RAPID MASS MICROPROPAGATION OF MUSA ACUMINATA CV BERANGAN (AAA): FROM LAB TO FIELD

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Banana is one of the priority fruit crop in Malaysia.

*Musa acuminata* cv berangan is one of the bananas grown commercially.

Mainly as dessert, now developed into food products.

Susceptible to biotic and abiotic stress.

Need for biotechnologies for crop improvement.

Embryogenic cell suspension is an ideal target system for micropropagation and adopting biotechnological method (transgenics).
Micropropagation via shoot meristem cultures:
- not efficient for plant production
- labour intensive
- not ideal target tissue for plant transformation

Embryogenic cell suspension:
- 10X more efficient for plant production
- Can be automated in bioreactors
- Single cell transformation
Current protocols for banana somatic embryogenesis are limited by low embryo germination, low plant regeneration rates and long culture period.

Optimisation of multiplication, development and regeneration to improve the number of plantlets recovery from somatic embryos.
Different concentrations of two amino acids (L-glutamine and L-proline) in developmental media (liquid and solid).

Different plating density of embryos for germination
Explants pre-treated on solid media with 23µM of 2, 4-D and subsequently transferred to media with 10 µM 2, 4-D for 3-4 months
Propagation of embryogenic cell suspension

Murashige and Skoog (MS) basal media
1.1 mg/L 2,4-D
0.25 mg/L zeatin
10 mg/L ascorbic acid
20 g/L sucrose
Different development media consisting of varying concentration of proline and glycine in liquid or solid media were tested.

Proline and glycine have promotive effects for embryo development.

High concentration of proline in liquid media however prove to induce abnormality in the embryo differentiation stage.

Abnormalities observed include formation of pseudo-radicle structure, extensive browning and stunted growth which eventually caused the embryos to be necrotic.
Abnormal embryos in proline-supplemented liquid M3 media. Embryos with long (A-B) and short (C-D) pseudo-radicle structure. E) retarded embryos 5 months after culture in development media. F) Embryos with extensive browning and browning couple with root-like formation (G). (H) A detached root-like structure and (I) fused root-like structure.
Developing somatic embryos on solidified M3 media were cultured with different embryo plating density. 200 µl of suspended embryogenic cell suspension were dispersed on M4GS media (MS basal medium, 400 mg/l L-glutamine, 30 g/l sucrose, 2 g/l Phytagel™) in three culture densities. Cells were either spread evenly throughout the petri dish (low density), or partially spread on 2/3 (moderate density) or 1/3 of the petri dish (high density).
Comparison between different embryo culture density on solid media and developmental stages of maturation after 3 months in culture.

Culture density studies showed that highly dense embryo inoculation developed poorer in term of duration taken to mature and time taken to subculture as compared to less dense inoculation.
The minimum duration needed to obtain mature embryos in liquid media was shown to be earlier (1.5 month) than that needed in solid media (2.5 months).

In all media tested, regenerated plantlets obtained in liquid M3 media was higher than that obtained in solid counterparts.
Comparison in the number of plantlets obtained in different development media
Morpho-histodifferentiation stages of banana (cv berangan) somatic embryos. Rooted berangan plantlet. Acclimatized berangan plantlets ready to be planted in the field.
Tissue culture derived plants in the field
6 months; oil palm
Open field; 2 months
The graphs show the number of hands per bunch for 'open field' treatment and control in percentage of plants (%).
IN BETWEEN OIL PALM TREES

OPEN FIELD

Graphs showing the percentage of plants (%), height (meter), treatment, and control for both in between oil palm trees and open field conditions.
IN BETWEEN OIL PALM TREES

OPEN FIELD

Percentage of plants (%)

Pseudostem diameter (cm)

- 9-11 cm
- 12-14 cm
- 15 cm

- Treatment
- Control
IN BETWEEN OIL PALM TREES

OPEN FIELD

Graph 1: Percentage of plants (%)

- Treatment
- Control

Graph 2: Bunch weight (kg)

- Treatment
- Control
IN BETWEEN OIL PALM TREES

OPEN FIELD

- Flowering time
- Percentage of plants (%)
- Flowering time (month)
- Treatment
- Control
IN BETWEEN OIL PALM TREES

OPEN FIELD

![Graphs showing percentage of plants over harvesting time for treatment and control groups in both in between oil palm trees and open field conditions.](image)
2,4d pulse important for embryogenic callus induction

Liquid media for development can enhance embryo production

Low to moderate embryo culture density can improve maturation period

Field assessment - based on annova of the average results showed insignificant difference between meristem and cell suspension derived plants for p-value more than 0.05 (at 95% confidence level)
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