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Field Observation of Clonal Oil Palms Irradiated with Gamma Rays

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ABSTRACT

Clonal palms that were derived from callus cultures were repeatedly exposed to gamma rays of 5Gy (Grays) for three and five times during subculture intervals. Field observations and data recording were conducted four years after field planting. Preliminary results from palms derived from the callus cultures periodically irradiated for five times during subculture intervals recorded highly significant differences in leaf area index (LAI) and biomass compared with the control and standard cross palms. A two-dimensional principle component analysis score plot showed a distinct cluster of the various irradiation exposures compared to the standard cross materials. In addition, from the score and loading plots, a higher dosage of gamma irradiation resulted in increased rachis length, LAI, trunk height, biomass and leaf chlorophyll (SPAD) content, with an inversely smaller value in leaf water potential.

ABSTRAK

Klon sawit dihasil melalui kultur kalus yang terdedah kepada sinar gamma 5Gy (Grays) berturutan selama tiga dan lima kali subkultur. Pemerhatian di ladang dan penghasilan data telah dijalankan selepas tahun keempat penanaman. Keputusan awal mendapati radiasi berkala sebanyak lima kali subkultur mencatatkan perbezaan yang amat ketara dalam indeks helaian daun (LAI) dan biojisim berbanding dengan sawit kawalan dan standard. Skor analisis komponen dalam plot prinsip dua dimensi menunjukkan satu kelompok yang berbeza daripada pelbagai pendedahan penyinaran berbanding dengan sawit standard. Di samping itu, dari pemarkahan dan plot muatan, gamma penyinaran dengan dos yang lebih tinggi menghasilkan peningkatan panjang rakis, LAI, ketinggian pokok, biojisim dan kandungan klorofil daun (SPAD), berbanding dengan nilai potensi air daun yang rendah.

Keywords: irradiation, gamma rays, field trials, mutation, principle component analysis.

INTRODUCTION

The oil palm genetic resources in Malaysia are based on the Deli *Dura* population derived from four palms introduced in Bogor in 1848 (Kushairi and Rajanaidu, 2000). Sizeable oil palm germplasm collection have been available since 1973 (Rajanaidu and Rao, 1987), evaluated and utilised for crop improvement. The breeding programme for oil palm carried out by conventional cross hybridisation is extremely time-consuming, taking more than 10 years to select for desirable economic traits. To speed up introgression of suitable genes into existing oil palm materials that currently have limited genetic variation, several methods have been employed with the aim of broadening this genetic base. An on-going genetic engineering effort at the Malaysian Palm Oil Board is one of the strategies to meet this objective. Alternatively, a non-genetically modified (GM) approach, the mutation breeding technique could be employed. In other crops, mutation breeding has been used to improve and increase the availability of genetic resources. It was suggested by Nagatomi *et al*. (2006) that chronic irradiation produced a wider variation than acute irradiation. It was also recommended that low doses of irradiation should be used for targeting *in vitro* cultures (IAEA, 1986). Therefore, in this study, the mutated clonal palms were created from *in vitro* cultures that had been exposed to 5GY of gamma rays three and five times at four to five days subculture intervals within a month.

MATERIALS AND METHODS

Mutation Induction

Callus cultures of Clone CX49 from a high-yielding ortet with a yield performance of fresh fruit bunch [(FFB): 175.78 kg palm^{-1} yr^{-1}, oil to bunch (O/B); 28.4%] were used for mutation induc-

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tion. The calli were kept on basal nutrient medium containing 7.5 x 10^{-5}M naphthalene acetic acid (NAA), 1.5 g $litre^{-1}$ of activated charcoal and were gelled with 7 g $litre^{-1}$ of agar media. One week before irradiation, each coin-shaped callus clump of 10 mm in diameter was spread thinly in the middle of a 90 mm diameter petri dish containing the culture medium. The calli were exposed to a low dose of gamma radiation (GR) given repeatedly at short intervals. Treatments O-C and O are both controls whereby calli in Treatment O-C did not undergo repeated subculture whilst Treatment O underwent five subcultures at five-day intervals. This treatment was to test the effect of repeated culturing at short intervals on the cultures. Calli in treatments 15GY and 25GY were repeatedly exposed to 5GY of GR for three and five times, respectively during subculture intervals. All the treated cultures including control Treatment O-C were transferred to fresh nutrient medium and maintained in the dark for eight weeks. Embryoids regenerated were bulked for shoot development and rooted before transplanting to nursery.

Field Planting

Rooted ramets from all the four experiments above (Treatments O-C, O, 15GY and 25GY) were transferred directly to a mixture of sand and soil as described by Rohani *et al.* (2003). In vegetative propagated plants like oil palm, it is difficult to distinguish materials with spontaneous mutations from the desired mutations *in vitro* or at the nursery stage except for those that show discernable variations as described by Meilina *et al.* (2005). Plantlets from these irradiation experiments were planted alongside standard crosses (SC) in trial 0.458 at Bukit Lawiang, MPOB Research Station Kluang in August 2006 (*Figure 1*).

Figure 1. Irradiated clonal palms planted in August 2006 at Bukit Lawiang, MPOB Research Station, Kluang.

Vegetative Measurement

Eight palms per treatment were chosen for the study. Vegetative measurement was based on Frond 17 which include measurements of leaf number (LFNO), petiole cross-section (PETIOLE), leaf area index (LAI), rachis length (RL), frond dry weight (FDW) and trunk dry weight (TDW). Leaf chlorophyll content (SPAD), chlorophyll fluorescent (FV/FM) and leaf moisture content measuring leaf water potential (LWP) were measured based on standard leaf disc sampling technique using chlorophyll fluorometer. Gas exchange measurements of photosynthesis rate (Photo), photosystem (PSII) and water-use efficiency (WUE) were carried out at three readings per palm using the LICOR Li-6400 Portable Photosynthesis System.

Analysis

Multivariate analysis on the recorded data was conducted using the statistical package, Unscrambler Version 10.1. A relationship between vegetative measurement and other physiological evaluations from numerous data were analysed using the principle component analysis (PCA) algorithms provided by the software. The above studied measurements are usually correlated, and therefore it may be interesting to find general regularities in the relations that occur between them. For finding the regularities, multidimensional analyses are used, one of which is PCA (Evgenidis *et al.*, 2011). PCA makes it possible to transform a given set of characteristics (variables), which are mutually correlated, into a new system of characteristics, known as principal components, which are not correlated. It is a multivariate statistical method for exploring and simplifying complex data sets. Each principal component is a linear combination of the original variables, and so it is often possible to ascribe the meaning to what the components represent (Lewis and Lisle, 1998).

RESULTS AND DISCUSSION

Preliminary results of a two-dimensional PCA score plot (*Figure 2)* showed distinct clustering of the various irradiation treatments applied. The PCA showed that two components explained 64% of the total variation among traits. The first PCA (PC-1) was assigned 39% and the second PCA (PC-2) was assigned 25% of total variation among traits. Standard cross materials (SC) were clustered at the top-left corner in a negative PC-1 quadrant, in comparison to the 25GY (which clustered at bottom – right) in a positive quadrant indicating an inversely correlation between both treatments. The 15GY irradiated palms were clustered at the bottom-left quadrant in negative of both PC-1 and PC-2 quadrants. Both controls (treatments O-C and O) were found nearer to the x- and y-axes.

In *Figure 3*, the correlation coefficients among the traits indicate that the plot currently shows the relationship among the traits that had relatively large loading on both PC-1 and PC-2 axes. Correlation plots of all measurements of these irradiated palms showed that LFNO, PETIOLE, LAI, RL, FDW, TDW and SPAD were closely related while measurements of LWP, photosynthesis rate (Photo) and WUE were negatively correlated to chlorophyll fluorescence (FV/FM) and photosystem (PSII) measurements.

In addition, from the score and correlations loading plots (*Figures 2* and *3*), that 25GY palms gave rise to high values of physiological characteristics, specifically LFNO, RL, LAI, PETIOLE, FDW, TDW and SPAD counts, with inversely low values in LWP as compared to the SC.

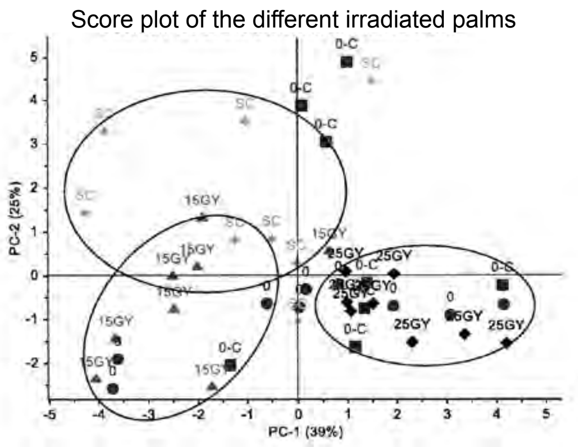

Figure 2. Score plot of palms derived from the different irradiation treatments.

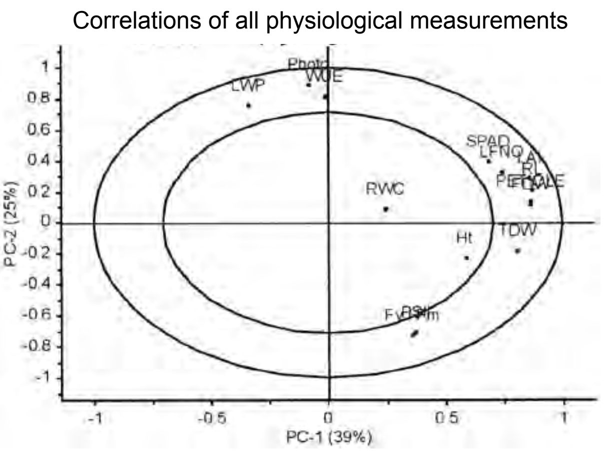

Figure 3. Correlation between vegetative and instrumentation measurement.

CONCLUSION AND RECOMMENDATIONS

This study provides a better understanding on the relationship between the vegetative and instrumentation measurements with regards to the growth of the oil palm in the field. Besides that, *in vitro* gamma irradiated callus cultures were shown to give rise to oil palm with alterations to certain physiological and vegetative characteristics such as biomass. This information will further enhance the understanding of the oil palm growth in relations to the conformity of clonal DxP materials.

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