DEVELOPMENT OF ORCHID VARIETIES BY NON-CONVENTIONAL TECHNIQUE

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Introduction

Orchid cut flowers has become an important export commodity in Malaysia, contributing to about 40% of total value of export of cut-flowers since 1995. Orchid gains its popularity for its wide range of varieties producing an array of shapes, colours and sizes. However, many of the existing hybrids have been in the market for more than 15 years, with some more than 20. With ever changing tastes and preferences of consumers, there is an urgent need for new or improved varieties with new colours or size. In orchids, conventional breeding and selection has been difficult or impossible, costly and time-consuming and often hampered by genetic sterility. Induction of mutation by gamma-irradiation has provided an avenue for creating variability in many plant species. The present study was undertaken to elucidate the effects of gamma-irradiation with the objective of determining the appropriate dose feasible for inducing desired mutation in already established varieties of orchids. The purpose of the study was to develop protocol for the creation of variability in orchids.

Materials and Methods

Protocorm-like bodies of *Mokara* Chark Kuan and *Dendrobium* Jacky, the plant materials used, were initiated through the standard culture of shoot tips in Vacin and Went medium. The culture of protocorm-like bodies was maintained in Vacin and Went liquid medium with 1.2% coconut water. The culture was irradiated in a series of different doses of 0 to 150 Grays in sterile environment (Briggs and Constantin, 1977). Reculture was accomplished immediately after irradiation in semi-solid medium. Survival and plantlet regeneration as well as dry weight accumulation were observed. Random Amplified Polymorphic Deoxyribonucleic Acid analysis was carried out according to Doyle and Doyle (1990) with some modifications to observe polymorphism in the genomic deoxyribonucleic acid of the plants after irradiation. Detected polymorphism was reconfirmed by Southern analysis.

Results and Discussion

Gamma-irradiation had resulted in various degrees of lethality to cultures of both varieties. Decrease in survival with increasing doses was significantly observed. Optimum dose of gamma-irradiation for *Mokara* Chark Kuan was in the range of 20-40 Grays. *Dendrobium* Jacky recorded an optimum at 60-70 Grays. Regeneration of protocorm-like bodies declined with increase in doses in both varieties. Fresh weight,dry weight and dry weight accumulation generally decreased with increase in doses.

Random Amplified Polymorphic Deoxyribonucleic Acid analysis carried out on 7 *Mokara* Chark Kuan samples using 17 primers from Operon Kit AE resulted in two primers (OPAE-08 and OPAE-11) producing 2 novel bands for each primer indicating polymorphism in the samples. The primers detected somaclonal variations in 2 non-irradiated samples probably initiated through repeated subculture of protocorm-like bodies. They also detected variations caused by gamma-irradiation. When Southern analysis was performed using the novel bands as probes, the results supported the findings obtained from Random Amplified Polymorphic Deoxyribonucleic acid analysis

Amplification using OPAE-11 as primers were cloned and sequenced. The sequences could be used for Sequenced Characterised Amplified Regions for the specific amplifications of a single band. Homology searches using pAL108 and pAL202 as query sequences revealed 56.6% and 63.4% similarity to *Lycopersicon esculuntum* copia-like retrotransposon and *L. esculuntum* gypsy-like retrotransposon respectively (Aung and Tan, 1996) . There is a possibility that both pAL108 and pAL202 are related to transposable elements. Both repeated subculture and irradiation could have activated these transposable elements which contributed to the variations. Southern analysis of genomic deoxyribonucleic acid showed that probe B and pAL108 were parts of the genomic deoxyribonucleic acid of *Mokara* Chark Kuan. It further confirmed the polymorphisms detected by Random Amplified Polymorphic deoxyribonucleic acid analysis. There might be some similarities between pAL108 and pAL202 that allowed pAL202 to hybridise to pAL108. When Random Amplified Polymorphic deoxyribonucleic acid analysis was performed on 8 *Dendrobium* Jacky samples using 20 primers from Operon Kit AE, no polymorphism was detected. It is possible that these primers were unable to detect any possible polymorphism that had occurred. Primers containing different combinations of oligonucleotides should be considered in future study.

Conclusions

Gamma-irradiation induced changes in the culture. It caused significant effects on fresh weight, dry weight and dry weight accumulation. Variations caused by repeated subculture of protocorm-like bodies and irradiation were detected by Random Amplified Polymorphic Deoxyribonucleic Acid analysis. Phenotypic expression of these variations could not be confirmed in this study as plantlets take at least another 2-3 years to flower.

References

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