
CHAPTER SIX

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SUMMARY

In vitro mutation induction by using gamma ray at 20, 30, 40 and 60 Gy was used to generate variability in Pisang Berangan (AAA), so as to provide the opportunity to select plants with desirable characters such as early fruiting, short stature and resistance to *Fusarium* wilt disease.

Modified MS medium supplemented with 4.5 mg/L of BAP was used for shoot-tip meristem culture to generate sufficient meristem pieces for *in vitro* mutagenesis by gamma-irradiation. Mutant meristems were sub-cultured to M_1V_4 before establishing plantlets for evaluation.

Mutation frequency increased with increased dosage whereas, survival and capacity to regenerate decreased with increased doses. The exposure of shoot-tip meristem pieces to radiation doses produced wide variation in growth and morphogenetic performance. Mutagenic treatments induced 2 to 3-fold increase in variability. The induced morphological and physiological changes include the desirable traits such as dwarf and earliness. The high dose of 60 Gy caused many plants to become short and stunted in growth (150-180 cm). At 40 Gy dose, the majority of plants showed a plant height between 270-310 cm. At 30 Gy about 10% of plant showed a height of less than 270 cm. High doses might also cause many mutational events per cell, which might increase risk of a favourable mutation being accompanied by one or more unfavourable genetic changes.

Mutation induction caused a wide range of variability in flowering time. Earliness to flowering was recorded for about 3% of the whole population, where the variants came to shooting as early as 6.0-6.5 months as compared to the control (7 – 8 months). Early flowering plants such as those less than 231 days were selected for further investigation.

A wide variation was also observed in bunch characters, mainly due to many deformed fruit bunches, causing reduced bunch weight, comb size and finger size. However, some selections were obtained for high bunch weight at 30 Gy and a higher number of comb/bunch at 40 Gy treatments.

Some regenerated plantlets via tissue culture technique also showed somaclonal variation at 3 –5%. However, the range of somaclonal variants was narrow and most of them undesirable. The most common off-type found was dwarfs, which could be detected early in the nursery before transplanting to the field. The other somaclonal variants observed in the nursery or the field includes foliage position, pseudostem colour, fruit shape and quality, which were inferior to the original clone.

Attempts were made to use exogenous gibberellic acid to discriminate between dwarf and normal plants. Banana plants (Pisang Berangan) micropropagated (mutated and control) exhibited a significantly greater leaf sheath elongation in response to GA_3. The response to GA_3 increased as the concentration increased. The differential growth induced by GA_3 to tall and dwarf plants could be the basis for selection and elimination of dwarfs from mutated-micropropagated plants.

Gibberellic acid is a useful technique to detect heritable changes, mainly dwarfism at early stages of *in vitro* development of bananas. The method would be simple and cheap, and

could prevent the waste of resources on non-productive dwarf plants. On the other hand, for commercial use this screening may be too expensive and may increase the cost of production.

The mutated plantlets of Pisang Berangan were screened for *Fusarium* wilt tolerance by using 'double-tray technique' and in *Fusarium* field 'Hot-spot'. A total of 1000 mutated plants were screened by 'double-tray' technique. All were found susceptible. Screening for *Fusarium* tolerant in the field (hot spot) showed that all plants (totaling 1798) also succumbed to the disease eventually.

Further characterization of mutants at DNA level was carried out by using flow cytometry and RAPD analysis. DNA content of mutated plants significantly different from control plants especially at high doses of 40 and 60 Gy. However, there were no significant differences between DNA content at 20 Gy and 30 Gy and also between 40 Gy and 60 Gy. The phenotypic variations observed at high doses were likely due to changes in the DNA sequences at the chromosomal level. In addition, higher doses may result in changes of cell cycle kinetics.

DNA amplification fingerprinting was successfully used to detect genetic polymorphism in the mutated plants. More RAPD bands were present in the somaclonal and irradiated samples compared to non-irradiated control. DNA concentration was a major factor affecting the amplification products. A decrease in the concentration resulted in few or no bands due to increase in stringency of the reaction. However, the concentration of DNA between 25 to 50 ng resulted in good amplification and product that was easy to score. The RAPD bands (from primers: OPA-03_{1700}, OPA-05_{1600}, OPA-07_{1500} and OPA-09_{1700}), which were consistently present in all normal and absent in all dwarfs might be useful for the

detection of dwarfism. The result of the study suggests that the primer identified can be used to determine accurately the rate of dwarfism in a population.

As a conclusion from the results obtained, RAPD analysis can be used to detect genetic variation in gamma-irradiation induced mutants in bananas specially Pisang Berangan. Furthermore, it can be concluded that in commercial scale the RAPD technique is very ideal to analyze the genetic integrity of banana plantlets to guarantee that there will be no abnormality on banana plants produced through tissue culture technique. Furthermore, RAPD markers, likewise other PCR techniques (Baurens *et al.*1996), are useful to detect genetic instability of *in vitro* propagated bananas.