APPENDIX

APPENDIX (A)

Preparation of Modified MS Medium

Materials

The materials used in media preparation were: hot plate/ stirrer, pH meter, balance, autoclave, one 2-liter or more Erlenmeyer flask, one 10-ml pipette, two medicine droppers, spatula, washed sterilized bottles (jars) with caps, dispenser, distilled water and stock solutions of major salts, minor salts, vitamins, iron, BAP, coconut, sucrose and agar or gelrite.

Procedure

According to the formula chosen, the above components from different stock solutions were dissolved in distilled water of about half the final volume of the media, except the gelling agents. For one-liter medium, 2-liter Erlenmeyer flask (conical flask) with ingredients was placed on the hot plate/ stirrer. The stir-bar slided into the flask and the stirrer was turned on.

The pH meter (CD740, WPA, Cambridge U.K.) was calibrated according to the manufacturer's instructions, and used to adjust pH of the medium to 5.5- 5.8 with 0.2 NaoH or 1N HCl. Finally, the medium was brought to its precise volume. Then 0.6-1.0% Agar (Sigma, St Louis, Missouri, U.S.A) or gelrite (0.1- 0.3%) was weighed and added to the mixture. Heating and stirring were continued until the medium boiled vigorously but was not boiled over. The media was dispensed 30 ml per bottle or 15 ml per test tube using dispenser COMPUPUMP, Tecnomara AG 8304 Wallisellen, Switzerland) the medium,. Steam sterilization or autoclaving for nutrient media was done. A pressure of 1.05 Kg/cm^2 or 103.4 K Pa at 121°C over 20- 30 minutes.

Appendix (B)

Preparation of Potato Dextrose Agar

Dehaydrated Potato Dextrose Agar (PDA) used for isolation of Fusarium oxysporum f. sp. *cubense* race 4 from diseased plant tissues (Singlenton *et al.*, 1992).

The medium was prepared from:

Difco potato dextrose agar	39 g
Distilled water	1 L

The mixture was autoclaved at 121°C for 30 minutes and distributed in Petri dishes.

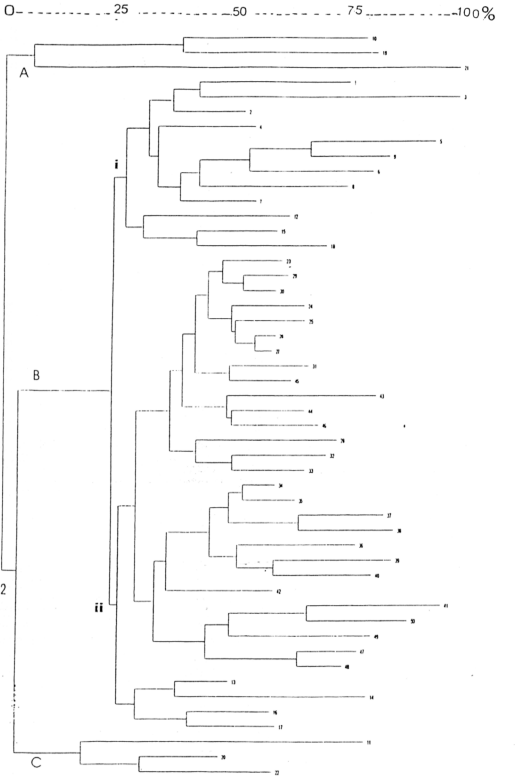


Figure 4.6-F- Dendrogram generated from RAPD fingerprinting profile in mutated "Pisang Berangan" based on RAPDistance Software Package (Version 1.04) - Phylogenetic tree of 50 different variables.

Appendix (E)

Statistical analysis

Table 1: Analysis of variance of DNA content for different variants induced by gamma-irradiation

Source of variability	df	SS	MS	F value
Variation within treatments	3	0.197	0.066	9.429**
Variation between treatments	29	7.588	0.262	37.34**
Error	87	0.585	0.007	
Total	**119**	**8.465**		

***Significantly at (P<0.01)*

Table 2: Analysis of variance of DNA content for all treatments of mutant Pisang Berangan (20, 30, 40 and 60 Gy's) and control.

Source of variability	df	SS	MS	Sig. of F
Between groups	4	0.247	0.180	**
DNA content	1	0.218	0.218	**
Within groups	3	2.948	9.827	**
Error	249			

***Significantly at (P<0.01)*

Table 3: Mean and Standard Error of DNA content of Pisang Berangan mutants and their control (Duncan`s Multiple Rang Test-DMRT)

Source of variation (Doses Gy)	Subset for alpha = 0.05		
	I	II	III
Control	1.840^{a}±(1.810)		
20 Gy		1.893^{b}± (4.517)	
30 Gy		1.898^{b}± (5.025)	
40 Gy			1.9327^{c}± (5.382)
60 Gy			1.9296^{c}± (9.304)

a, b & c different superscripts within column, within DNA content significant between means (P <0.05)

Growth cabinet experiment: *In vitro* stage

Table 4: ANOVA for leaf sheath of mutated, non-mutated and dwarf plants at 0, 29 and 59 µmol/L of GA_3.

Source of variation		df	SS	MS	Sig. of F
0	Treatment	2	41.486	20.743	**
	Error	27	8.057	0.298	
	Total	29	49.543		
29	Treatment	2	63.242	31.621	**
	Error	27	12.766	0.473	
	Total	29	76.008		
59	Treatment	2	53.321	26.660	**
	Error	27	10.212	0.378	
	Total	29	63.533		

** *Significantly differences at (P<0.01)*

Table 5: ANOVA for leaf petiole of mutated, non-mutated and dwarf plants at 0, 29 and 59μmol/L of GA_3.

Source of variation		df	SS	MS	Sig. of F
0	Treatment	2	3.731	1.865	**
	Error	27	5.144	0.191	
	Total	29	8.875		
29	Treatment	2	20.099	10.049	**
	Error	27	52.996	1.963	
	Total	29	73.095		
59	Treatment	2	28.501	14.250	**
	Error	27	22.473	0.832	
	Total	29	50.974		

** *Significantly different at (P<0.01)*

Table 6: ANOVA for pseudostem length of mutated, non-mutated and dwarf plants at 0, 29 and 59μmol/L of GA_3.

Source of variation		df	SS	MS	Sig. of F
0	Treatment	2	52.605	26.302	**
	Error	27	7.230	0.268	
	Total	29	59.835		
29	Treatment	2	133.736	66.868	**
	Error	27	70.576	2.614	
	Total	29	204.312		
59	Treatment	2	150.493	75.246	**
	Error	27	82.317	3.049	
	Total	29	232.810		

** *Significantly different at (P<0.01)*

Growth cabinet experiment (deflasking stage)

Table 7: ANOVA for leaf sheath – leaf 1 – at GA_3 289μ mol/L

Source of variation	df	SS	MS	Sig. of F
Treatment	2	162.812	81.406	**
Error	39	70.010	1.795	
Total	41	232.822		

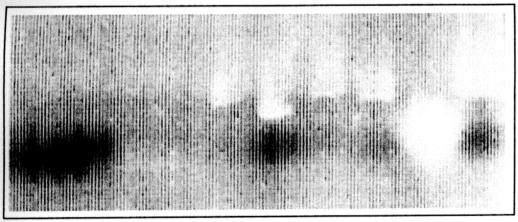
** *Significantly different at (P<0.01)*

Table 8: ANOVA for leaf sheath, leaf 2 at GA_3 289μ mol/L

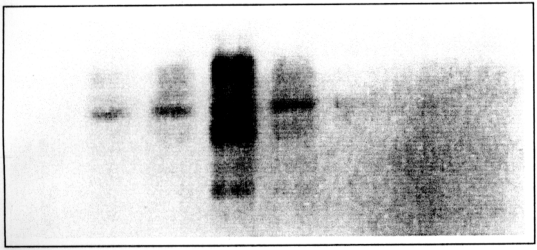
Source of variation	df	SS	MS	Sig. of F
Treatment	2	322.358	161.179	**
Error	39	49.801	1.277	
Total	41	372.159		

** *Significantly different at (P<0.01)*

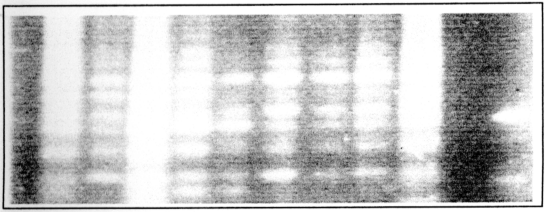
Appendix-F



5ng/ul



25ng/ul



50ng/ul

The result of verification experiments for optimization and reproducibility of DNA amplification were showed no amplification or non-reproducible bands for 5 ng/ul of DNA, while to some extent reproducible bands appeared at 25 ng and good amplification at 50 ng/ul.