Severson, J. & Owen, J. L. (1994). Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple sequence repeat. *Theoretical and Applied Genetics*, 89(7), 998-1006.

Gupta, P. K., Balyan, H. S., Sharma P. C. & Ramesh, B. (1996). Microsatellites in plants: a new class of molecular markers. *Current Science*, 45(1), 45–54.

Guptav, P. K. & Varshney, R. K. (2000). The development and use ofmicrosatellite markers for genetic analysis and plant breedingwith emphasis on bread wheat. *Euphytica*, 113(3), 163-185.

Gustafson, A. *Mutation in plant breeding: A glance backward and a look forward*. Lecture presented at 5th International Congress on Radiation Research, Seatle, Academic Press, New York, July 1994.

Hagidimitriou, M. (2005). Genetic diversity of major greek olive cultivars using molecular (AFLP and RAPD) markers and morphological traits. *Journal of American society of horticulture science*, 130 (2), 211-217.

Hagiladi, A., Umiel, N., Gilad, Z., &. Yang, X. H. (1997).*Curcuma alismatifolia* I. Plant morphology and the effect of tuberous root number on flowering date and yield of inflorescence. *Acta Horticulture*, 430, 747-753.

Hall, A. T. (1999). BioEdit: a user friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Oxford University Press*. 41, 95-98.

Hegde, R. K. (2006). Studies *on Induced Mutagenesis and in vitro Regeneration in Turmeric* (*Curcuma longa L.*).Unpublished doctoral dissertation.University of Agricultural Sciences, India.

Harada, T., Matsukawa, K., Sato, T., Ishikawa, R., Niizeki, M. & Saito, K. (1993). DNA-RAPD detect genetic variation and paternity in *Malus*. *Euphytica*, 65(2), 87–91.

Hemalatha, K. (1998). Induction *of mutation in carnation (Dianthus caryophyllus L.) through gamma rays and ethyl methane sulphonate*.Unpublished doctoral dissertation, University of Agricultural Sciences, India.

Herrmann, M. G., Durtschi, J. D., Wittwer, C. T. & Voelkerding, K. V. (2007). Expanded instrument comparison of amplicon DNA melting analysis for mutation scanning and genotyping. *Clinical Chemistry*, 53(8), 1544-1548.

Hongpakdee, P., Ohtake, N., Sueyoshi, K., Ohyama, T. & Ruamrungsri, S. (2010). Effects of low night temperature and short day length on some phytohormones and nutrient status in *Curcuma alismatifolia* Gagnep. *Thai Journal of Agricultural Science*, 43(3), 163-173.

Houle, D. (1989). Allozyme associated heterosis in *Drosophila melanogaster*. *Genetics*, 123(4),1467-1483.

Ichikawa, S. (1970). Relative biological efficiency of 14.1 MeV fast neutrons and 137Cs gamma rays in the stamen hairs of Tradescantia reflexa Rafin. Japanese Journal Genetics, 45, 205-216.

Ichikawa, S. & Takahashi, C. (1977). Somatic mutation frequencies in the stamen hairs of stable and mutable clones of *Tradescantia* after acute gamma-ray treatments with small doses. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 45(2), 195-204.

Ichikawa, S. (1992). *Tradescantia* stamen-hair system as an excellent botanical tester of mutagenicity: its responses to ionizing radiation and chemical mutagens, and some synergistic effects found.*Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 270(1), 3-22.

Ikushima, T. (1987). Somatic mutation in cereals.*Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 181(1), 199–207.

IAEA (International Atomic Energy Agency), *Mutant Varieties Database*.Retrieved fromhttp://mvgs.iaea.org.

Jabbarzadeh, Z., Khosh-khui, M., Salehi, H. & Saberivand, A. (2010). Inter simple sequence repeat (ISSR) markers as reproducible and specific tools for genetic diversity analysis of rose species. *African Journal of Biotechnology*, 9(37), 6091-6095.

Jain, S. M. (2002). A review of induction of mutations in fruits of tropical and subtropical regions.*Acta Horticulture*, 575, 295-302.

Jain, S. M. (2006). Mutation-assisted breeding for improving ornamental plants. *Acta Horticulturae,* 714, 85-98.

Jain, S. M. (2007). Recent advances in plant tissue culture and mutagenesis. *Acta Horticulturae,* 736, 205-211.

Jan, S. J. K. (2002).PIC calculator.http://www.liv.ac.uk/~kempsj/pic.html.

Jan, S., Parween, T. & Siddiqi, T. O.(2011). Gamma radiation effects on growth and yield attributes of Psoralea corylifolia L. with reference to enhanced production of psoralen. *Plant Growth Regulator*, 64(2), 163–171.

Jayachandran, B. K. & Mohankumar, N. (1992). Effect of gamma ray irradiation on ginger.*South Indian Horticulture*, 40, 283-288.

Jeong, H. J., Jo, Y. D. & Kang, B. C. (2010). Identification of Capsicum species using SNPmarkers based on high resolution melting analysis. *Genome*, 53(12), 1029–1040.

Jones, N., Ougham, H., Thomas, H. & Pašakinskien, I. (2009). Markers and mapping revisited: finding your gene. *New Phytologist,* 183(4), 935-966.

Joshi, S. P., Gupta, V. S., Aggarwal, R. K., Ranjekar, P. K. & Brar, D. S. (2000). Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus *Oryza*. *Theoretical and Applied Genetics,* 100 (8), 1311-1320.

Kadkhodaei, S., Shahnazari, M., Nekouei M. K. (2011).A comparative study of morphological and molecular diversity analysis among cultivated almonds (*Prunus dulcis*). *Australian Journal of Crop Science,* 5(1), 82–91.

Khan, S. & Goyal, S. (2009). Improvement of mungbean varieties through induced mutations. *African Journal of Plant Science*, 3(8), 174-180.

Kahriz, Z. A., Jafarkhani Kermani, M. & Amiri, M. (2012).Effect of gamma rays on nuclear DNA content in different rose genotypes.*International Research Journal of Applied and Basic Sciences*, 3(6), 1155-1160.

Kalia, R. K., Rai, M. K., Kalia, S., Singh, R. & Dhawan, A. K. (2011). Microsatellite markers: an overview of the recent progress in plants. *Euphytica*, 177(3), 309–334.

Kamenetsky, R. & Okubo, H. (2013).*Ornamental geophytes; from basic science to sustainable production.*CRC press. 597 Pages.

Karuri, H. W., Ateka, E. M., Amata, R., Nyende, A. B., Muigai, A. W. T., Mwasame, E. & Gichuki, S. T. (2010). Evaluating diversity among Kenyan sweet potato genotypes using morphological and SSR markers. *International Journal of Agriculture Biology*, 12, 33-38.

Ketmaro, S. (2007).Improvement of Dracaena (*Dracaena godseffiana*) Varieties by Using Gamma Rays.Unpublished master's dissertation.Kasetsart University. Bangkok.

Khai, T. H.& Lang, N. T. (2005).Microsatellite markers to identify allele variation of somaclonal mutants in indica rice. *Omonrice*, 13, 121-125.

Khuankaew, T., Ruamrungsri, S., Ito, S., Sato, T., Ohtake, N., Sueyoshi, K., & Ohyama, T. (2010). Assimilation and translocation of nitrogen and carbon in *Curcuma alismatifolia* Gagnep.*Plant Biology*, 12(3), 414–423.

Kikuchi, O. K. (2000). Orchid flowers tolerance to gamma-radiation. *Radiation Physics and Chemistry*, 57(3), 555-557.

Kim, J. H., Baek, M. H., Chung, B. Y., Seung, G. W. & Kim, J. S. (2004). Alterations in the photosynthetic pigments and antioxidant machineries of red pepper (*Capsicum annuum* L.) seedlings from gamma irradiated seeds.*Journal of Plant Biology*, 47 (4), 314-321.

Kim, J. H., Lee, M. H., Moon, Y. R., Kim, J. S., Wi, S. G., Kim, T. H. & Chung, B. Y. (2009). Characterizationof metabolic disturbances closely linked to the delayed senescenceof Arabidopsis leaves after g-irradiation. *Environmental and Experimental Botany*, 67, 363–371.

Klein, R. M. & Klein. D. T. (1971). Post-irradiation modulation of ionizing radiation damage to plants.*The Botanical Review*, 37(4), 397-436.

Kon, E., Ahmed, O. H., Saamin, S. & Majid, N. M. (2007). Gamma Radiosensitivity Study on Long Bean (*Vigna sesquipedalis*). *American Journal of Applied Sciences*, 4 (12), 1090-1093.

Korbie, D. J. & Mattick, J. S. (2008). Touchdown PCR for increased specificity and sensitivity in PCR amplification. *Nature Protocols*, 3(9), 1452–1456.

Kovalchuk, O., Arkhipov, A., Barylyak, I., Karachov, I., Titov, V., Hohn, B. & Kovalchuk, I. (2000). Plants experiencing chronic internal exposure to ionizing radiation exhibit higher frequency of homologous recombination than acutely irradiated plants. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 449(1), 47–56.

Kovacs, E. & Keresztes, A. (2002). Effect of gamma and UV-B/C radiation on plant cells. *Micron*, 33(2), 199-210.

Kozgar, M. I., Goyal, S. & Khan, S. (2011). EMS induced mutational variability in *Vigna radiata* and *Vigna mungo*. *Research Journal of Botany*, 6, 31-37.

Kuehny, J. S., Sarmiento, M. J., & Branch, P. C. (2002). Cultural studies in ornamental ginger. In ed. J. Janick, and E. Whipkey.*Trends in new crops and new uses* (pp. 477-482). Alexandria, VA, ASHA Press.

Kumar, V. & Bhagwat, S. G. (2012). Microsatellite (SSR) Based Assessment of Genetic Diversity among the Semi-dwarf Mutants of Elite Rice Variety WL112. *International Journal of Plant Breeding and Genetics*, 6: 195-205.

Kumar, P., Gupta, V. K., Misra, A. K., Modi, D. R. & Pandey, B. (2009). Potential of Molecular Markers in Plant Biotechnology. *Plant Omics Journal*, 2(4), 141-162.

Kumar, S., Prasad, K. V.& Choudhary, M. L. (2006). Detection of genetic variability among chrysanthemum radiomutants using RAPD markers. 90(8), 1108-1113.

Kumara, V. (2008). *Morphological and molecular characterization of induced mutants in Groundnut*. Unpublished doctoral dissertation, University of Agricultural Sciences, India.

Labajová, M., Senková, S., Žiarovská, J., Ražná, K., Bežo, M., Štefúnová, V. & Zeleňáková, L. (2011). The potential of ISSR markers in Amaranth Gamma–radiance

mutants genotyping. *Journal of Microbiology, Biotechnology and Food Sciences*, 1(4), 507-521.

Lagercrantz, U., Ellegren H. & Andersson, L. (1993). The abundance of various polymorphic microsatellite motifs differs betweenplants and vertebrates. *Nucleic Acids Research*, 21(5), 1111–1115.

Lamseejan, S., Jompuk, P., Wongpiyasatid, A., Deeseepan, S.& Kwanthammachart, P. (2000). Gamma-rays Induced Morphological Changes in *Chrysanthemum* (*Chrysanthemum morifolium*). *Kasetsart Journal (Nat. Sci.),* 34(3), 417 – 422.

Larsen, K., Ibrahim, H., Khaw, S. H. & Saw, L. G. (1999). Gingers ofPeninsular Malaysia and Singapore. Kota Kinabalu: *Natural History Publications (Borneo).*135 pp.

Lee, M. H., Moon, Y. R., Chung, B. Y., Kim, J. S., Lee, K. S., Cho, J. Y. & Kim, J. H. (2009). Practical use of chemical probes for reactive oxygen species produced in biological systems by gamma irradiation. *Radiation Physics and Chemistry*, 78, 323-327.

Lee, S. Y., Fai, W. K., Zakaria, M., Ibrahim, H., Othman, R. Y., Gwag, J. G., Rao, V. R. & Park, Y. J. (2007). Characterization of polymorphic microsatellite markers, isolated from ginger (*Zingiber officinale Rosc*.). *Molecular Ecology Notes*, 7(6), 1009–1011.

Lee, F. C. (2007). *Effect of light intensity and daylength on growth and flowering of Siam tulip Curcuma alismatifolia var. Chiang Mai Pink*.Unpublished master's thesis, University Putra Malaysia.

Lee, G. J., Chung, S. J., Park, I. S., Lee, J. S., Kim, J. B., Kim, D. D. & Kang, S.Y. (2008). Variation in the phenotypic features and transcripts of color mutants of chrysanthemum (*Dendranthema grandiflorum*) derived from gamma ray mutagenesis. *Journal of Plant Biology*, 51(6), 418- 423.

Lehmensiek, A., Sutherland, M. & McNamara, R. (2008). The use of high resolutionmelting (HRM) to map single nucleotide polymorphism markers linked toa covered smut resistance gene in barley. *Theoretical and Applied Genetics*, 117(5), 721–728.

Lekawatana, S. & Pituk, O. (1998). New floricultural crops in Thailand. *Acta Horiculture*, 454, 59-63.

Lewontin, R.C. (1984). Detecting population differences in quantitative characters as opposed to gene frequencies.*American Naturalist*, 123,115-124.

Li, Y. D., Chu, Z. Z., Liu, X. G., Jing, H. C., Liu, Y. G. & Hao, D. Y. (2010). A cost effective high-resolution melting approach using the EvaGreen dye for DNA polymorphism detection and genotyping in plants.*Journal* of Integrative *Plant Biology,* 52(12), 1036-1042.

Liao, M., Wang, Y., Rong, X., Zhang, Z., Li, B., Wang, L. & Chen, G. (2011). Development of new microsatellite DNA markers from Apostichopus japonicus and their cross-species application in Parastichopus parvimensis and Pathallus mollis. *International Journal of Molecular Sciences*, 12(9), 5862–5870.

Liew, M., Pryor, R., Palais, R., Meadows, C., Erali, Lyon, E. &Wittwer, C. (2004). Genotyping of single-nucleotide polymorphisms by high-resolution melting of small amplicons.*Clinical Chemistry*, 50(7), 1156–1164.

Lin J. T. & Yang, D. J. (2008). Determination of steroidal saponins in different organs of yam (*Dioscorea pseudojaponica* Yamamoto). *Food Chemistry*, 108(3), 1068–74.

Lochlainn, S., Amoah, S., Graham, N. S., Alamer, K., Rios J. J., Kurup, S., Stoute, A., Hammond, J. P., Stergaard, L., King, G. J., White, P.J. & Broadley, M.R. (2011). High Resolution Melt (HRM) analysis is an efficient tool to genotype EMS mutants in complex crop genomes. *Plant Methods*, 7(1), 43.

Luckey, T. D. (1980). *Hormesis with ionizing radiation* (p. 222). Boca Raton, FL: CRC press.

Luckey, T. (2003). Radiation for health.*Radio Protection Management*, 20:13-21.

Lynch, M. (1996). A quantitative-genetic perspective on conservation issues. In J. C. Avise and J.L. Hamrick, *Conservation genetics: case histories from nature* (pp. 471-501). New York: Chapman and Hall.

Ma, T. H., Xu, C., Liao, S., McConnell, H., Jeong, B. S. & Won, C. D. (1996). In situ monitoring with the *Tradescantia* bioassays on the genotoxicity of gaseous emissions from a closed landfill site and an incinerator. *Mutation research*, 359 (1), 39 –52.

Mackay, J. F., Wright, C. D. & Bonfiglioli, R. G. (2008). A new approach to varietal identification in plants by microsatellite high resolution melting analysis: application to the verification of grapevine and olive cultivars. *Plant Methods* ,4 (1), 8.

Mader,E., Lukas, B. & Novak, J. (2008). A strategy to setup codominant microsatellite analysis for high-resolution-melting-curve-analysis (HRM). *BMC Genetics*, 9 (1), 69.

Mac, C., Teoh, S. B. & Ratnam, A. (1986). The Influence of Gamma-rays on the Injury and Chromosomal Aberrations of Long Bean *(Vigna sesquipedalis*, Fruw.). *Pertanika*, 9(1), 109 -117.

Mallet, J. (2005). Hybridization as an invasion of the genome. *Trends in Ecology and Evolution*. 20(5), 229–237.

Maluszynski, M., Ahloowalia, B. S. & Sigurbjörnsson, B. (1995). Application of *in vivo* and *in vitro* mutation techniques for crop improvement. *Euphytica*, 85: 303–315.

Maluszynski, M., Nichterlein, K., van Zanten, L. & Ahloowalia, B. S. (2000). Officially released mutant varieties – the FAO/IAEA Database. *Mutation Breeding Review*, 12, 1–84.

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research*, 27(2 part 1), 209-220.

Masumbuko, L. I. & Bryngelsson, T. (2006). Inter simple sequence repeat (ISSR) analysis of diploid coffee species and cultivated *Coffee Arabica* L. from Tanzania. *Genetic Resources and Crop Evolution*, 53(2), 357-366.

Mato, M., Onozaki, T., Ozeki, Y., Higeta, D., Itoh, Y., Yoshimoto, Y., Ikeda, H., Yoshida, H. & Shibata, M. (2000). Flavonoid biosynthesis in white-flowered Sim carnations (*Dianthus caryophyllus*). *Scientia Horticulture*, 84(3), 333-347.

Maurya, D. K. & Nair, C. K. K. (2006). Preferential radioprotection to DNA of normal tissues by ferulic acid under ex vivo and in vivo conditions in tumor bearing mice. *Molecular and Cellular Biochemistry*, 285(1-2), 181-190.

McGregor, C. E., Lambert, C. A., Greyling, M. M., Louw, J. H.& Warnich, L. (2000).A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum*.L.) *germplasm. Euphytica*, 113(2), 135–144.

Mednic, I. G. & Usmanov, P. D. (1985). Influence of gamma-rays on the number of initial cell in *Arabidopsisi thaliana*. *Arabidopsis Information Service.* 22, 65–70.

Meiselman, N. G. (1956). *The effects of chronic gamma radiation upon two species of Nicotiana and their interspecific hybrid*. Unpublished doctoral dissertation, The State University. USA.

Mejri, S., Mabrouk, Y., Voisin, M., Delavault, P., Simier, P., Saidi, M. & Belhadj, O. (2012). Variation in quantitative characters of faba bean after seed irradiation and associated molecular changes. *African Journal of Biotechnology*, 11(33), 8383-8390.

Menezes, I. C., Cidade, F. W., Souza, A. P. & Sampaio, I. C. (2009). Isolation and characterization of microsatellite loci in the black pepper, *Piper nigrum* L. (piperaceae). *Conservation Genetics Resources*,1(1), 209-212.

Métais, I., Aubry, A., Hamon, B. & Jaluozot, R. (2000). Description and analysis of genetic diversity between commercial bean lines (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics*, 101(8), 1207-1214.

Meyer, W., Michell, T. G., Freedman, E. Z. & Vilgalys, R. (1993). Hybridization probes for conventional DNA fingerprinting used as single primers in polymerase chain reaction to distinguish strain of *Cryptococcus neoformans*. *Journal of Clinical Biology*, 31(9), 2274–2280.

Mikaelsen, K. & Aastveit, K. (1957). Effects of neutrons and chronic gamma radiation on growth and fertility in oats and barley. *Hereditas,* 43(2), 371-380.

Misset, M. T. (1992).Meiotic abnormalities during microsporogensis and low fertility in prostrate ecotypes of *Ulex* species (Papilionoideae, Genisteae). *Canadian Journal of Botany*, 70(6), 1223-1227.

Morgante, M. & Oliveri, A. M. (1993). PCR-amplified microsatellites as markers in plant genetics. *The Plant Journal*, 3(1), 175–182.

Morishita, T., Yamaguchi, H., Degi, K., Shikazono, N., Tanaka, A. & Abe, T. (2003). Dose response and mutation induction by ion beam irradiation in buckwheat.*Nuclear Instruments and Methods in Physics Research Section B*, 206, 565–569.

Mostafa, G. G. (2011). Effect of sodium azide on the growth and variability induction in *Helianthus annuus* L. *International Journal of Plant Breedingand Genetics*, 5(1), 76-85.

Mulcahy, D. L., Cresti, M., Sansavini, S., Douglas, G. C., Linskens, H. F., Mulcahy,G. B., Vignani R.& Pancaldi, M. (1993). The use of random amplified polymorphic DNAs to fingerprint apple genomes. *Scientia Horticulture*, 54(2), 89–96.

Muleo, R., Colao, M. C., Miano, D., Cirilli, M., Intrieri, M. C., Baldoni, L. & Rugini, E. (2009). Mutationscanning and genotyping by high-resolution DNA melting analysis in olivegermplasm. *Genome*, 52(3), 252–260.

Nagatomi, S., Mitsui, K., Miyahara, K., Nakagawa, K. & Yamagishi, T. (1998).A new variety of Manila grass, "Winter Field". Frost resistance and dwarf mutant. *Technical News Institute of Radiation Breeding*, 63, 1-2.

Nagatomi, S., Mitsui, K. & Miyahara, K. (1993).Selection of evergreen mutant lines in Manila grass (Zoysia matrella MERR.). *Technical News***,** 44, 1-2.

Nagatomi, S., Miyahira, E. & Degi, K. (2000). Induction of flower mutation comparing with chronic and acute and gamma irradiation using tissue culture technique in *Chrysanthemum morifolium* RAMAT. *Acta Horticulture*. 508, 69-74.

Nagatomi, S. & Degi, k. (2009). Mutation Breeding of *Chrysanthemum* by Gamma Field Irradiation and In Vitro Culture.In Q.Y. Shu.*Induced Plant Mutations in the Genomics Era (*PP. 285-261). Rome: Food and Agriculture Organization of the United Nations.

Naito, K., Kusaba, M., Shikazono, N., Takano, T., Tanaka, A.& Tanisaka, T. (2005). Transmissible and nontransmissible mutations induced by irradiating Arabidopsis *Thaliana pollen* with gamma-rays and carbon ions. *Genetics*, 169(2), 881-889.

Nakano, M., Amano, J., Watanabe, Y., Nomizu, T., Suzuki, M., Mizunashi, K., Mori, S., Kuwayama, S., Han, D.S., Saito, H., Ryuto, H., Fukunishi, N., & Abe, T. (2010). Morphological variation in *Tricyrtis hirta* plants regenerated from heavy ion beam-irradiated embryogenic calluses. *Plant Biotechnology*, 27(2), 155–160.

Nakatsuka, T., Nishihara, M., Mishiba, K. & Yamamura, S. (2005). Two different mutations are involved in the formation of white-flowered gentian plants. *Plant Science*, 169(5), 949-958.

Nazari, L. & Pakniyat, H. (2008). Genetic diversity of wild and cultivated barley genotypes under drought stress using RAPD markers. *Biotechnology*, 7(4), 745-750.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences*, 70(12), 3321-3323.

Nwachakwu, E. C., Ene, L. S. O. & Mbanaso, E. N. A. (1994). Radiation sensitivity of two ginger varieties (*Zingiber officinale* Rose) to gamma irradiation. *Tropenlandwirt*, 95(1), 99-103.

Nybom, H. & Schaal, B. A. (1990). DNA 'fingerprints' applied to paternity analysis in apples (Malus × domestica). *Theoretical and Applied Genetics*, 79(6), 763–768.

Oliver, R. E., Lazo, G. R., Lutz, J. D., Rubenfield, M. J., Tinker N. A., Anderson, J. M., Wisniewski Morehead, N. H., Adhikary, D., Jellen, E. N., Maughan, P. J., Brown Guedira, G. L., Chao, S., Beattie, A. D., Carson, M. L., Rines, H. W., Obert, D. E., Bonman, J. M. &Jackson, E. W. (2011). ModelSNP development for complex genomes based on hexaploid oat using high-throughput454 sequencing technology. *BMC Genomics*, 12, 77.

Oswaldo, W. (2007). Gamma rays and carbon ion beam irradiation for mutation induction to breed banana (*Musa* spp.) especially on response to black Sigatoka disease .unpublished doctoral dissertation, University of Tsukoba. Japan.

Paisooksantivatana, Y. & Thepsen, O. (2001). Phenetic relationship of some Thai *Curcuma* species (Zingiberaceae) based on morphological, palynological and cytological evidence. T*hai Journal of Agricultural Science*, 34, 47–57.

Palai, S. K. & Rout, G. R. (2011). Characterization of new variety of *Chrysanthemum* by using ISSR markers. *Horticultura Brasileira*, 29(4), 613-617.

Pancaldi, M., Weeden, N. F., Sansavini, S. & Mulcahy, D. L. (1999). Molecular analysis of mutant clones in apple. *Acta Horticulturae*, 484, 311–317.

Panda, M. K., Mohanty, S., Subudhi, E., Acharya, L. & Nayak, S. (2007). Assessment of genetic stability of micropropagated plants of *Curcuma* L. by cytophotometery and RAPD analysis. *International journal of integrative biology*, 1(3), 189-195.

Paradiz, J., Skrk, J. & Druskovic, B. (1992). Cytogenetic effects of ionizing radiation on meristem. *Acta Pharmaceutica*, 42, 397–401.

Parker, K. E. & Horsley, R. J. (1972). The ultraviolet radiosensitivity of *Oedogonium cardiacum* cells at various stages of the cell cycle. *Radiation Botany*, 12(4), 239-248.

Patterson, C., Williams, D. M. & Humphries. C. J. (1993). Congruence between molecular and morphological phylogenies.*Annual Review* of *Ecology, Evolution, and Systematics*, 24, 153-188.

Pate, J. S., Dixon, R. K. (1982).*Tuberous, Cormous and Bulbous plants: Biology of an Adaptive Strategy in Western Australia*, 268 pages. Nedlands, Western Australia: University of Western Australia Press.

Patzak, J. (2001). Comparison of RAPD, STS, ISSR and AFLP molecular methods used for assessment of genetic diversity in hop (*Humulus lupulus* L.). *Euphytica*,121 (1), 9–18.

Paz, M. D. P. (2003). *Rhizome manipulation affects growth and development of ornamental gingers*. MSc Thesis. Louisiana State University.

Peakall, R. & Smouse, P. E. (2006).Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6(1), 288-295.

Pimonrat, P., Suraninpong, P. & Wuthisuthimethavee, S. (2012). Acute effect of gamma radiation on stable characteristics of *Spathoglottis plicata* blum. *Acta Horticulturae*, 953,173-180.

Pivoriene, O., Pasakinskiene, I., Brazauskas, G., Lideikyte, L., Jensen, L. B. & Lubberstedt, T. (2008). Inter-simple sequence repeats (ISSR) loci mapping in the genome of perennial ryegrass. *Biologia*, 54(1), 17–21.

Pongchawee, K., Pradissan, R. & Pipatcharoenchai, W. (2007). Induce mutation in *Anubias* spp. through *in vitro* Irradiation. *Thai Fisheries Gazette*, 60, 493–497.

Powell, W., Machray, G. C. & Provan, J. (1996). Polymorphism revealed by simple sequence repeats**.** *Trends in plant science*, 1(7), 215–222.

Preussa, S. B. & Britta, A. B. (2003). A DNA-damage-induced cell cycle checkpoing in *Arabidopsis*.*Genetics*, 164(1), 323–334.

Provaznikova, D., Kumstyrova, T., Kotlin, R., Salaj, P., Matoska, V., Hrachovinova, I. &Rittich S. (2008). High-resolution melting analysis for detection of MYH9 mutations. *Platelets*, 19(6), 471-5.

Puchooa, D. & Venkatasamy, K. (2005). Detection of genetic diversity in *Trochetia boutoniana* using random amplified polymorphic DNA (RAPD) markers. *Biotechnology*, 4(4), 267-274.

Puchooa, D. (2005). *In vitro*mutation breeding of *Anthurium* by gamma radiation.*International Journal of Agriculture Biology*, 7(1), 11-20.

Purseglove, J. W. (1972).*Tropical Crops: Monocotyledons*. Halsted Press Division, Wiley, London: Longman.

Quastler, H., Schertiger, A. M. & Stewart, W. N. (1952). Inhibition of growth by irradiation. IV. Growth arrest vs. effects on mitotic activity. *Journal of Cell Comparative Physiology*, 39(3), 357-369.

Raina, S. N., Rani, V., Kojima, T., Ogihara, Y., Singh, K. P. & Devarumath, R. M. (2001). RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification, and phylogenetic relationships in peanut (*Arachis hypogaea*) cultivars and wild species. *Genome,* 44 (5), 763–772.

Rajadurai, K. (2001). *Enhancing the Bioproductivity ofGloriosa superba L. through Mutatic Genetic Manipulation.*Unpublished doctoral dissertation.Tamil Nadu Agriculture University, Coimbatore.

Ramachandran, M. & Goud, J.V. (1983). Mutagenesis in safflower (*Carthamus tinctorius*).I Differential radiosensitivity. *Genetic Agraria,* 37 (2), 309–18.

Ramanna, M. S. & Nataraja, A. T. (1965). Studies on relative mutagenic efficiency of Alkylating agents under different conditions of treatment. *Indian Journal of Genetics and Plant Breeding*, 25 (1), 24-26.

Ramesh, H. L. & Murthy Yogananda,V. N. (2012). Effect of gamma radiationon morphological and growth parametersof Mulberry variety M5. *International Journal of Science & Nature*. 3(2), 447-452.

Ramesh, H. L. & Murthy Yogananda,V. N. & Munirajappa. (2012). Effect of different doses of gamma radiation on growth parameters of Mulberry (Morus) variety Kosen. *Journal of Applied and Natural Science,* 4 (1), 10-15.

Ramulu, K. S. (1970). Induced chlorophyll chimeras and mutations in Sorghum. *The Madras Agriculture Journal*. 57, 727-732.

Reddy, P. M., Sarla, N. & Siddiq, E. A. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica*, 128(2), 9-17.

Reja, V., Kwok, A., Stone, G., Yang, L., Missel, A., Menzel, C. & Bassam, B. (2010). ScreenClust: Advanced statistical software for supervised and unsupervised high resolution melting (HRM) analysis. *Methods*, 50(4), S10-4.

Robertson, J. L., Russel, R. M., & Savin, N. E. (1980). *A user's guide to Probit Or LOgit analysis*. Pacific Southwest Forest and Range Experiment Station.

Roh, M. S. & Lawson, R. H. (1993). *Curcuma*.Grower Notebook.A step-by-step guide to success.*Greenhouse Manager*, 12, 10.

Roldàn-Arjona, T. & Ariza, R. R. (2009). Repair and tolerance of oxidative DNA damage.*Mutation research*, 681(2), 169-179.

Rohlf, F. J. (2002). *NTSYS-Pc: Numerical Taxonomy System, Version 2. 1*, Exeter Publishing, Setauket, New York, NY, USA.

Roychowdhury, R. & Jagatpati, T. (2013). Mutagenesis—A Potential Approach for Crop Improvement. In K.R. Hakim, P. Ahmad, M. Ozturk (Eds.), *Crop improvement* (pp.149-187). West Benga: Springer US.

Ruamrungsri, S., Ohtake, N., Sueyoshi, K., Suwanthada, C., Apavatjrut, P. & Ohyama, T. (2001). Changes in nitrogenous compounds, carbohydrates and abscisic acid in *Curcuma alismatifolia* Gagnep.during dormancy. *Journal of Horticulture Sciences and Biotechnology*, 76, 48–51.

Ruamrungsri, S., Ohtake, N., Sueyoshi, K., Suwanthada, C., Ohyama, T. & Apavatjrut, P. (2005). Effect of nitrogen and potassium on growth and development of *Curcuma alismatifolia* Gagnep. *Acta Horticulture*, 673, 443-448.

Rusli, I. *Malaysian Nuclear Agency Gamma Greenhouse*; International Atomic Energy Agency (IAEA), Vienna, Austria,*Plant Mutation Reports*, Vol. 2, No. 2, June 2010.

Saensouk, S. & Chantaranothai, P. (2003).The family Zingiberaceae in Phu Phan National Park.*In Proceedings of the 3rd Symposium on the family Zingiberaceae, Khon Kaen, Thailand*. *Applied Taxonomic Research Center, Khon Kaen University* (pp. 16-25).

Saghai-Maroof, M. A., Biyashev, R. M., Yang, G. P., Zhang, Q. & Allard, R. W. (1994). Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations and population dynamics. *Proceedings of the National Academy of Sciences*, 91(12), 5466-5470.

Saini, N., Jain, N., Jain S. & Jain, R. K. (2004). Assessment of genetic diversity within and among Basmati and non-Basmati rice varieties using AFLP, ISSR and SSR markers. *Euphytica*, 140(3), 133–146.

Sato, Y., Shirasawa, K., Takahashi, Y., Nishimura, M. & Nishio, T. (2006). Mutant selection from progeny of gamma-ray-irradiated rice by DNA heteroduplex cleavage using brassica petiole extract. *Breeding Science*, 56(2), 179-183.

Sax, K. (1963). The stimulation of plant growth by ionizing radiation. *Radiation Botany*, 3(3), 179-186.

Sax, K. (1955.). The effect of ionizing radiation on plant growth. *American Journal Botany*, 42, 360-364.

Schevchenko, V. V. & Grinikh, L. I. (1981). Spectrum of chlorophyll deficient mutations induced by gamma-irradiation of *Arabidopsis thaliana* at different stages of development and scored with embryo test. *Arabidopsis Information Service*, 18, 127–129.

Schilthuizen, M., Hoekstra, R. F. and Gittenberger, E. (1999). Selective increase of a rare haplotype in a land snail hybrid zone, *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 266 (1434), 2181–2185.

Sen, L.A. (1979). Speciality of the effect of chemical mutagen on several barley varieties. *Soviet Genetics*, 15, 301-306.

SenthamizhSelvi, B., Ponnuswami, V. & Sumathi, T. (2007). Identification of DNA polymorphism induced by gamma ray irradiation in Amla (Emblica Officinalis Gaertn.) grafts of M_1V_1 and M_1V_2 generation. *Journal of Applied Sciences Research*, 3(12), 1933-1935.

Sharma, J. R. (1998). *Statistical and Biometrical Techniques in Plant Breeding*, New Age International, New Delhi, India.

Shehata, S. M, Ammar, M. H, Abdelkalik, F. A. & Zayed, B. A.(2009). Morphological, molecular and biochemical evaluation of Egyptian jasmine rice variety and its M5 derived mutants. *African Journal of Biotechnology*, 8 (22), 6110-6116.

Sherif, F. E., Khattab, S., Ghoname, E., Salem, N. & Radwan, K. (2011). Effect of gamma irradiation on enhancement of some economic traits and molecular changes in *Hibiscus Sabdariffa* L. *Life Science Journa*l, 8(3).

Shikazono, N., Yokota, Y., Kitamura, S., Suzuki, C., Watanabe, H., Tano, S. & Tanaka, A. (2003). Mutation rate and novel tt mutants of Arabidopsis thaliana induced by carbon ions.*Genetics*, 163(4), 1449-1455.

Shiran, B., Amirbakhtiar, N., Kiani, S., Mohammadi, S. H., Sayed-Tabatabaei, B. E.& Moradi, H. (2007). Molecular characterization and genetic relationship among almond cultivars assessed by RAPD and SSR markers.*Scientia Horticulture*, 111(3), 280–292.

Shirley, B. W, Hanley, S. & Goodman, H. M. (1992).Effects of ionizing radiation on a plant genome: Analysis of two *Arabidopsis thaliana* testa mutations. *Plant Cell*, 4(3), 333–347.

Shu, Q. Y. & Lagoda, P.J.L. (2007). Mutation techniques for gene discovery and crop improvement.*China academic Journal of Molecular Plant Breeding*, 5(2), 193-195.

Shu, G.Y. (2009). *Induced Plant Mutations in the Genomics Era*. Rome: Food and Agriculture Organization of the United Nations.

Sigrist, M. S., Pinheiro, J.B., Filho, J. A. & Zucchi, M. I. (2011). Genetic diversity of turmeric germplasm *(Curcuma longa*; Zingiberaceae) identified by microsatellite markers. *Genetics and Molecular Research*, 10 (1), 419-428.

Sigrist, M. S., Pinheiro, J. B., Azevedo-Filho, J. A., Colombo, C. A., Sandhu, S., Souza, A. P. & Zucchi,M. I. (2010). Development and characterization of microsatellite markers for turmeric (*Curcuma longa* L.). *Plant Breeding*, 129(5), 570-573.

Siju, S., Dhanya, K., Syamkumar, S., Sheeja, T. E., Sasikumar, B., Bhat, A. I. & Parthasarathy, V. A. (2010). Development characterization and utilization of genomic microsatellite markers in turmeric (*Curcuma longa* L.). *Biochemical Systematics and Ecology*,38(4), 641-646.

Singh, S., Panda, M. K. & S. Nayak (2012). Evaluation of genetic diversity in turmeric (*Curcuma longa* L.) using RAPD and ISSR markers. *Industrial Crops and Products*, 37(1), 284-291.

Sirisawad, T., Sirirugsa, P., Suwanthada, C. & Apavtjrut, P. (2003). Investigation of chromosome numbers in 20 taxa of *Curcuma*, In *Proceedings of the 3rd Symposium on the family Zingiberaceae. Khon Kaen, Thailand*, July 7-12, 2002. *Applied Taxonomic Research Center, Khon Kaen University (pp. 54-62).*

Skornickova, J. (2006). *Cucurma*-stunning beauty, hidden treasure.Gardenwise, 27, 4-4.

Smith, S. D. & Baum. D. A. (2006). Phylogenetics of the florally diverse *Andean clade Iochrominae (Solanaceae).Amereican Journal of Botany*, 93(8), 1140–1153.

Smith, P. F., Konings, A. & Kornfield, I. (2003). Hybrid origin of a cichlid population in Lake Malawi: Implications for genetic variation and species diversity. *Molecular Ecology*, 12(9), 2497–2504.

Song, Q. G., Jia, Y., Zhu, D., Grant, R.T., NelsonHwang, E.Y., Hyten, D.L. & Cregan, P.B. (2010). Abundance of SSR motifs and development of candidate polymorphic SSR markers (BARCSOYSSR_1.0) in soybean.*Crop Science*, 50(5), 1950-1960.

Soulè, M. E. & Zegers. G. P. (1996). Phenetics of natural populations. V. Genetic correlates of phenotypic variation in the pocket gopher (*Thomomys bottae*) in California. *Journal of Heredity*, 87, 341-350.

Sparrow, A. H. & Woodwel, G. M. (1962).Prediction of sensitivity of the plants to chronic gamma irradiation.*Radiation Botany,* 2, 9-26.

Sparrow, A. H. & Singleton, W. R. (1953). The use of radio-cobalt as a source of gamma rays and some effects of chronic irradiation on growing plants. *American Naturalist,* 117, 29-48.

Sparrow, A. H. (1954). Stimulation and inhibition of plant growth by ionizing radiation. *Radiation Research,* 1, 562.

Sung, W. C. (2005). Effect of gamma irradiation on rice and its food products. *Radiation Physics and Chemistry*, 73(4), 224–228.

Sawangmee, W., Taychasinpitak, T., Jompuk, P., & Kikuchi, S. (2011). Effects of Gamma-ray Irradiation in Plant Morphology of Interspecific Hybrids between *Torenia fournieri* and *Torenia baillonii*.*Kasetsart Journal (Nat. Sci.),* 45, 803-810.

Suwanseree, V. W., Teerakathiti, T., Wongchaochant, S., & Taychasinpitak, T. (2011). Petal color and petal form mutations observed in Torenia hybrida following gamma irradiation in vitro. *Kasetsart Journal (Nat Sci),* 45, 656-665.

Savva, D., Depledge, M., Atienzar, F. A., Evenden, A., & Jha, A. (2000). Optimized, RAPD analysis generates high-quality genomic DNA profiles at high annealing temperature. *Biotechniques*, 28(1), 52–54.

Syamkumar, S. & Sasikumar, B. (2007). Molecular marker based genetic diversity analysis of *Curcuma* species from India. *Scientia Horticulturae,* 112(2), 235–241.

Tah, P. R. (2006). Induced macromutation in mungbean (*Vigna radiata* (L.) Wilczek). *International Journal of Botany*, 2(3), 219-228.

Tangpong, P., Taychasinpitak, T., Jompuk, C.& Jompuk, P. (2009). Effects of Acute and Chronic Gamma Irradiations on *In vitro* Culture of *Anubias congensis* N.E. Brown.*Kasetsart Journal- Natural Science*, 43(3), 449 – 457.

Tautz, D. & Renz, M. (1984). Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic acids research*, 12 (10), 4127–4138.

Terato, H., Tanaka, R., Nakaarai,Y., Nohara, T., Doi, Y., IWai, S., Hirayama, R., Furusawa, Y. & Ide, H. (2008). Quantitative analysis of isolated and clustered DNA damage induced by gamma-rays, carbon ion beam, and iron ion beams. *Journal of Radiation Research*, 49(2), 133–146.

Tertivanidis, K., Koutita, O., Papadopoulos, I. I., Tokatlidis, I. S., Tamoutsidis, E. G., Pappa-Michailidou, V. & Koutsika-Sotiriou, M. (2008). Genetic

diversity in bean populations based on random amplified polymorphic DNA markers. *Biotechnology*, 7, 1-9.

Thohirah, L. A, Flora, C. L. S. & Kamalakshi, N. (2010). Breaking Bud Dormancy and Different Shade Levels for Production of Pot and Cut *Cucurma alismatifolia. American Journal of Agricultural and Biological Sciences*, 5 (3), 385-388.

Tian, H. L., Xue, J. H., Wen, J., Mitchell, G. & Zhou, S. L. (2008). Genetic diversity and relationships of Lotus (Nelumbo) cultivars based on allozyme and ISSR markers. *Scientia Horticulture*, 116(4), 421-429.

Toth, G., Gaspari, Z. & Jurka, J. (2000). Microsatellites in Different Eukaryotic Genomes: Survey and Analysis. *Genome Research*, 10(7), 967–981.

Tuteja, N., Singh, M. B., Misra, M. K., Bhalla, P. L. & Tuteja R. (2001). Molecular mechanisms of DNA damage and "repair": Progress in plants. *Critical Reviews in Biochemistry and Molecular Biology*, 36(4), 337–397.

Van Gastel, A. J. G. & De Nettancourt, D. (1974). The effects of different mutagens on self-incompatibility in *Nicotiana alata* Link and Otto: I. Chronic gamma irradiation.*Radiation Botany*, 14 (1), 43–50.

Van Harten, A. M. (1998). *Mutation Breeding: Theory and Practical Applications.* United Kingdom: Cambridge University Press.

Vainstein, A. (2002). *Breeding for ornamental: classical and molecular approaches*. Rehovot, Israel: Kluwer Academic Publishers.

Voisine, R., V´ezina, L. P. Willemot, C. (1991). Induction of senescence-like deterioration of microsomal membranes from cauliflower by free radicals generated during gamma irradiation. *Plant Physiology*, 97(2), 545–550.

Vos, P., Hogers, R., Bleeker, M., Reijans, M. & Van de Lee, T., Hornes, M.(1995). AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research*, 23(21), 4407-4414.

Waldmann, P. & Andersson, S. (1998). Comparison of quantitative genetic variation and allozyme diversity within and between populations of *Scabiosa canescens* and *S. columbaria*.*Heredity*, 81(1)**,** 79-86.

Wang, H. Z., Wu, Z. X. Lu, J. J., Shi, N. N., Zhao, Y., Zhang , Z. T. & Liu, J. J. (2009). Molecular diversity and relationships among *Cymbidium goeringii* cultivars based on inter-simple sequence repeat (ISSR) markers. *Genetica*, 136(3), 391–399.

Wang, J. L., Gao,Y. B., Zhao, N. X., Ren, A. Z., Ruan, W. B., Chen, L., Liu, J. L. & Li, C. L. (2006). Morphological and RAPD analysis of the dominant species Stipa krylovii Roshev.in Inner Mongolia steppe. *Botanical Studies*, 47(1), 23-35.

Wang, Y., Wang, F., Zhai, H. & Liu, Q. (2007). Production of a useful mutant by chronic irradiation in sweetpotato. *Scientia Horticulturae*, 111(2), 173–178.

Wang, Z., Weber, J. L., Zhong G. & Tanksley, S. D. (1994).Survey of plant short tandem repeats.*Theoretical and Applied Genetics*, 88(1), 1–6.

Watanabe, M. & Horikawa, M. (1977). Analyses of differential sensitivities of synchronized HeLa $3 cells to radiations and chemical carcinogens during the cell cycle.*Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 44(3), 413-425.

Weir, B. (1990). *Genetic data analysis: methods for discrete population genetic data*. Massachusetts: Sinauer Assoc. Sunderland.

Weising, K., Winter, P., Huttel, B.& Kahl, G. (1998). Microsatellite markers for molecular breeding. *Journal of Crop Production*, 1(1), 113–143.

White, H. E., Hall, V. J. & Cross, N. C. P. (2007). Methylation-sensitive high resolution melting-curve analysis of the SNRPN gene as a diagnostic screen for Prader-Willi and Angelman syndromes.*Clinical Chemistry*, 53(11), 1960–1962.

Wi, S. G., Chung, B. Y., Kim, J. H., Baek, M. H., Yang, D. H., Lee, J. W.& Kim, J. S. (2005). Ultrastructural changes of cell organelles in arabidopsis stems after gamma irradation. *Journal of Plant Biology*, 48(2), 195-200.

Wi, S. G., Chung, B. Y., Kim, J. S., Kim, J. H., Baek, M. H., Lee, J. W. & Kim, Y. S. (2007). Effects of gamma irradiation on morphological changes and biological responses in plants. *Micron*, 38(6), 553–564.

Wilkinson, J. Q. & Crawford, N. M. (1991). Identification of the Arabidopsis CHL3 gene as the nitrate reductase structural gene *Nia2*. *Plant Cell*,3(5), 461–471.

Williams, G. K., Kubelik, A. R., Livak, K. L., Rafalski, J. A. & Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, 18(22), 6531-6535.

Wittwer, C. T., Reed, G. H., Gundry, C. N., Vandersteen, J. G. & Pryor, R. J. (2003). High-resolution genotyping by amplicon melting analysis using LCGreen. *Clinical Chemistry*, 49(6), 853–860.

Wittwer, C. T. (2009). High-Resolution DNA Melting Analysis: Advancements and Limitations. *Human Mutation*, 30(6), 857–859.

Wolfe, A. D. & Liston, A. (1998). Contributions of PCR-based methods to plant systematics and evolutionary biology.In P.S. Soltis, D. E. Soltis, & J. J. Doyle.*Molecular systematics of plants: DNA sequencing (pp. 43-86)*. Kluwer, New York: Springer US.

Wongpiyasatid, A., Jompuk, P., Topoonyanoon, N., Taychasinpitak, T., & Teerakathiti, T. (2009).Improvement of *Curcuma* hybrids by mutation induction. Abstract. *8th national Horticulture congress*, 68.

Wongpiyasatid, A., Thinnok, T., Taychasinpitak, T.,Jompuk, P., Chusreeaeom, K.& Lamseejan, S. (2007). Effects of acute gamma irradiation on adventitious plantlet regeneration and mutation from leaf cuttings of African Violet (*Saintpaulia ionantha*). *Kasetsart Journal-Natural Science*, 41(4), 633 – 640.

Wu, S. B., Wirthensohn, M. G., Hunt, P., Gibson, J. P. & Sedgley, M. (2008). Highresolution melting analysis of almond SNPs derived from ESTs. *Theoretical and Applied Genetics*, 118(1), 1–14.

Wu, D., Qingyao, S., Zhonghua, W. & Yingwu, X. (2002). Effect of gamma irradiation on starch viscosity and physico-chemical properties of different rice. *Radiation Physics and Chemistry*, 65(1), 79–86.

Wu, S. B., Tavassolian, I., Rabiei, G., Hunt, P., Wirthensohn, M., Gibson, John, P., Ford, Christopher, M. & Sedgley, M. (2009). Mapping SNP-anchored genes using high-resolution melting analysis in almond. *Molecular Genetics and Genomics*, 282 (3), 273–281.

Xiaoming, S., Ke, L., Yanli, N. & Genfa, Z. (2006). Biochemistry and genetic analysis of bioeffects of low energy N^+implantation and -radiation on *Arabidopsis thaliana*. *Frontiers in Biology in China*, 1(1), 41–45.

Yamakawa, K. (1966). Relative radiosensitivities of three developmental stages, *i.e*. floral development, gametogenesis, and embryogenesis under chronic gamma irradiation.In *GammaField Symposium*, No. 5, 91-122. Institute of Radiation Breeding, Japan.

Yasmin, S., Islam, M. S., Nasiruddin K. M. & Alam, M. S. (2006). Molecular characterization of potato germplasm by random amplified polymorphic DNA markers.*Biotechnology*, 5(1), 27-31.

Yeh, F. C., Yang, R., Boyle, T. B. J., Ye, Z. & Mao, J. X. (2000).*Popgen ver. 1.32: the user-friendly shareware for population genetic analysis*.Molecular Biology and Biotechnology Center, University of Alberta, Canada.

Yu, R. H., Shan, X. H., Wang, S., Li,X. H., Jiang, Y., Tan, H.& Li, Y. D. (2011). A screening method for detecting simple sequence repeat (SSR) polymorphism of *Zea mays* using highresolution melting-curve analysis. *African Journal of Biotechnology*, 10(73), 16443-16447.

Youssef, A. A., Aly, M. S. Hussein, M. S. (2000). Response of geranium (*Pelargonium graveolenus* L.) to gamma irradiation and foliar application of Speed Grow. *Egyptian Journal of Horticulture*, 27(1), 41-53.

Zhang, J. X., Huang, J. P. & Lin, L. M. (1995). A new favorite in flower markets: cultivation technique and regulation of flowering of *Curcuma alismatifolia*. *Taiwan Flower Industry*, 92(3), 36-40.

Zaka, R., Chenal, C. & Misset, M.T. (2004). Effects of low doses of short-term gamma irradiation on growth and development through two generations of *Pisum sativum.Science of the Total Environment*, 320 (2), 121-129.

Zalewska, M., & Jerzy, M. (1997). Mutation spectrum in *Dendranthema grandiflora* Tzvelev after in vivo and in vitro regeneration ofplants from irradiated leaves. *Acta Horticulture*, 447, 615-618.

Zhiyi, R. & Haowen, Y. (2004). A method for genotoxicity detection using random amplified polymorphism DNA with Daniorerio. *Ecotoxicology Environment Safty*, 58(1), 96–103.

Zhou, Y., Gao, W., Wang, F. & Gu, F. (2007). Assessment of genetic diversity of *Rehmannia glutinosa* Libosch based on ISSR markers. *Geneticâ si Biologie Moleculara*, 8(1), 141–9.

Ziekiewicz, E., Rafalski, A. & Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. *Genome*, 20(2), 176-83.

Zivkovic, B., Radovic, J., Sokolovic, D., Siler, B., Banjanac, T. & Strbanovic, R. (2012). Assessment of genetic diversity among alfalfa (*Medicago sativa* L.) genotypes by morphometry, seed storage proteins and RAPD analysis. *Industrial Crops and Products*, 40, 285–291.