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IN VITRO MUTATION INDUCTION BY GAMMA IRRADIATION FOR THE IMPROVEMENT OF A TRIPLOID BANANA PISANG BERANGAN (AAA)

By

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DEDICATED TO:

MY PARENTS

&

MY HUSBAND

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ABSTRACT

Tissue culture and induced mutation techniques were used to generate variation for the improvement of Pisang Berangan cv. 'Intan' (AAA). Best field performing banana in *vitro*-plants were multiplied and evaluated for better field performance, which included improved agronomic traits such as earliness in fruiting, short plant stature, high yielding capacity with desirable fruit quality and tolerance to *Fusarium* wilt.

A suitable micropropagation medium for Pisang Berangan was established through modification of MS basic medium, which resulted in significant increase in shoot multiplication. Shoot-tip meristems of Pisang Berangan were then irradiated at 20, 30, 40 and 60 Gy to induce mutations. The cultivar exhibited differences in dose responses and post-irradiation recovery based on the number of shoots produced per explant. The irradiated explants were multiplied *in vitro* to produce M_1V_4 plants before being field planted for evaluation.

Gamma irradiation caused many mutagenic changes in both qualitative and quantitative traits. Variants of morphological traits include pseudostem: size, length and pigment; leaf: deformation, colour and size and deformation of bunch/ finger etc. attributed to the bulk of the variation accounting for 25 – 30% while agronomic traits (plant height, bunch weight and fruit characters) accounting for only 3 –4%. There were also somaclonal variations observed in tissue cultured plants (without irradiation). However, frequency of somaclonal variation was much lower than mutagenic changes.

Nuclear DNA content of mutated plants was determined by using flow cytometric techniques. The results showed differences in DNA content between variants indicating the effect of gamma-irradiation on the genotype of these plants. Variants of short plant stature or stunted growth showed great differences in DNA content compared to control (non-irradiated). Generally, nuclear DNA contents decreased with an increase of gamma-dose from 20 Gy to 60 Gy.

Random amplified polymorphic DNA (RAPD) analysis was used to examine changes in the mutants. Four out of 10 primers tested showed polymorphism, which could differentiate between normal and dwarf variants. Among 450 mutant tested, only 50 samples showed clearly reproducible bands suitable for further analysis. Primer OPA-03 and OPA-05 showed major bands of 1.5 Kbp and 0.7, 0.5 and 0.4 Kbp respectively, which were absent in 40 Gy samples when compared with control and plants treated at 20 and30 Gy, while the major bands of 1.5 Kbp and 1.0 Kbp were absent in 60 Gy treated plants. Primers OPA-03 and OPA-09 detected a higher range of variability at 40 and 60 Gy doses which produced variants that were either very short or with very low bunch weight or no bunching.

Detection of dwarf off-types caused by somaclonal variation and mutation induction of Pisang Berangan was examined by using Gibberellic Acid (GA_3) at 29 µmol/L and

59μmol/L concentrations at *in vitro* culture stage and at 289 μmol/L for plants at deflasking stage. Differences in response between dwarf variants and control plants were not constant for treatment at the *in vitro stage.* However, at the deflasking stage elongation of the sheath of the first emerged leaf after GA_3 treatment was about 2-fold greater in control plants compared with dwarf mutants, which is useful in discriminating between normal and dwarfs. The method is simple and cheap, and could prevent the waste of resources on non-productive dwarf plants.

Somaclonal variants and induced mutants at 20, 30, 40 and 60 Gy were evaluated for tolerance to *Fusarium* wilt disease by using 'double-tray' technique as well as testing in heavily infested field (Hot-spot). In double-tray screening, the results showed that all inoculated plantlets succumbed to disease infection gradually, ranging from 10 days to one month. In field evaluation, all suckers tested did not survive more than 6 months, though some mutants survived longer period than others.

ABSTRAK

Teknik kultur tisu dan aruhan mutasi digunakan untuk menghasilkan variasi dalam meningkatan prestasi Pisang Berangan kultivar Intan (AAA). Pokok-pokok pisang *in vitro* yang mempunyai prestasi ladang terbaik digandakan dan dinilai untuk menghasilkan prestasi ladang yang lebih baik termasuk ciri-ciri agronomi seperti pembuahan awal, pokok rendah, hasil kualiti buah yang tinggi serta ketahanan terhadap penyakit layu *Fusarium*.

Suatu media mikropropagasi yang sesuai untuk Pisang Berangan menggunakan medium asas MS yang diubahsuai memberikan peningkatan ketara dalam penggandaan pucuk. Meristem pucuk Pisang Berangan diiradiasikan pada dos 20,30, 40 dan 60 Gy untuk mengaruh mutasi. Kultivar berkenaan memberikan perbezaan di dalam tindakbalas terhadap dos radiasi dan pemulihan selepas radiasi berdasarkan bilangan pucuk yang terhasil setiap eksplan. Eksplan teraruh digandakan secara *in vitro* bagi menghasilkan pokok-pokok M_1V_4 sebelum ditanam di ladang untuk penilaian.

Radiasi gamma mengakibatkan banyak perubahan mutagenik bagi ciri-ciri kualitatif dan kuantitatif. Variasi ciri morfologi termasuk pseudostem: saiz, panjang dan pigmen, daun: keabnormalan, warna dan saiz serta buah: saiz, keabnormalan tandan/sikat dan sebagainya merupakan variasi tertinggi iaitu 25 – 30% sementara ciri-ciri agronomi (tinggi pokok, berat tandan dan ciri buah) hanya kira-kira 3 – 4% sahaja. Variasi somaklon juga didapati pada pokok-pokok kultur tisu normal (tanpa radiasi). Bagaimanapun frekuensi variasi somaklon berbeza dan ianya sangat rendah berbanding perubahan mutagenik.

Kandungan DNA nuklear mutan ditentukan menggunakan teknik aliran sitometri. Perbezaan kandungan DNA antara mutan menunjukkan kesan radiasi gamma ke atas genotip pokok berkenaan. Variasi bagi ketinggian pokok dan tumbesaran bantut menunjukkan perbezaan kandungan DNA yang besar berbanding kawalan (tidak teraruh). Secara amnya, kandungan DNA nuklear menurun dengan peningkatan dos gamma dari 20 ke 60 Gy.

Analisis amplifikasi rawak DNA polimorfik (RAPD) digunakan untuk mengkaji perubahan-perubahan DNA pada mutan. Empat dari 10 primer menunjukkan polimorfisme yang boleh membezakan di antara pokok normal dan bantut. Daripada 450 mutan yang diuji, hanya 50 sampel menunjukkan jalur-jalur yang jelas dan sesuai untuk analisis. Primer-primer OPA-03 dan OPA-05 menunjukkan jalur-jalur utama 1.5 dan 0.7, 0.5 dan 0.4kbp masing-masing dalam pokok yang dirawat pada dos 40 Gy berbanding kawalan dan pokok-pokok yang dirawat pada dos 20 dan 30 Gy. Jalur-jalur utama 1.5kbp dan 1.0 kbp pula wujud dalam pokok-pokok yang dirawat pada dos 60 Gy. Primer-primer OPA-03 dan OPA-09 dikesan menghasilkan variabiliti yang tinggi pada dos 40 dan 60 Gy yang menghasilkan variasi pokok renek, berat tandan yang rendah serta tiada tandan.

Pengenalpastian pokok renek hasil variasi somaklon dan aruhan mutasi Pisang Berangan dilakukan menggunakan Asid Gibberalik (GA_3) pada kepekatan 29μmol/L dan 59μmol/L pada peringkat *in vitro* dan 289μmol/L pada peringkat deflask. Perbezaan tindakbalas antara variasi renek dan kawalan tidak mantap untuk rawatan pada peringkat *in vitro*.

Bagaimanpun, pada peringkat deflask, pemanjangan berkas daun pertama muncul selepas rawatan GA_3 kira-kira dua kali ganda lebih dari kawalan berbanding mutan renek di mana ia berjaya membezakan diantara normal dengan yang renek. Kaedah ini adalah mudah dan murah disamping berkesan mengelakkan pokok renek yang tidak produktif.

Variasi somaklon dan aruhan mutasi pada dos-dos 20, 30, 40 dan 60 Gy disaring terhadap ketahanan terhadap penyakit layu *Fusarium* menggunakan teknik `double-tray' dan ujian ladang yang terinfeksi `Hot Spot'. Keputusan daripada kaedah `double tray', menunjukkan semua pokok yang diinokulasi mati akibat infeksi penyakit antara 10 hari hingga sebulan. Daripada penilaian ladang pula, semua pokok yang disaring tidak dapat hidup melebihi 6 bulan. Bagaimanapun terdapat beberapa mutan yang hidup lebih lama berbanding yang lain.

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ABBREVIATIONS AND ACRONYMS

B(M)	-	Berangan mutant
B(N)	-	Berangan normal
bp	-	base pair
BW	-	bunch weight
°C	-	degrees Celsius
$CaCl_2$	-	Calcium chloride
C/ b	-	Comb per bunch
cm	-	centimeter
CW	-	Comb weight
dATP	-	deoxyadenosine triphosphate
dCTP	-	deoxycytidine triphosphate
DES	-	diethyl sulphate
dGTP	-	deoxyguanosine triphosphate
dNTPs	-	deoxyribonucleoside triphosphates
DNA	-	deoxyribonucleic acid
DMSO	-	dimethyl sulfoxide
DMRT	-	Duncan Multiple Range Test
dTTP	-	deoxythymidine triphosphate
EDTA	-	ethylene diaminetetra acetate
EMS	-	Ethylmethane sulfonate
g	-	gram
G	-	Girth
GA_3	-	Gibberellic acid
GML	-	Ground magnesium limestone
Gy	-	Gray-unit of radiaotion rate
HCl	-	hydrochloric acid
IAA	-	indole-3-acetic acid
INIBAP	-	International Network for the Improvement of Banana and Plantain
kb	-	kilobases
kbp	-	kilobases pair
KCl	-	potassium chloride
L	-	litre
M	-	Molar
Mbp	-	Mega base pair
mg	-	milligram
min	-	Minute
ml	-	Mililitre
mM	-	Millimolar
mmol	-	millimoles
MNU	-	N- methyl- N- nitrosourea
MS	-	Murashige and Skoog media
M.W	-	Molecular weight
NaCl	-	Sodium chloride
NaN_3	-	Sodium azide

NaOH	-	Sodium hydroxide
Na_2SO_4	-	disodium phosphate
ng	-	nanogram
PCR	-	Polymerase Chain Reaction
PDA	-	Potato Dextrose Agar
pg	-	picogram
PPFD	-	Photosynthetic photon flux density
RAPD	-	Random Amplified Polymorphic DNA
RFLP	-	Restriction Fragment Length Polymorphism
Rnase	-	Ribonuclease
Sec	-	second
TBE	-	Tris- borate- EDTA
TE	-	Tris – EDTA
µg	-	microgram
µl	-	microliter
µ mol / L	-	micromol per litre
UV	-	ultra violet
VCG	-	Vegetative Compatibility Group
v / v	-	volume per volume
w / v	-	weight per volume