Genetic Modification of Oil Palm and Its Biosafety

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MPOB

PRESENTATION OUTLINE

1. Introduction
2. Oil Palm Transformation Methods
3. Selection agent for transformation
4. Gene constructs for transformation
5. Transformation and Regeneration
   Transgenic oil palm
6. Biosafety of transgenic oil palm
7. Conclusion

Global Area of Biotech Crops

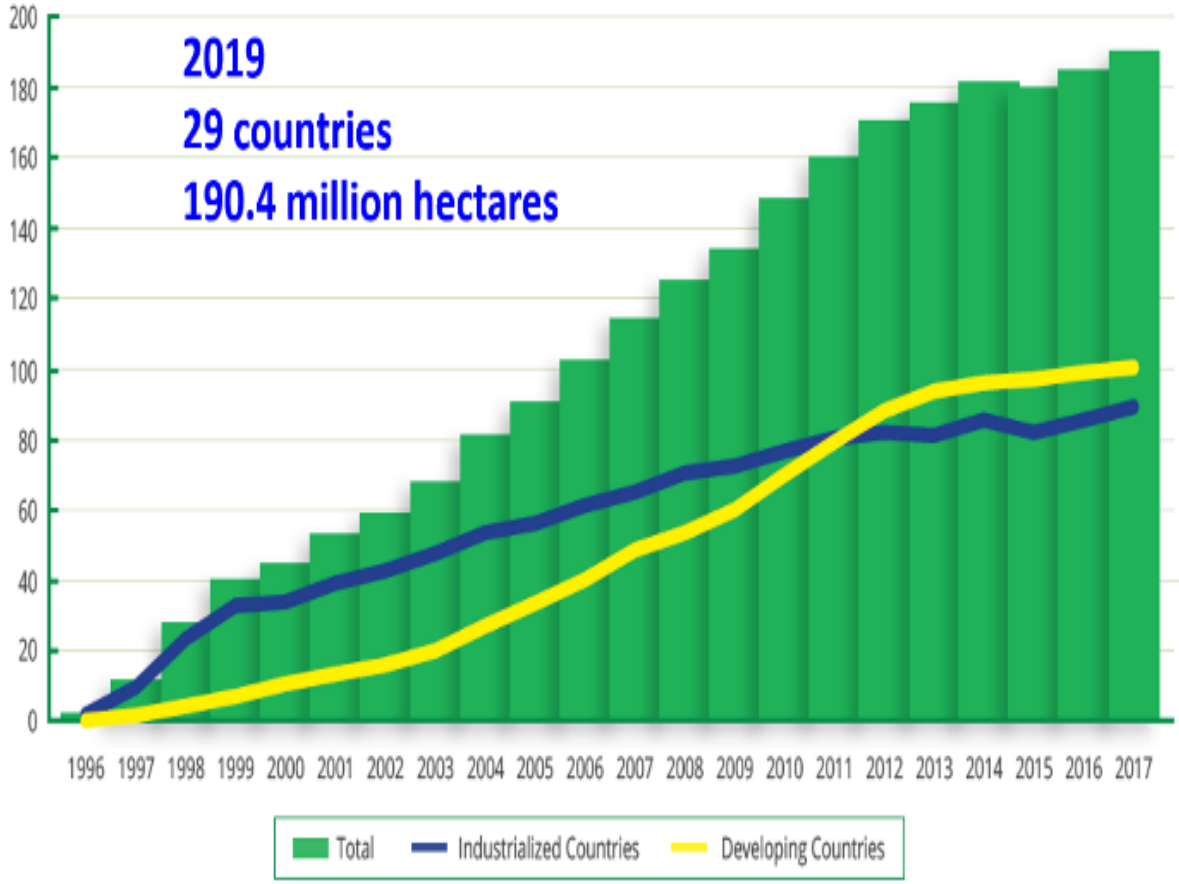


Figure 1. Global Area of Biotech Crops, 1996 to 2017: Industrialized and Developing Countries (Million Hectares)

Source: ISAAA, 2017

Four GM crops dominating world

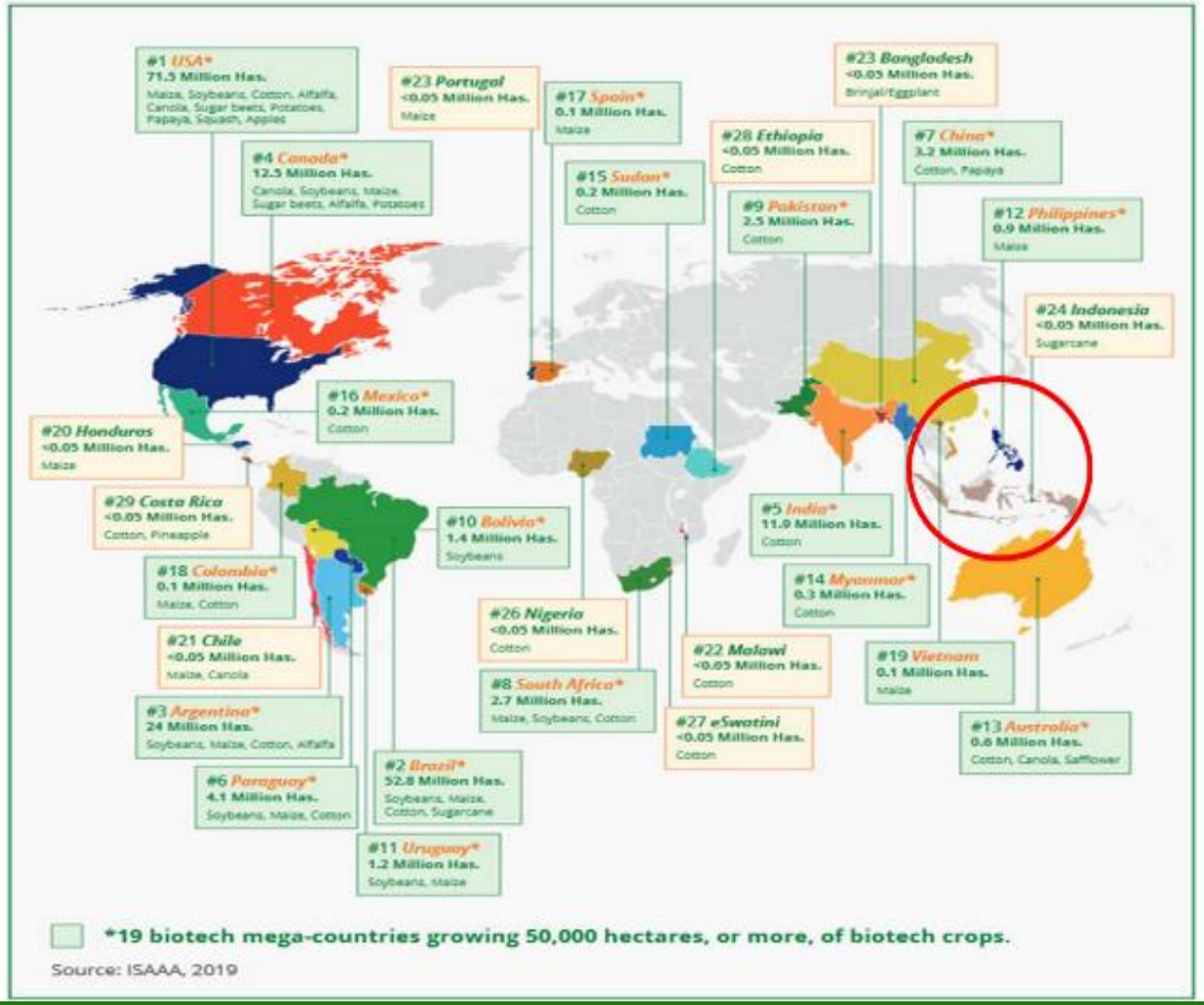
CORN

SOYBEAN

COTTON

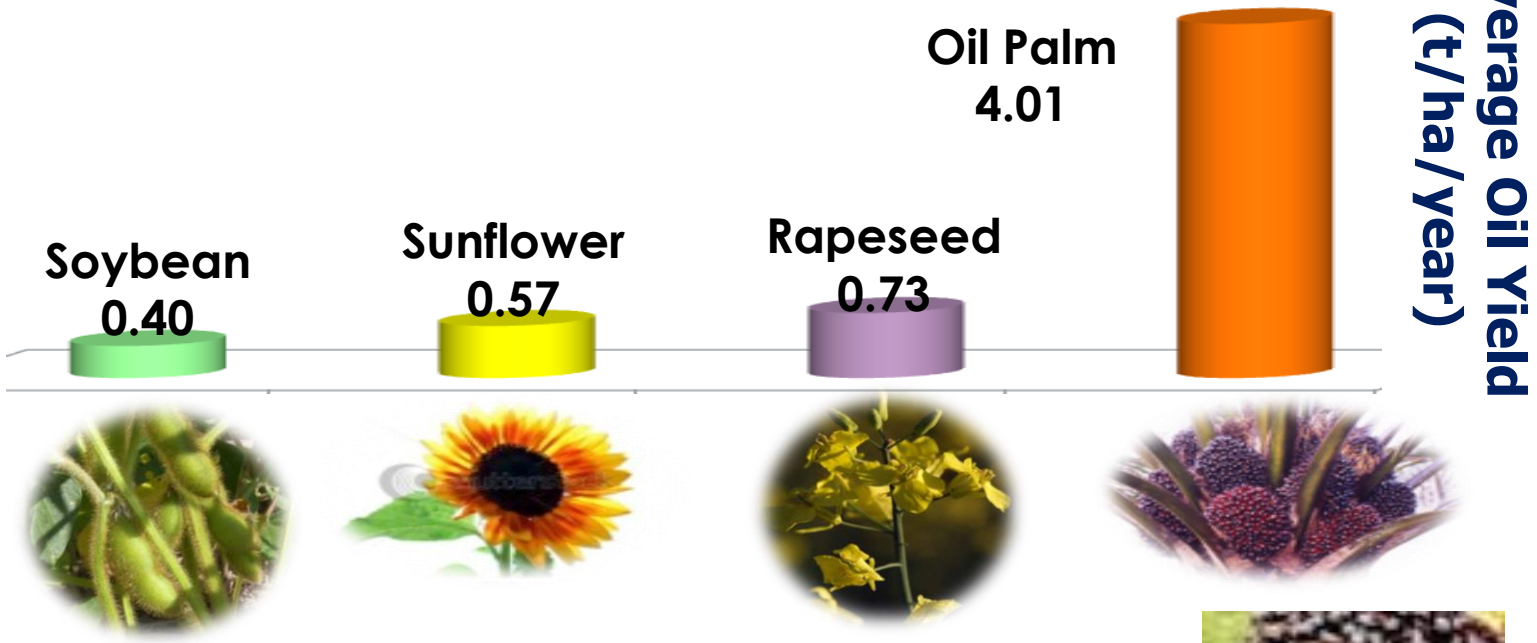
RAPESEED

Global Area of Biotech Crops



Adoption by 29 countries, but rates highly variable due to social stigma

OIL PALM – Most Productive Oil Crop



Planted on just 5% of land occupied by oil crops

YET

Contributes to ~ 40% of world's edible oil

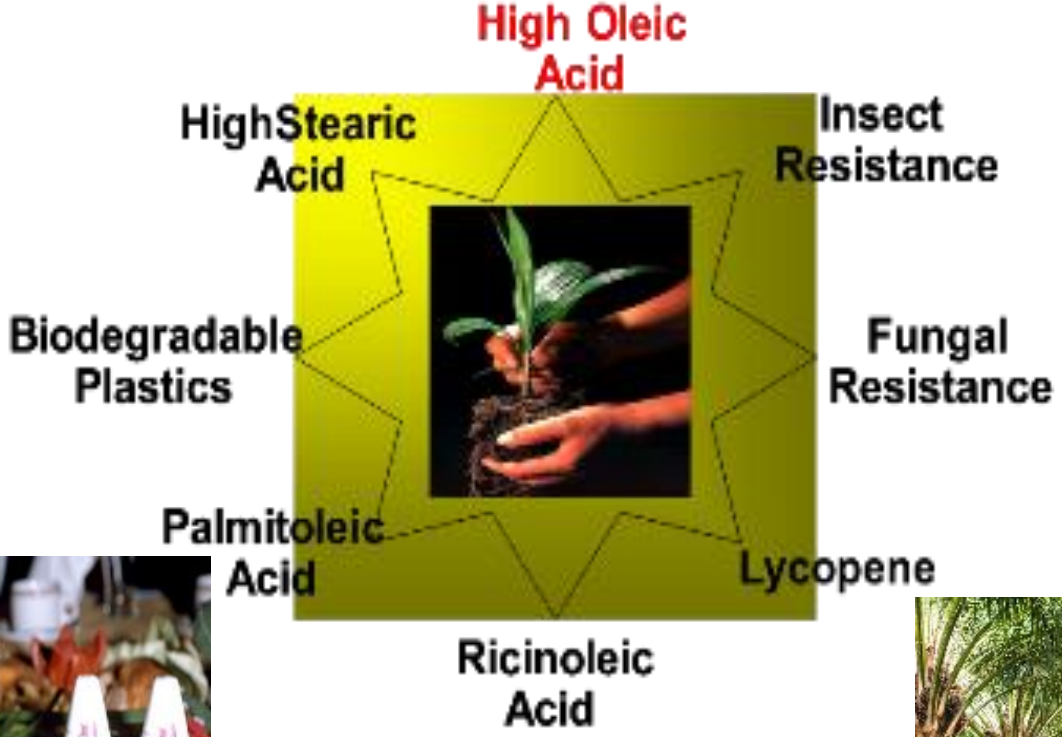
Genetic Modification of Oil Palm

Justification:

- Limited land resource and labour shortage
- Conventional breeding – long regeneration time

Genetic Modification of Oil Palm

Inherent high productivity of the oil palm to be channeled towards the production of high value specialty oils and industrial feedstock



Industrial Feedstock
Liquid oil market

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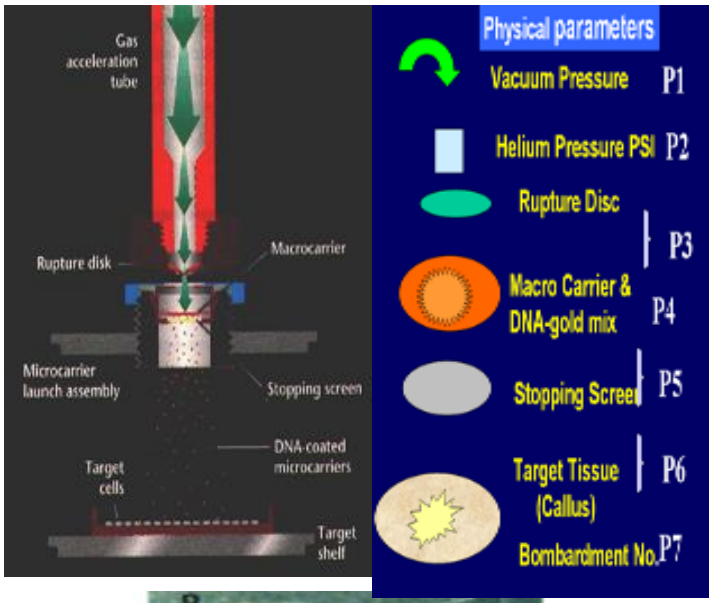
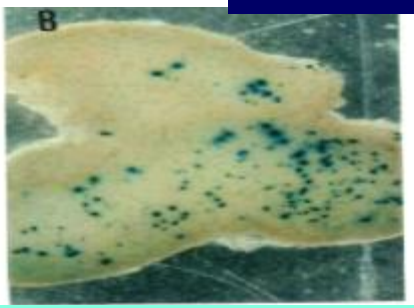
Biolistic Transformation of Oil Palm -Physical Parameters

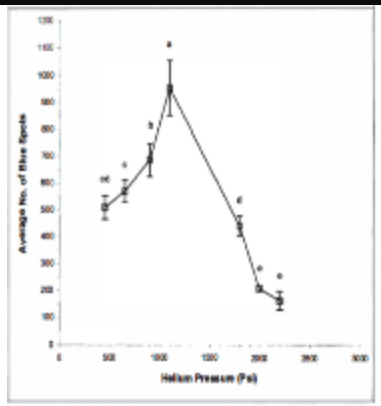


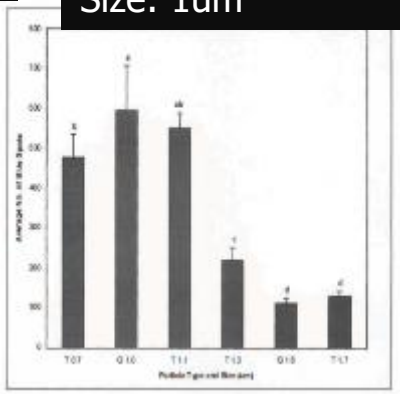


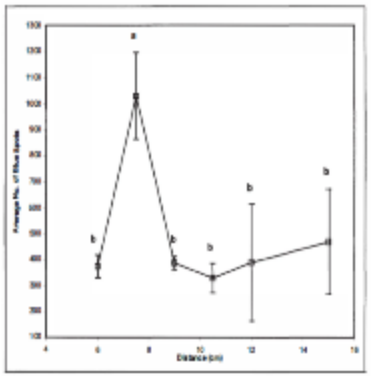
Gus staining for bombarded calli derived from Immature embryo

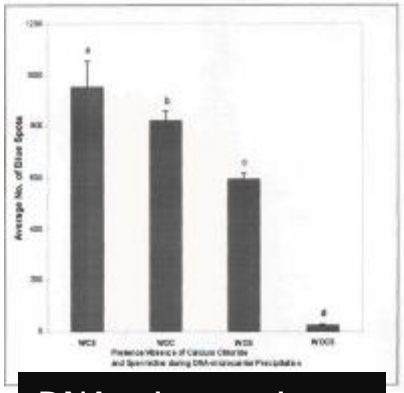
Helium pressure:1100 psi

Particle type: gold
Size: 1um







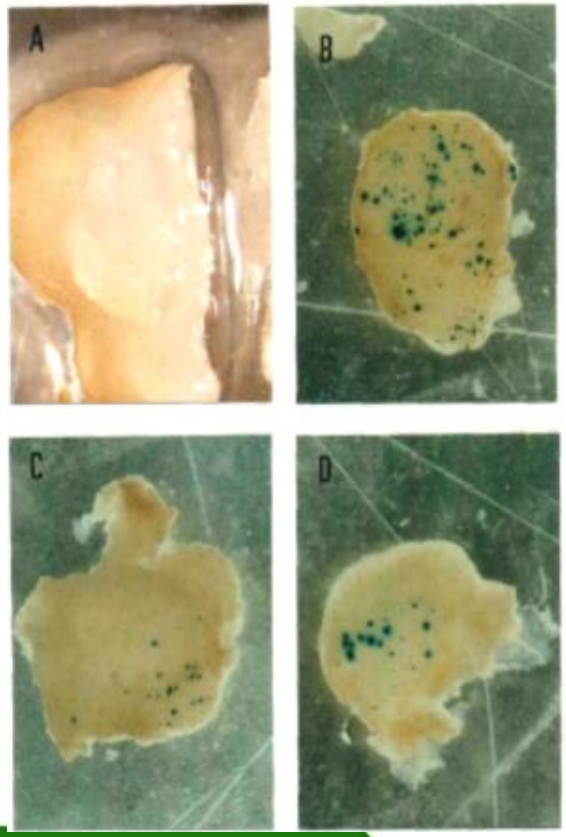


Distance to target tissue: 7.5 cm

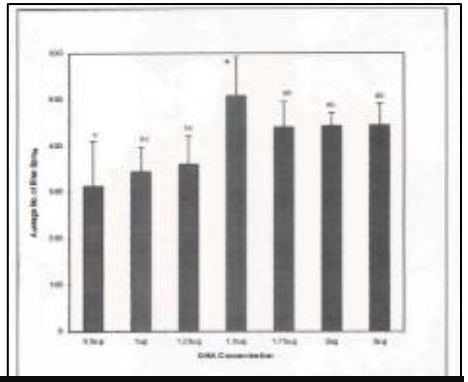
DNA-microcarrier precipitation: Calcium chloride Spermidine

Parveez et al (1997). Ind. Crops Prod. 6: 41-50.

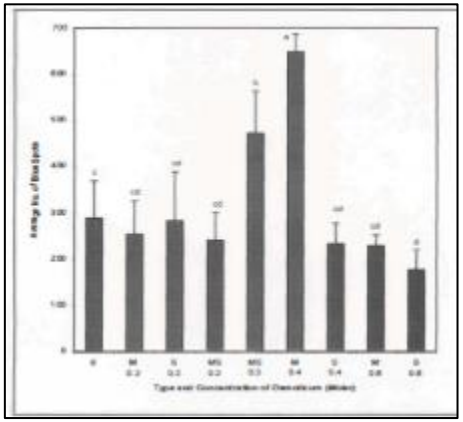
Biolistic Transformation of Oil Palm -Biological Parameters



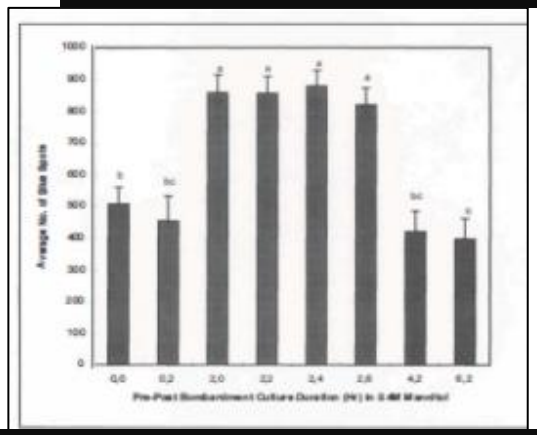
Gus staining for bombarded calli derived from Immature embryo



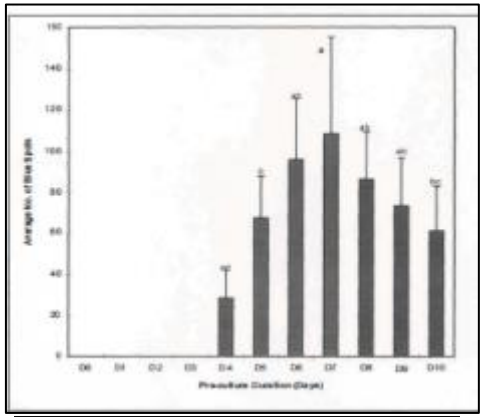
DNA concentration: 1.5ug



Osmoticum: 0.4 M Mannitol



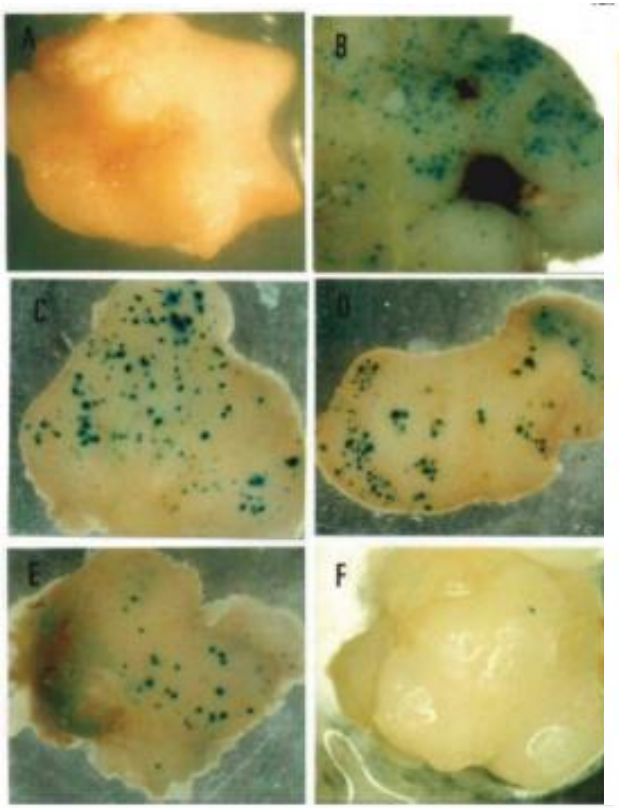
Osmoticum: 2h:4h in 0.4M Mannitol



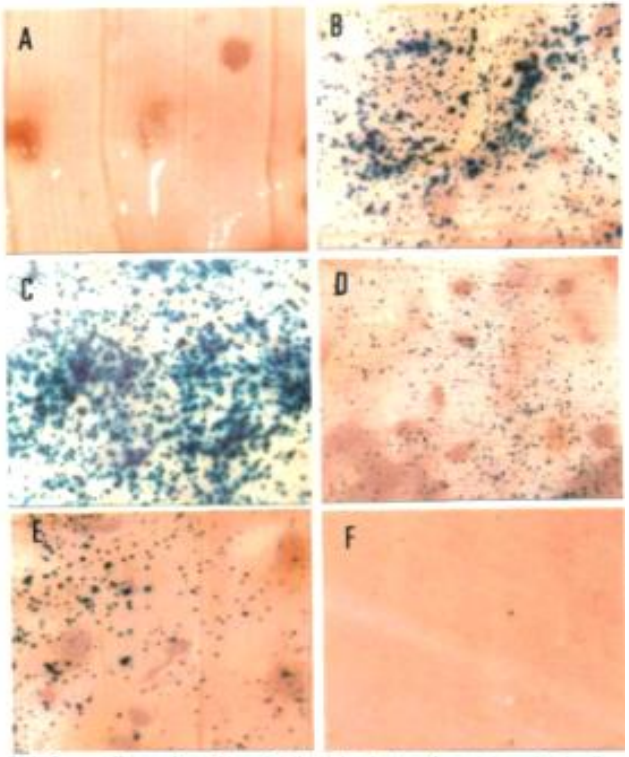
Preculture duration: 7 days

Parveez et al (1998). Ind. Crops Prod. 8:17-27.

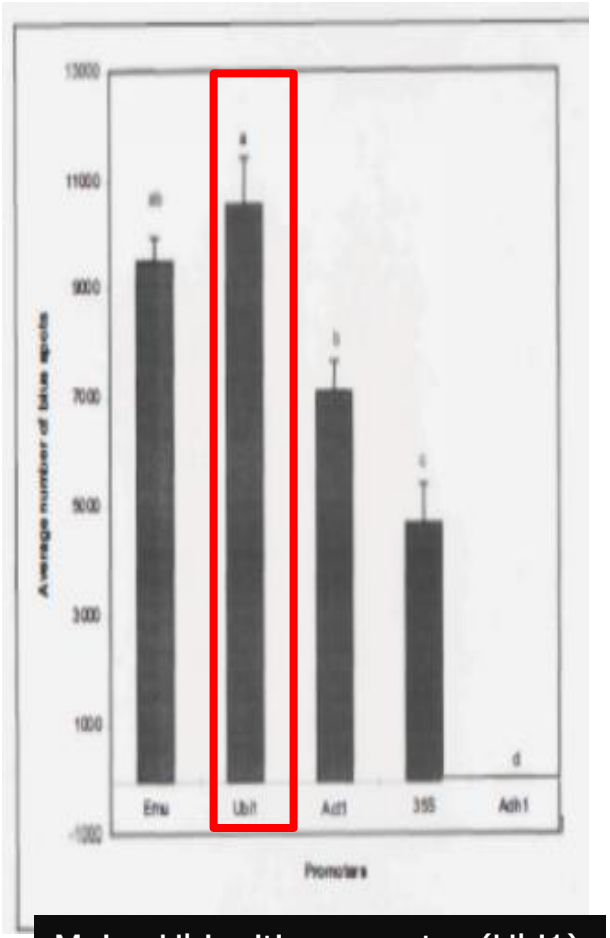
Biolistic Transformation of Oil Palm -Evaluation of Five Promoters



Gus staining: bombarded calli derived from Immature embryo



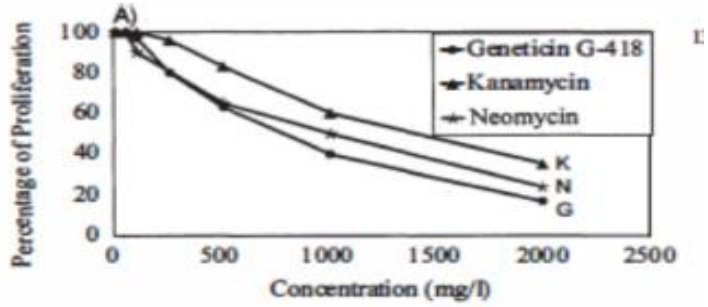
Gus staining: bombarded oil palm tissues

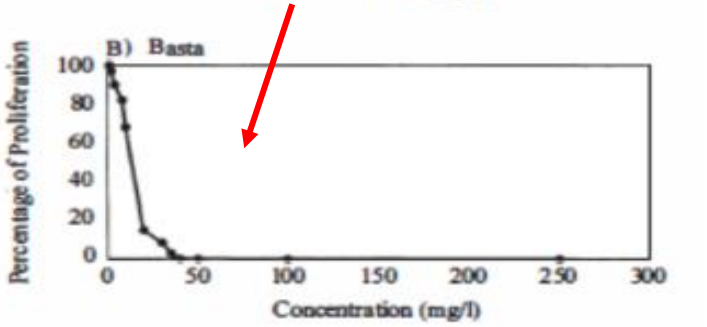


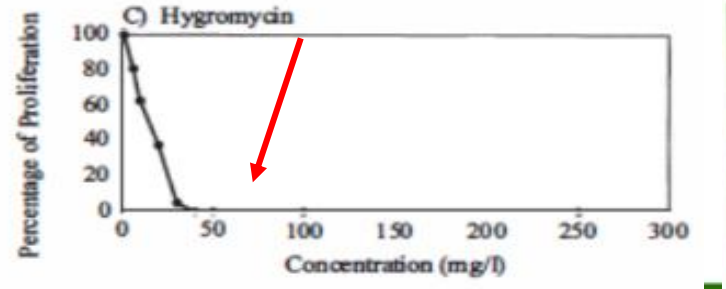
Maize Ubiquitin promoter (Ubi1)

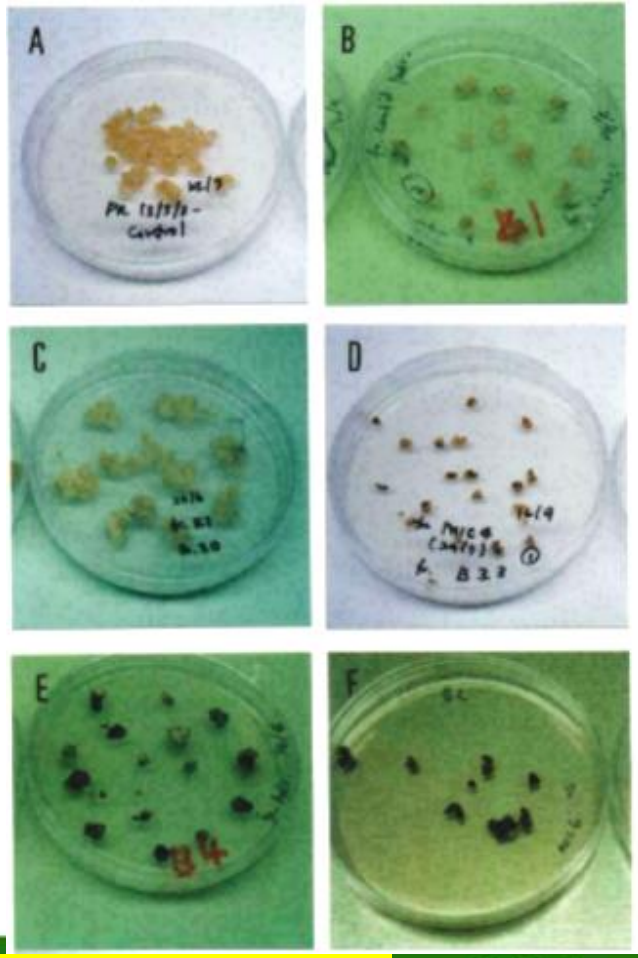
Chowdhury et al (1997). Plant Cell Rep. 16:277-281.

Biolistic Transformation of Oil Palm - Selection Agents For Oil Palm









MIC for compact calli derived from immature embryo:
40 mg/L Basta
40 mg/L Hygromycin

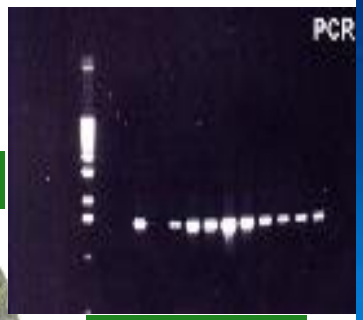
Parveez et al (1996). Asia-Pac. J. Mol. Biol. Biotechnol., 4: 219-228.

Biolistic Transformation of Oil Palm

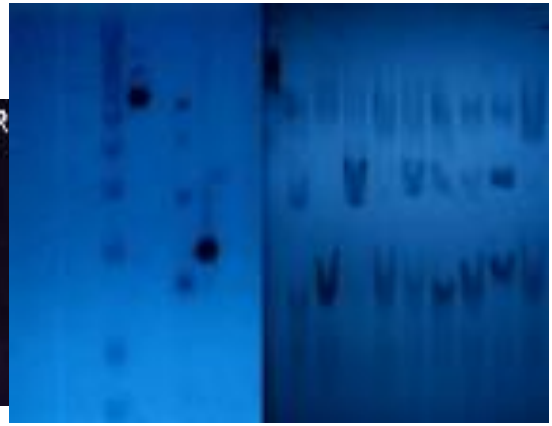
Basta resistant embryoid

Transgenic shoot

Rooting

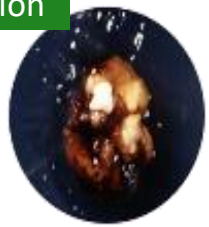


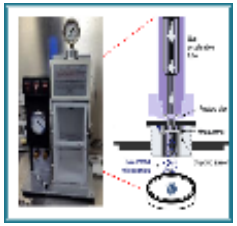
PCR analysis



Southern and protein analyses

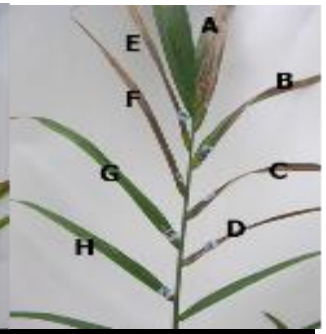
Selection





Biolistic

Mature transgenic oil palm



Control Transgenic

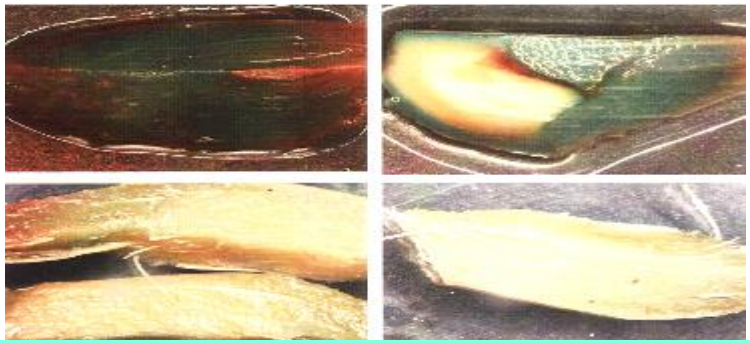
A:1000 ug/ml
B:500 ug/ml,
C:300 ug/ml,
D:250ug/ml,
E:200 ug/ml,
G:100ug/ml ,
H:50 ug/ml.

Parveez and Christou, P (1998). J. Oil Palm Res. 10: 29-38.
Parveez et al (2000). Biochem. Soc. Trans. 28 (6) 969-972.
Parveez, GKA (2000) Kluwer Academic Publishers 2:327-350.

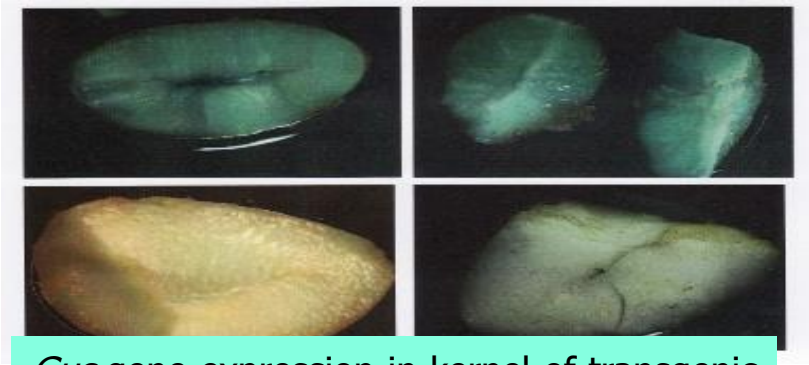
Regeneration of Basta-resistance Transgenic Oil Palm

Transgenic oil palm in biosafety nursery

The transgenic oil palm were proven to be fertile



Gus gene expression in mesocarp of transgenic oil palm (top) and in non-transgenic oil palm / control (bottom)



Gus gene expression in kernel of transgenic oil palm (top) and in non-transgenic oil palm / control (bottom)

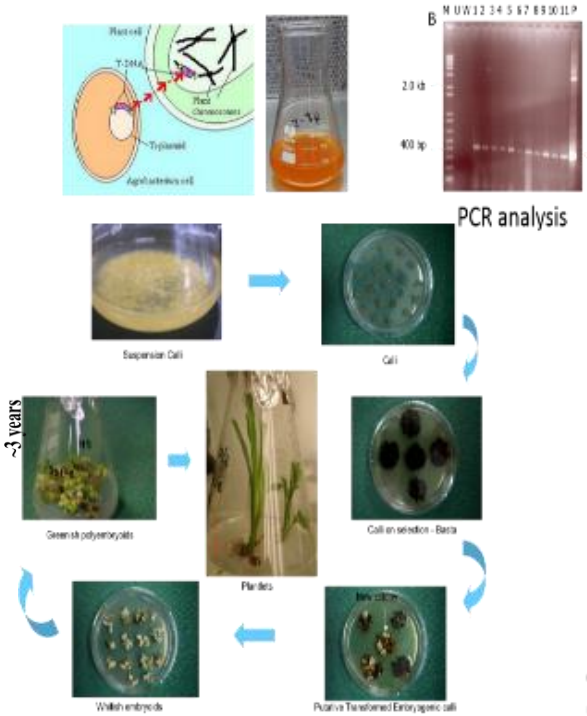
Parveez et al (2000). Biochem. Soc. Trans. 28(6):969-972
Parveez (2003). AgBiotechNet.5(ABN113):1-8

Agrobacterium-mediated Transformation of Oil Palm

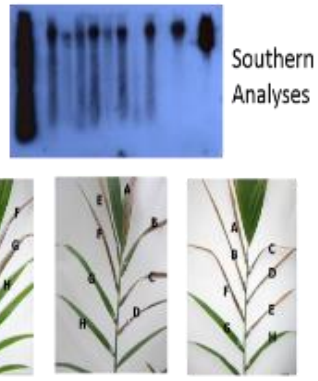
Transformed with pCambia1300-UBA
LBA4044 strain
Basta-resistance transgenic oil palm produced

Stage and procedure	Media	Duration
Starting material: Suspension embryogenic calli (EC)		
Transfer EC onto solid medium, incubate in the dark at 27 °C	EC agar medium	Overnight
Pre-plasmolysis; Transfer EC into agar medium, incubate at 27 °C	PM agar medium +acetosyringone	1 hour
Physical injury; Bombardment of EC	PM agar medium	
Agroinfection of EC; Shake 100 rpm at 28 °C	*Agrobacterium* suspension culture	2 hours
Post-plasmolysis; Transfer EC onto solid medium	PM agar medium	1 day
Co-cultivation of EC; Incubate in dark at 27 °C	CM agar medium +acetosyringone	2 days
Inhibit *Agrobacterium* growth; Transfer EC onto solid medium Incubate in dark at 27 °C	EC agar medium + 400 mg l^{-1} cefotaxime	4 weeks
Selection; Subculture EC onto selection medium monthly, Incubate in 16h light/8h dark at 28 °C	EC agar medium + 200 mg l^{-1} cefotaxime + 50 mg l^{-1} Basta	6-10 months
Development of roots; Subculture shoots onto solid medium in test tube	RIM medium	4-6 months
Transfer plantlets into jiffy peat	Jiffy peat pellet	3-6 months
Transfer plants into polybag	Soil	

Optimal Protocol



Selection and regeneration of Basta® resistant oil palm plants.



Comparison between transgenic plants and untransformed plant after application of Basta solution with different concentrations

Izawati et al (2009). J. Oil Palm Res. 21: 643-652.
Izawati et al (2012) Methods and Protocols Humana Press.177-188.

Optimum Protoplast Isolation From Oil Palm Suspension Culture

Oil palm suspension culture

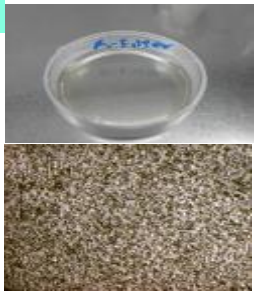
filtration through a 300-µm nylon mesh

Callus on solid medium

incubated in dark at 26ºC for 14 h

Complete digestion is confirmed under microscope

Digestion mixture is filtered through a double layer of miracloth



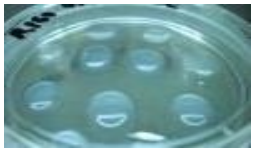
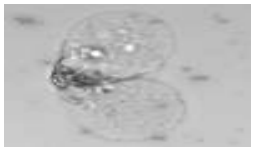
Clean and vital protoplasts are observed under microscope

- **Enz Sol VI**:
2% (v/v) Celluclast, 1% (v/v) Pextinex 3XL, 0.1% (w/v) Pectolyase Y23, 0.5% (w/v) Cellulase onuzuka R10, 3% KCl, 0.5% $CaCl_2.2H_2O$, 3.6% D-Mannitol
- **Washing Solution**:
3% KCl, 0.5% $CaCl_2.2H_2O$, 3.6% D-Mannitol
- **pH solution**: 5.6
- **Incubation time**: 14 hours
- **Incubation temperature**: 26°C
- **Filtration**: 22um (miracloth)
- **Centrifugation**
Speed: 100xg
Temperature: 22°C
Time: 5 minutes

!!! Yield of 1.14 x 10 ^{6} / ml with viability of 82% !!!

Masani et al (2013) Plant Sci. 210:118–127.
Masani et al (2022) Methods Mol Biol. 2022;2464:187-202.

Plant Regeneration of Oil Palm Protoplasts


Agarose beads

9-12 days

1st Cell division

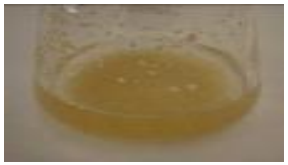
17-21 days

2nd Cell division

3-4 mth


Microcolony

5-6 mth



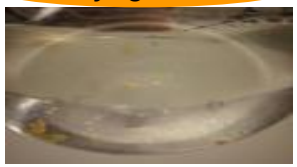

Microcalli

7-9 mth

Macrocalli

10-12 mth

Embryogenic calli



13-15 mth

Embryoids

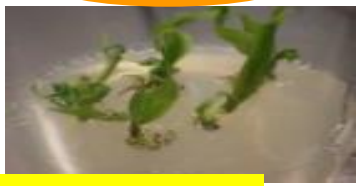
Greenish embryoids

15-18 mth

Shoots

19-23 mth

Plantlets



~2 years

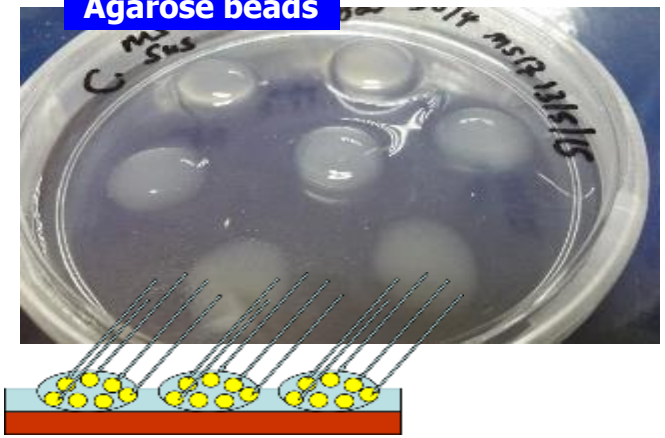
!!!Long regeneration process !!

Masani et al (2013) Plant Sci. 210:118–127.
Masani et al (2022) Methods Mol Biol. 2022;2464:187-202.

DNA microinjection of oil palm protoplasts

Platform and Injection Time

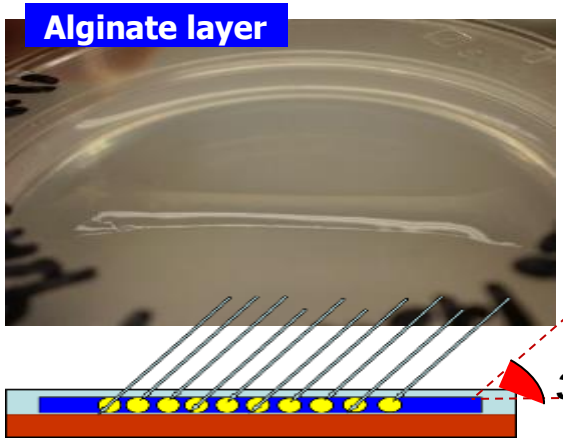
Agarose beads



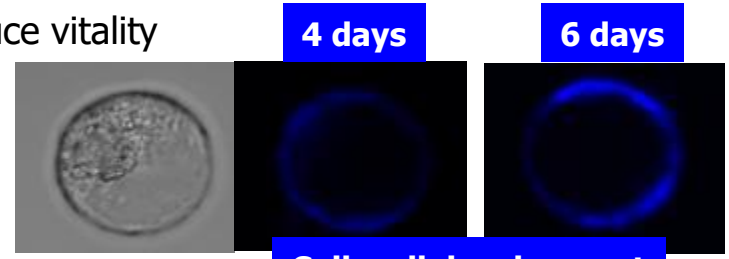
- Curve shape-limit movement of needle
- Light brown-Difficult to identify protoplast
- Agarose particle-clogge the needle tips
- only 5–10 cells/h
- Heat stress (~45°C)-reduce vitality

Platform: Alginate layer
Injection time: 3 days

Alginate layer



- flat shape-facilitate injection
- Transparent-easy to identify protoplast
- No heat stress-maintain vitality
- 50-100 cells/h
- Dissolve in Sodium acetate

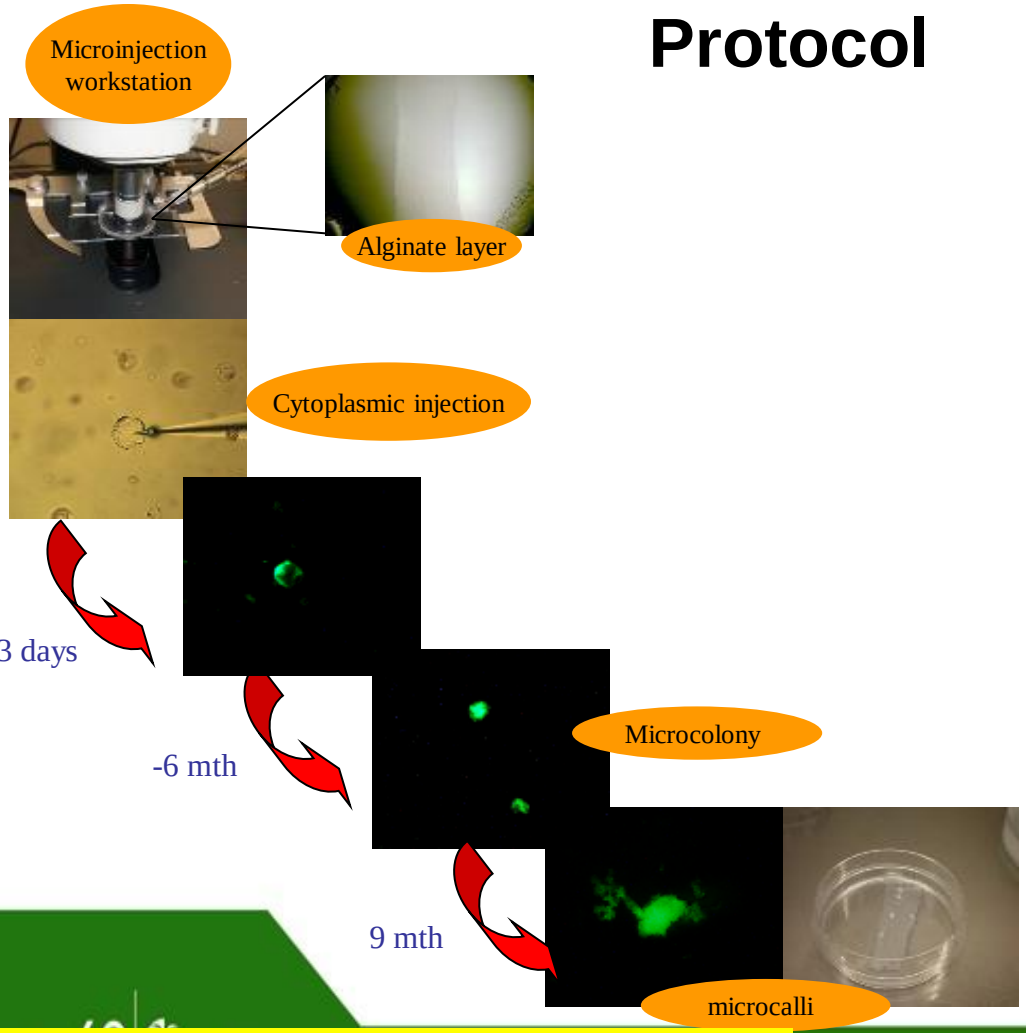


Cell wall development

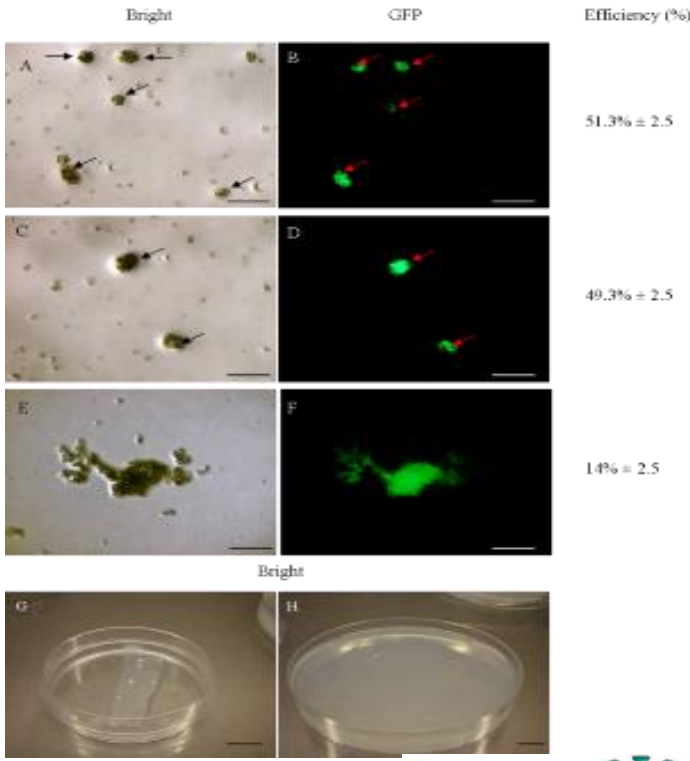
- 3-4 days ideal time for injection

Masani et al (2014) PLoS ONE 9(5): e96831
Masani et al (2022) Methods Mol Biol. 2022;2464:187-202.

DNA Microinjection of Oil Palm Protoplasts: Optimal Protocol

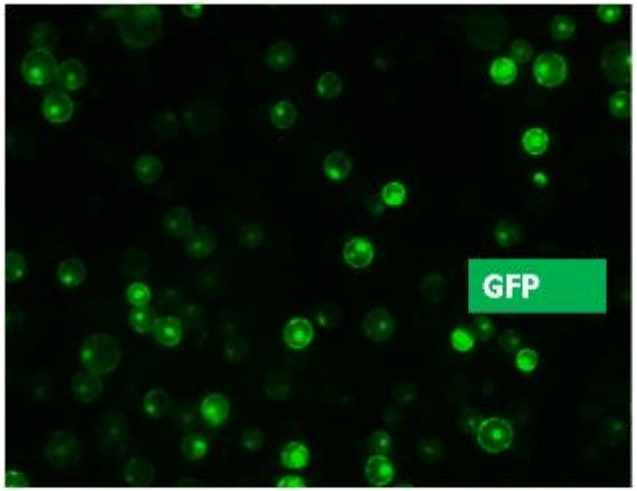


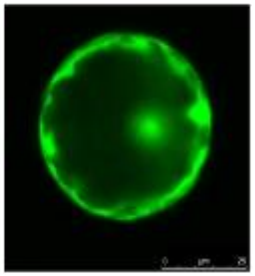
Development of microcalli expressing GFP

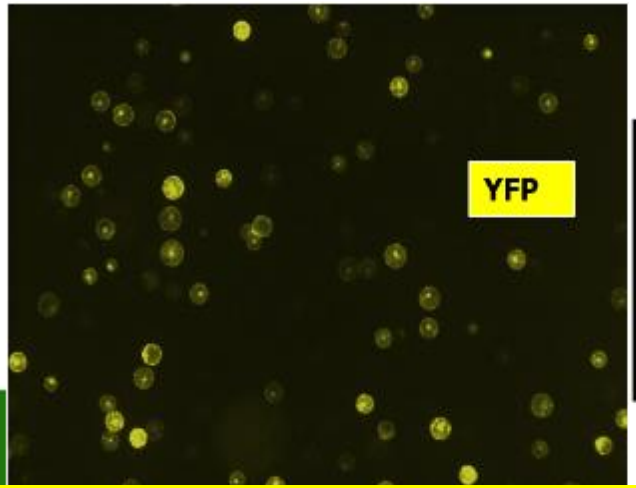


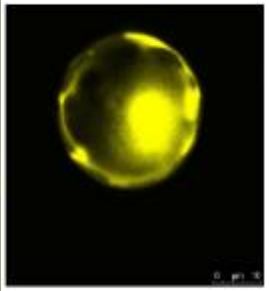
Masani et al (2014) PLoS ONE 9(5): e96831
Masani et al (2022) Methods Mol Biol. 2022;2464:187-202.

PEG-mediated Transfection of Oil Palm Protoplasts

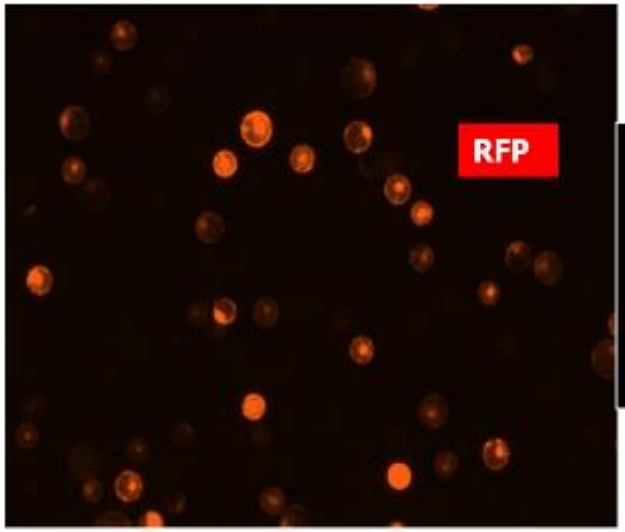


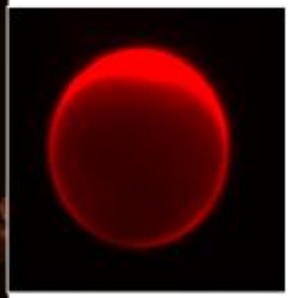






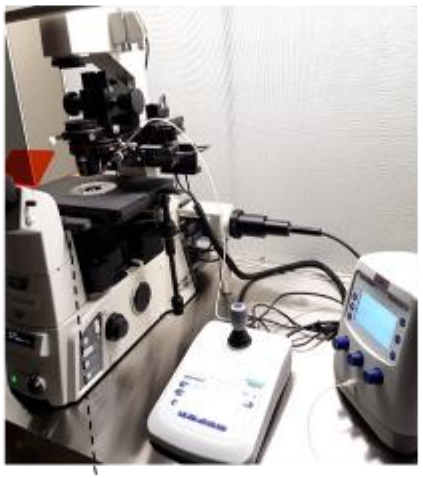
Transfection efficiency
4-20%



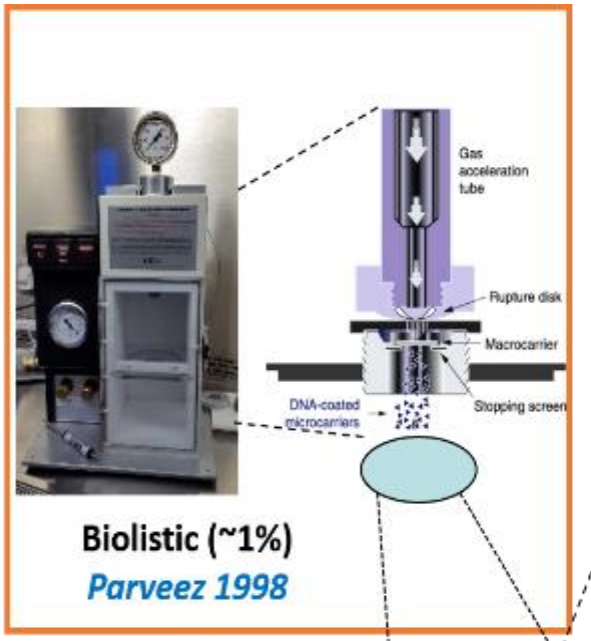


Masani et al (2014) PLoS ONE 9(5): e96831
Masani et al (2022) Methods Mol Biol. 2022;2464:187-202.

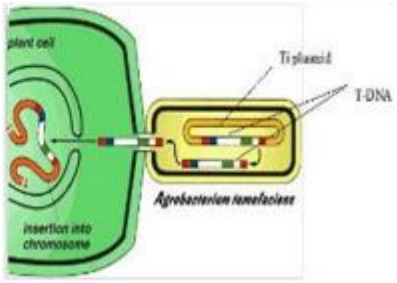
Oil Palm is Recalcitrant to Genetic Manipulation



DNA Microinjection (~14%)
Masani et al., 2014

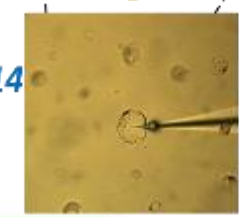


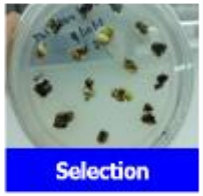
Biolistic (~1%)
Parveez 1998



Agrobacterium (0.7%)
Masli et al, 2009

PEG-mediated transformation (~20%)
Masani et al., 2014





Low transformation efficiency

Parveez et al (2015) Plant Cell Rep. 34:533-543.
Masura et al (2017) J. Oil Palm Res. 29(4):469-486.
Masani, et (2018). Biocatal. Agric. Biotechnol. 15:335-347

Fine Tune: Oil Palm Transformation Methods

Biolistics-mediated transformation- optimization in progress.

***Agrobacterium*-mediated transformation- optimization in progress**

Protoplast isolation from leaf and mesocarp tissues

Fine Tune Biolistic Parameters

- Optimization of some parameters for stable transformation have been determined.
- The study for others parameters are still ongoing.

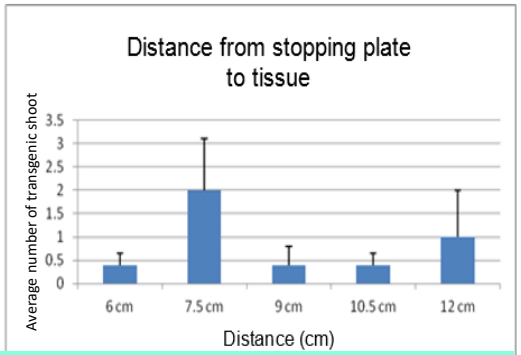
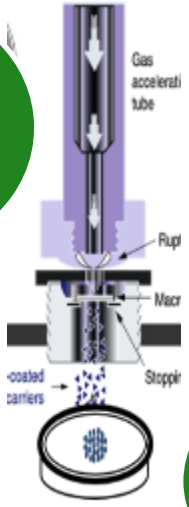


Figure 5. Effects of distance from stopping plate to tissue on transformation and regeneration of oil palm transformant. Data presented is the mean of five replicates.

Distance from stopping screen to tissue
7.5 cm



Helium pressure
1100psi

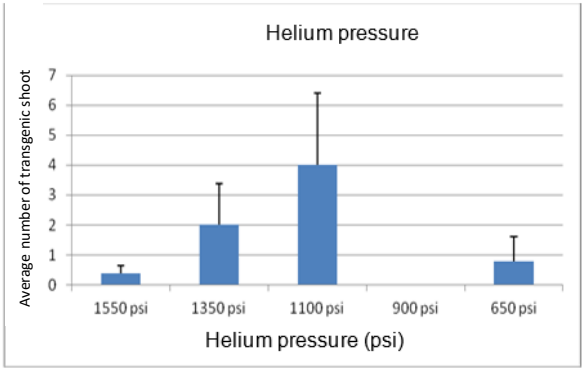


Figure 3. Effects of helium pressure on transformation and regeneration of oil palm transformant. Data presented is the mean of five replicates.

DNA quantity
2 µg

Number of bombardment
1X

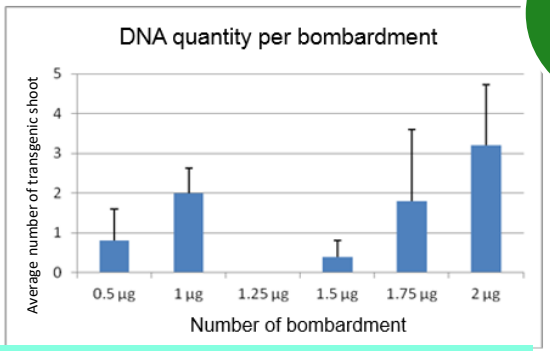
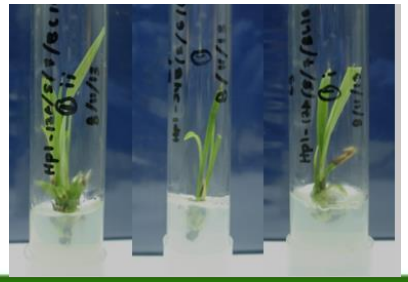


Figure 7. Effects of DNA quantity per bombardment on transformation and regeneration of oil palm transformant. Each value is the means of five replicates.



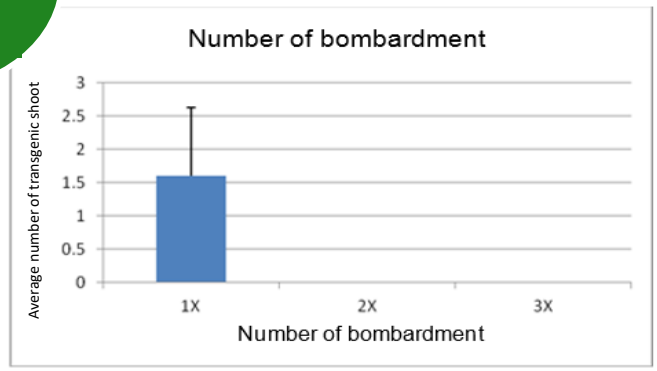
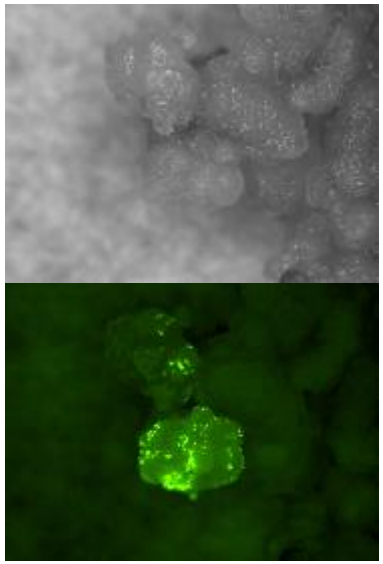


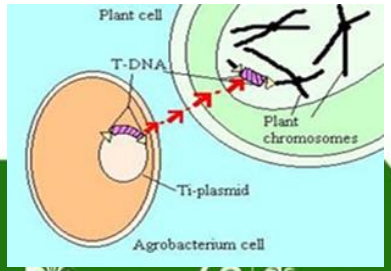
Figure 6. Effects of bombardment number on transformation and regeneration of oil palm transformant. Each value is the means of five replicates.

Nurfahisza et al. (2020) J. Oil Palm Res. 32(4):696-704.

Fine Tune *Agrobacterium* Parameters

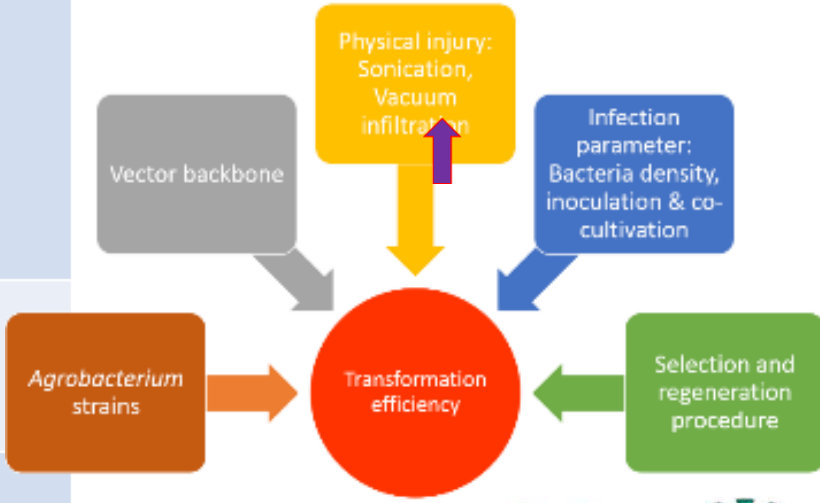
Based on number of GFP spots





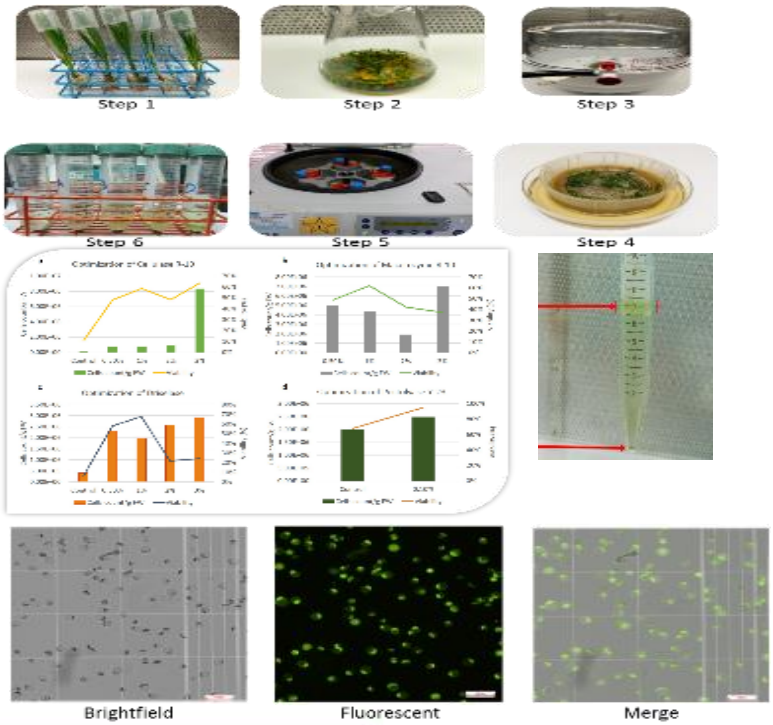
Transformation procedure	Standard protocol	Optimization
Plant materials preparation	8-mth old calli	Age of calli: 5, 6, 7, 8, 9, 10 Phenotype of calli: Yellowish, light-yellowish, brownish, blackish
Calli pretreatment	Bombardment L-1	Physical injury: bombardment, sonication, non-treated Preculture media: MSB, L-1, Y3A
Agrobacterium preparation	EHA105 1.0 0 hour 200 µM	Strain: AGL-1, EHA105, GV3101, LBA4404 Bacterial growth phase: 0.1, 0.3, 0.5, 0.7, 0.9, 1.1 Virulence activation: 0, 6hrs, 12hrs, 24hrs AS concentration: 0, 50, 100, 200, 300 & 400 µM
Agrobacterium infection	0.5 1 hour	Density: 0.1, 0.3, 0.5, 0.7, 0.9, 1.1 Infection time: 15, 30, 45, 60, 120 and 240 hours
Co-cultivation	3 days 28°C	Co-cultivation period: 1, 2, 3, 4, 5, 6, 7 days Co-cultivation temperature: 22°C @ 28°C

Optimal parameters Identified for increasing *Agrobacterium*-transformation efficiency



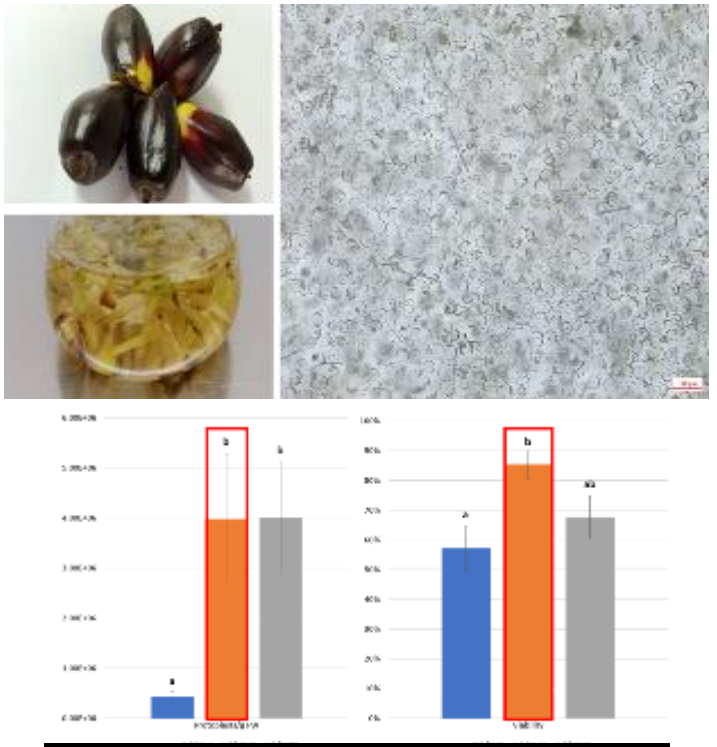
Protoplast Isolation From Oil Palm In Vitro Leaf and Mesocarp

Invitro leaf



2.5 × 106 cells protoplast with more than 90% viability

Mesocarp



3.98 × 106 protoplasts with 85% viability

Fizree et al (2021) Sci. Horti. 290:110522

PRESENTATION OUTLINE

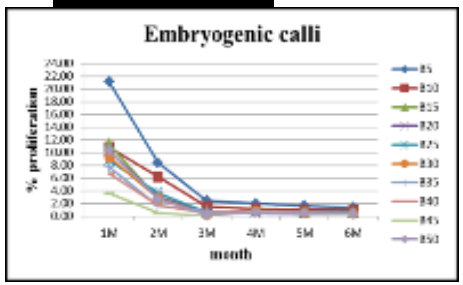
1. Introduction
2. Oil Palm Transformation Methods
3. **Selection agent for transformation**
4. Gene constructs for transformation
5. Transformation and Regeneration Transgenic oil palm
6. Biosafety of transgenic oil palm
7. Conclusion

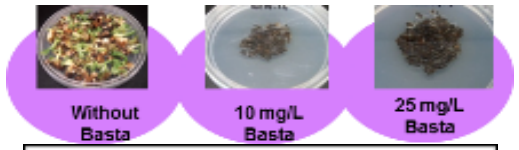
Selection Agents

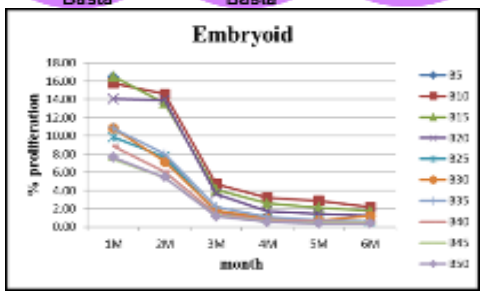
- ✓ Basta / Biolaphos / Glufosinate ammonium
- ✓ Hygromycin
- ✓ 2-Deoxyglucose (2-DOG)
- ✓ Mannose (PMI)
- ✓ Green Fluorescence Protein (GFP)
- ✓ Red Fluorescence Protein (RFP)

Evaluation On The Effectiveness of Bar Gene-based Selection Agents For Oil Palm Transformation

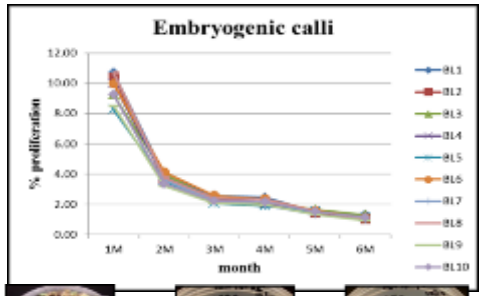
BASTA

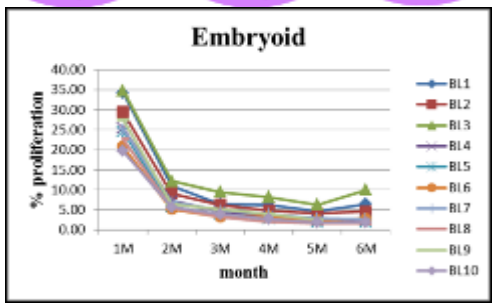




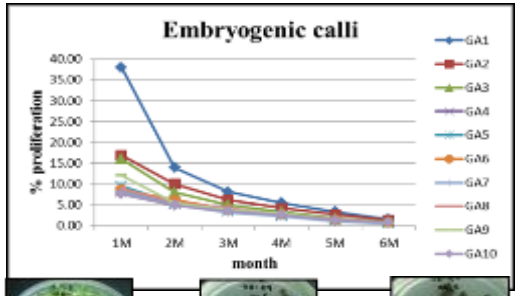


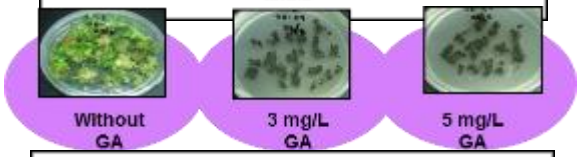
BIALAPHOS

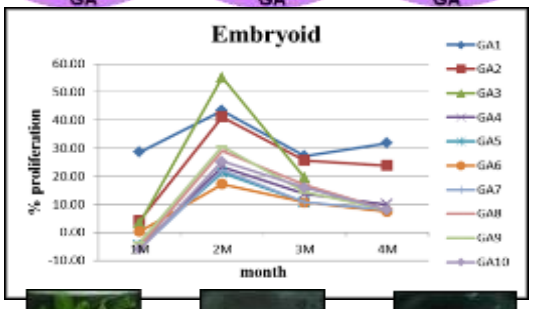




GLUFOSINATE AMMONIUM (GA)



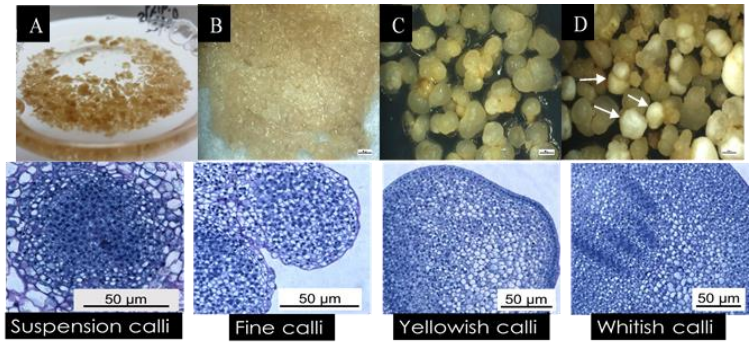




10 mg/l of Basta, 3 mg/l of bialaphos and GA for selection of transformed calli.
20mg/l Basta, 5mg/l of Biolaphos and GA for selection of embryoid.

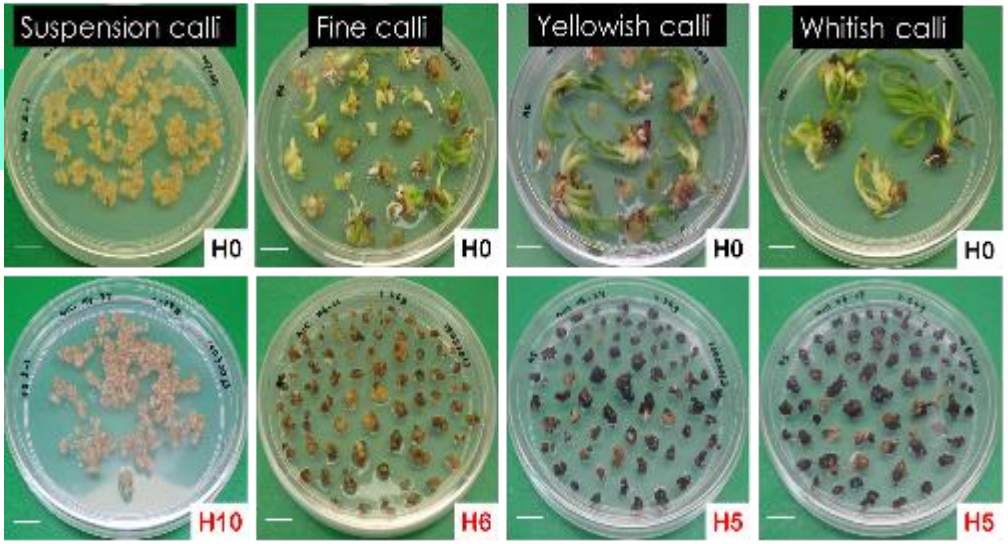
Nurfahisza et al. (2016) J. Oil Palm Res. 28(3):247–255

Minimal Inhibitory Concentration of Hygromycin For Selecting Transformed Oil Palm Embryogenic Calli



Four types of oil palm calli used in this study. A: suspension calli; B: fine calli; C: yellowish calli; D: whitish calli (arrow)

- Fine, yellowish and whitish calli- 5-6 mg/L Hygromycin
- Suspension calli-10 mg/L hygromycin



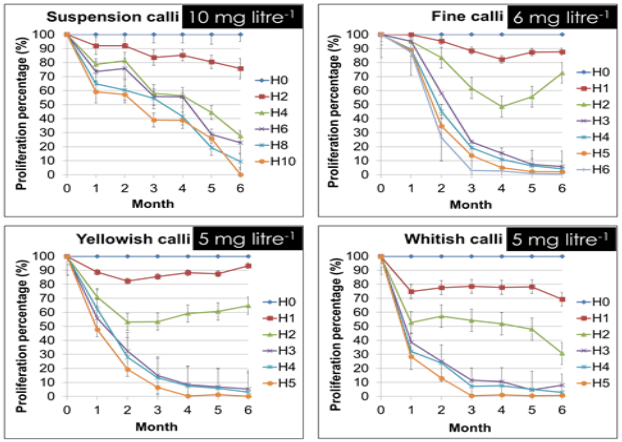
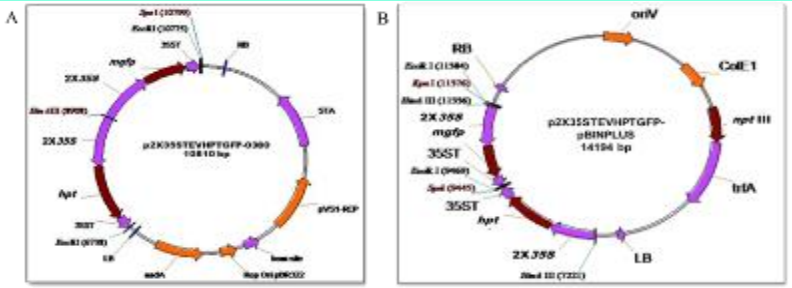


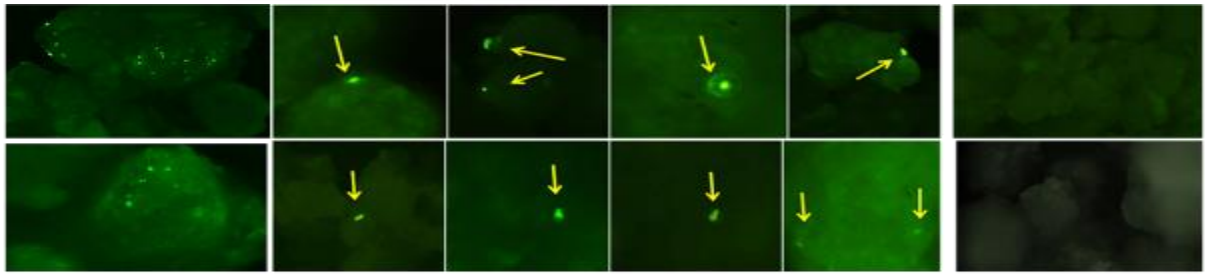
Figure 1. Proliferation percentage of embryogenic calli after being cultured for six months on EC media containing different concentrations of hygromycin

- To effectively select and regenerate the transformed oil palm embryogenic calli, the concentration of selection agent used during the selection should depend on the types of embryogenic calli used

NorFakhrana et al (2019). J. Oil Palm Res. 31(1):14-27

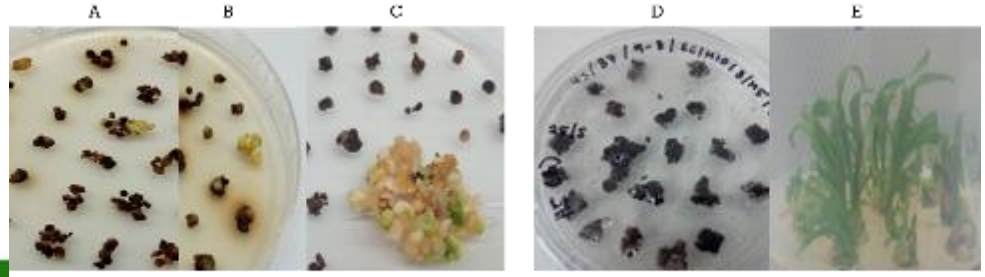
Genetic Transformation of Oil Palm Based On Selection With Hygromycin



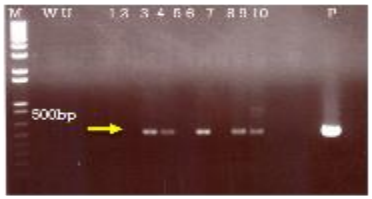


24 hours after bombardment | 2 weeks after bombardment | Non bombarded EC as Control

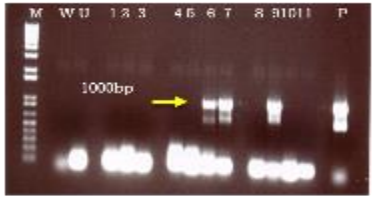
- Friable calli were bombarded with hpt and gfp genes
- Selection on 10mg/L Hygromycin for calli, then on 5 mg/L for embryoid
- Transgenes confirmed by GFP and PCR



A, B: Bombarded calli at eight month in 10 mg/l hygromycin selection; C: at three month in 5 mg/l hygromycin after nine months in 10 mg/l hygromycin selection; D: non-bombarded calli in selection medium and E: non-bombarded calli on EC medium without selection.



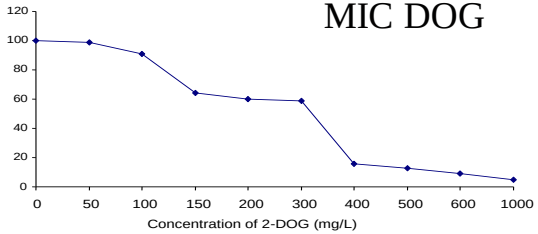
PCR analysis using Hygecre primers (~500bp). Five out of ten samples obtained band, from sample number 3, 4, 6, 8 and 9.

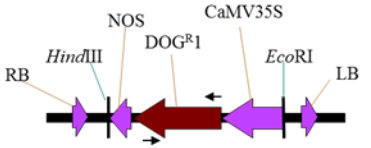


PCR analysis using Hpt1 primers (~1000bp). Bands obtained from sample number 6, 7 and 9.

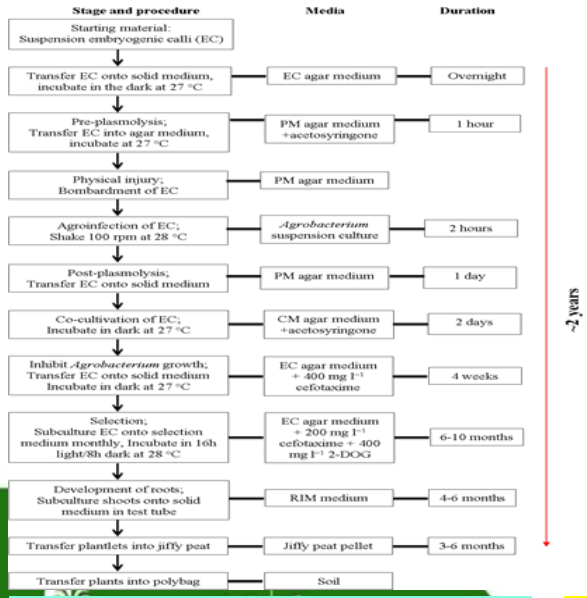
Bahariah et al (2017). J. Oil Palm Res. 29:180-188
Bahariah, et al (2021). J. Oil Palm Res. 33(4):577-587

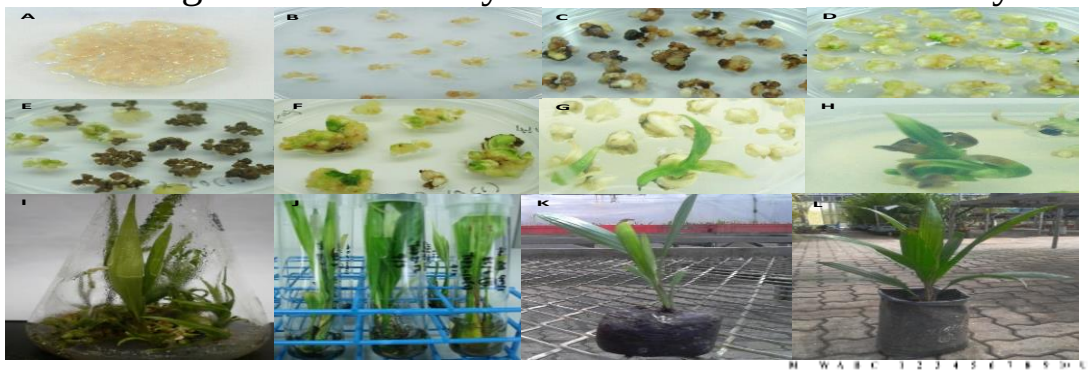
2-Deoxyglucose-6-phosphate phosphatase (DOGR1) Gene As a Selectable Marker for Oil Palm

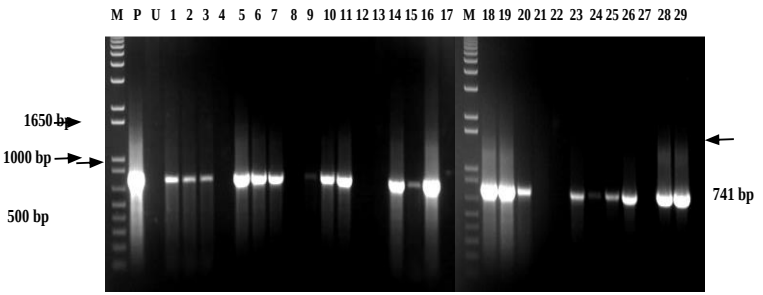


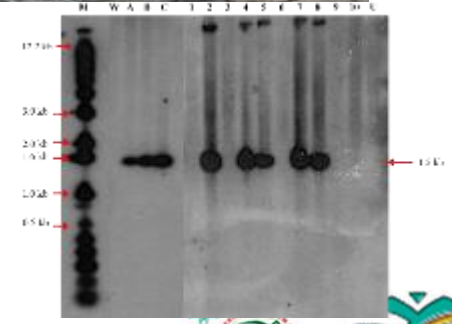


- *Agrobacterium*-mediated transformation-pBIDOG/LBA4404
- Selection on 400mg/L DOG
- Transgenic plants produced
- Transgene confirmed by PCR and Southern blot analyses





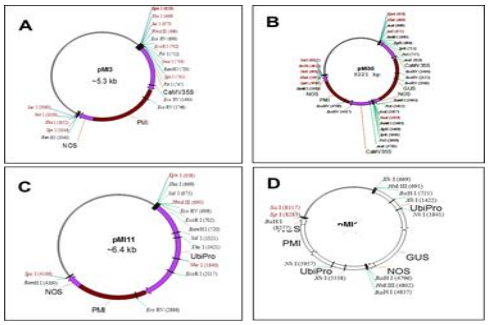




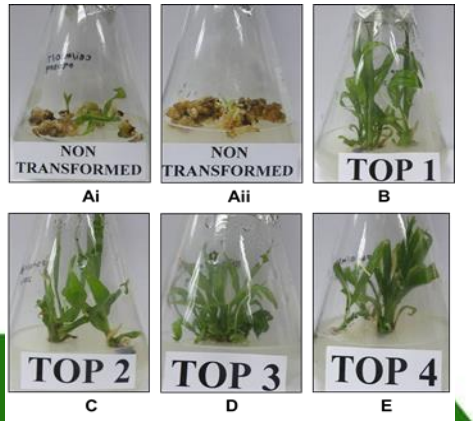
Agrobacterium-mediated transformation protocol

Izawati et al (2012) J. Oil Palm Res. 24:1296-1302
Izawati et al (2015). Front. Plant Sci. 6:727.

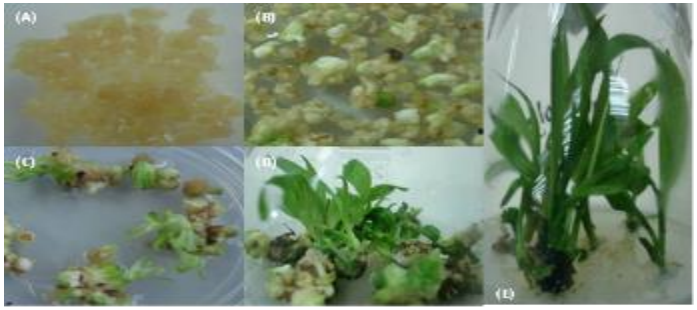
Phosphomannose Isomerase, A Novel Selectable Marker For Oil Palm Transformation



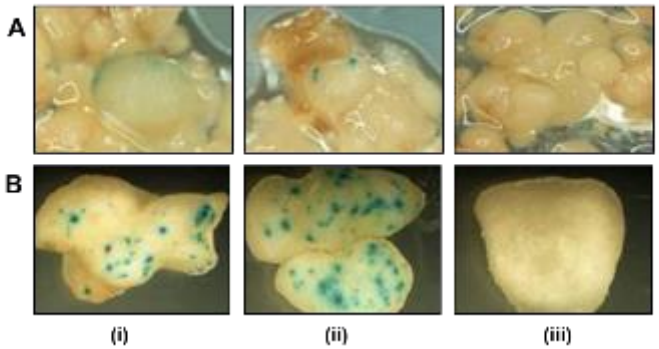
Biolistic Transformation of Oil Palm with PMI Transformation Vectors



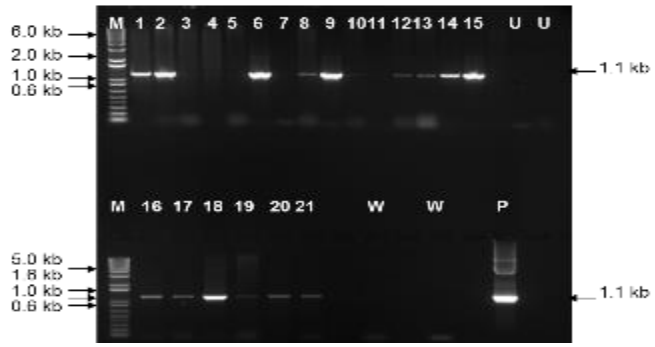
Comparison of transgenic oil palm plantlets carrying *pmi* gene on MS media containing 30 g/l mannose. Ai, Aii) Non-bombarded EC, EC bombarded with different construct B) pMI3, C) pMI3G, D) pMI11 and E) pMI11G vector.

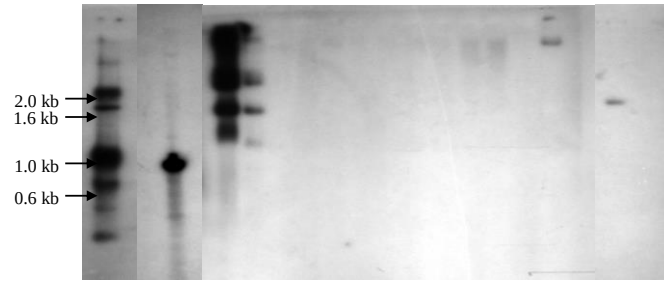


GUS HISTOCHEMICAL ASSAY



PCR ANALYSIS

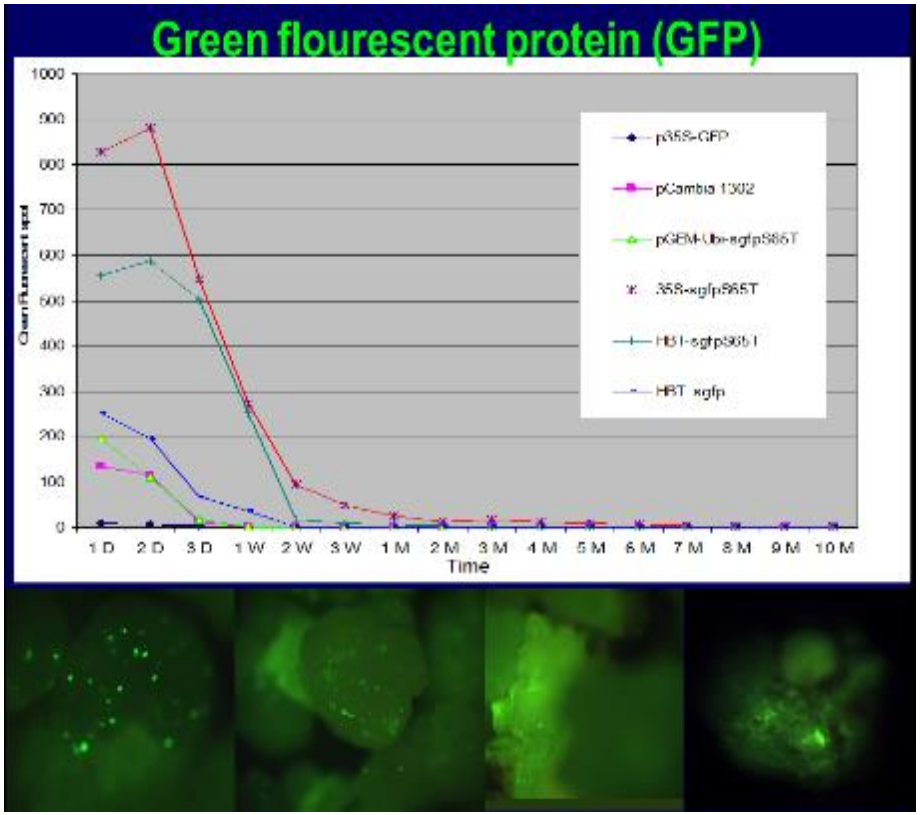




- Eliminated the problem of using negative selection systems, such as herbicides or antibiotic resistance genes
- 3% Mannose was used as selection agent
- Mannose selection system can be used in oil palm transformation

Bahariah et al (2012). Bioinformation 8(3)151-157.
Bahariah et al (2012) J. Oil Palm Res. 24:1250-1259
Bahariah et al (2013). Biocatal. Agric. Biotechnol., 2:295-304.

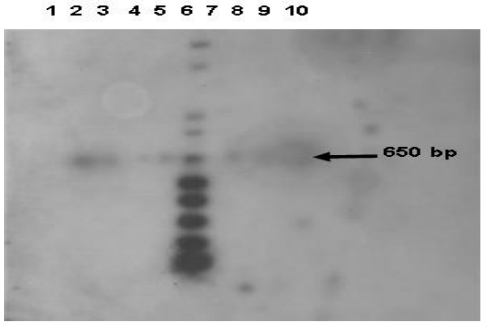
Green Fluorescent Protein As A Visual Selection Marker For Oil Palm Transformation



Stable expression of the GFP gene could be monitored up to 8 months after bombardment

Majid & Parveez (2007) Asia Pac. J Mol Bio Biotech. 15(1):1-8.
Parveez & Majid (2008) J Oil Palm Res. 20: 495-507.

Regenerated plantlets - regardless of GFP gene, GFP expression observed only in parts such as root and leaf
-Stable integration, chimeric

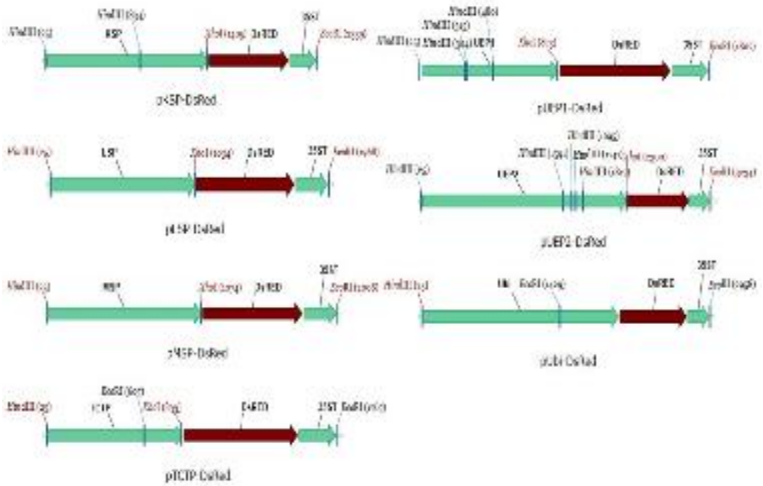


Southern blot analysis of putative GFP transgenic oil palm plantlets

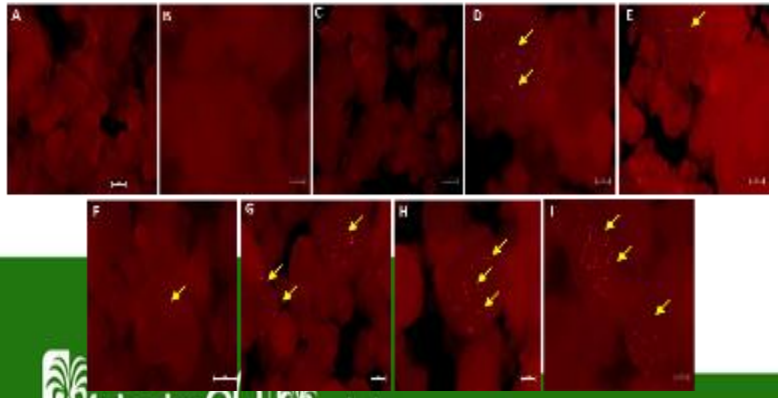
Failure to visualize GFP is due to the inability of the *gfp* gene to express in the regenerated whole transgenic plants

Parveez & Majid (2018) Ind. Crops Prod. 115: 134–145

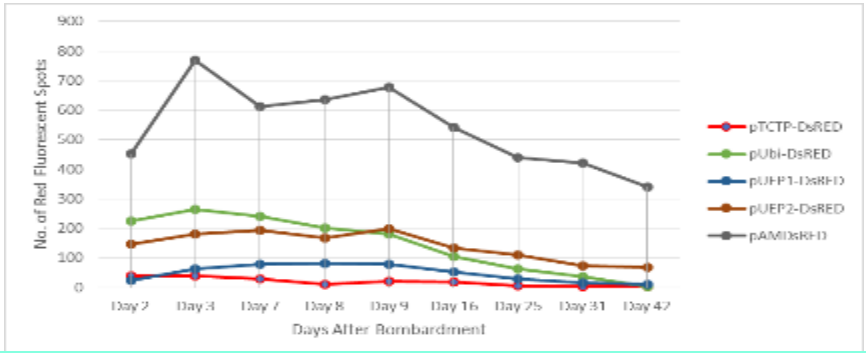
Evaluation of transient DsRED gene expression in oil palm embryogenic calli



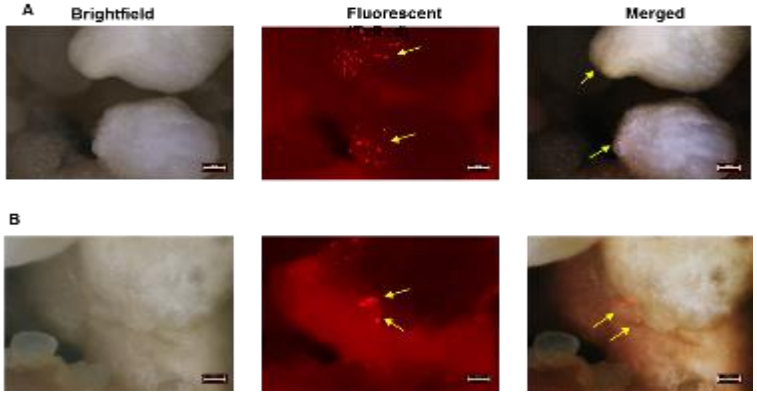
Seven DsRED constructs



Calli bombarded with DsRED constructs



The CaMV35S: most efficient promoter for driving the expression of DsRED gene in calli



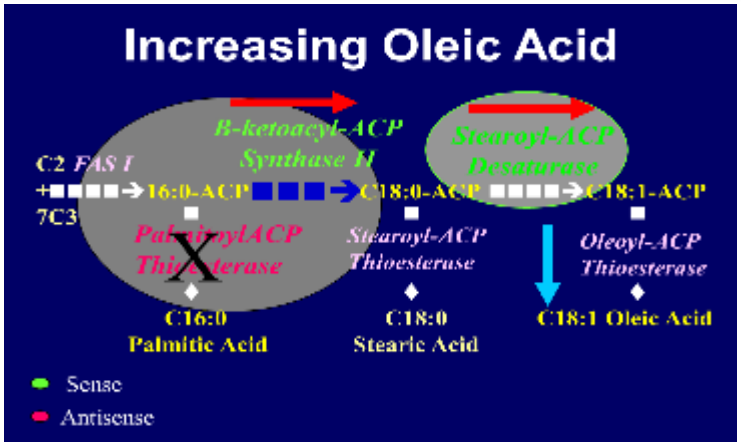
DsRED produced distinct bright signals in comparison with the pale background in non-transformed samples and showed improved signals retention

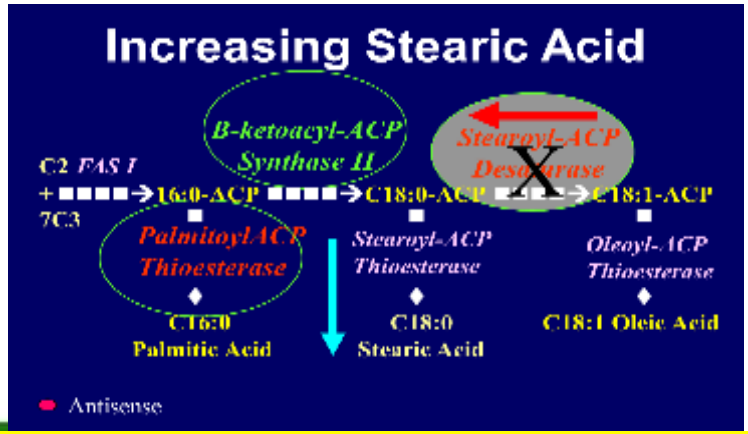
Fizree et al (2019) Sci. Horti. 257: 108679

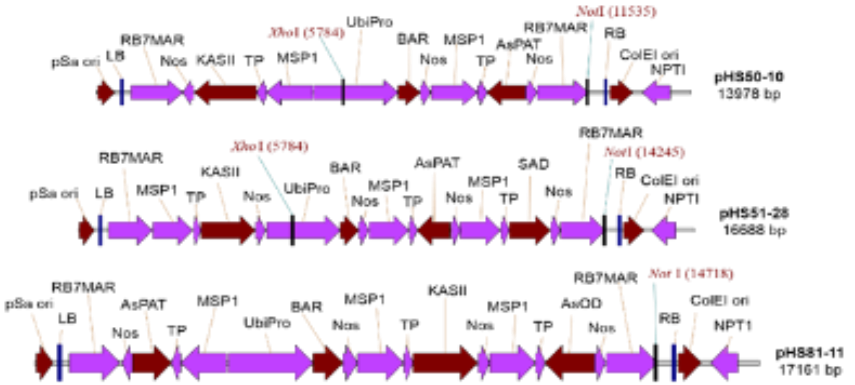
PRESENTATION OUTLINE

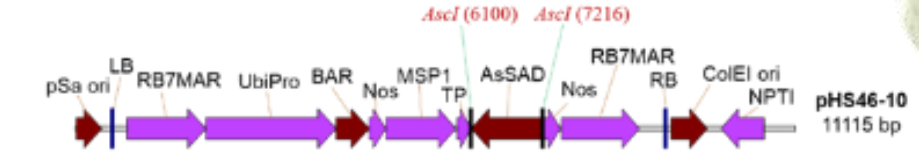
1. Introduction
2. Oil Palm Transformation Methods
3. Selection agent for transformation
4. **Gene constructs for transformation**
5. Transformation and Regeneration Transgenic oil palm
6. Biosafety of transgenic oil palm
7. Conclusion

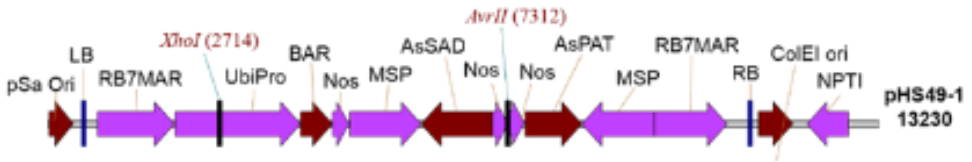
Gene Construct For Transformation





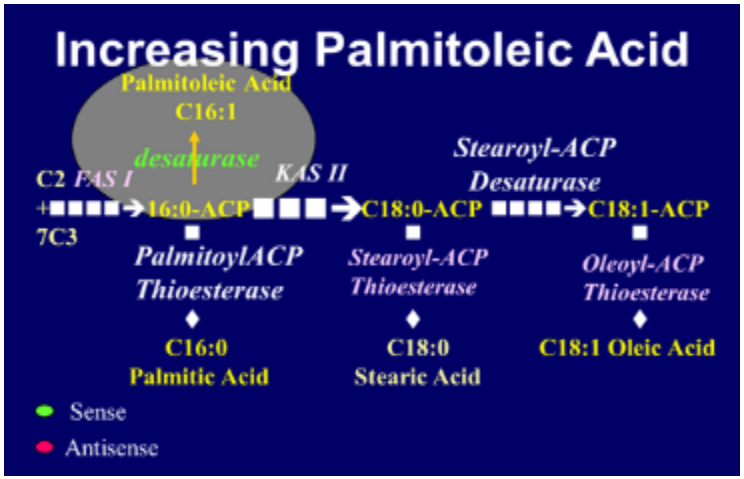


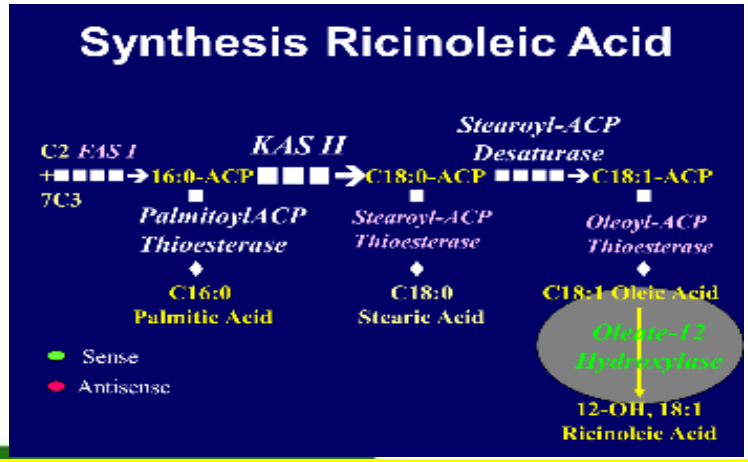


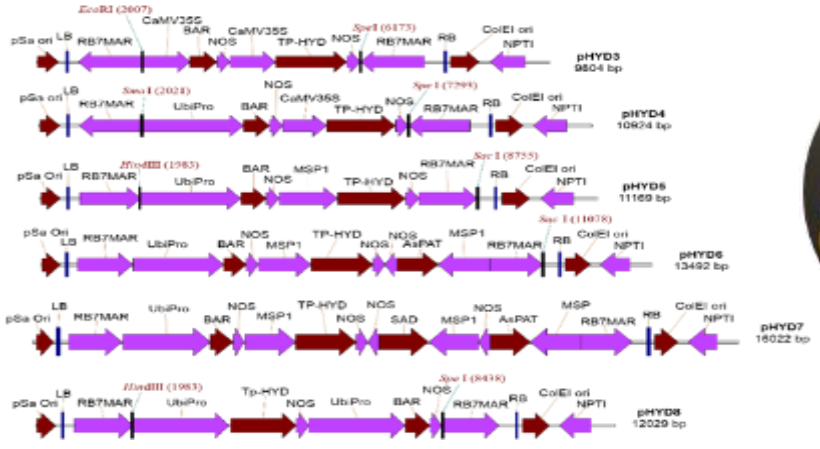


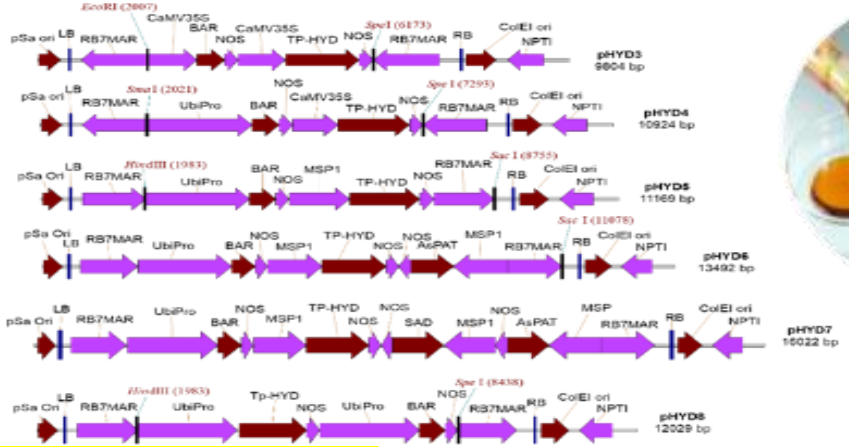
Masani, et (2018). Biocatal. Agric. Biotechnol. 15: 335-347
Masani et al. (2009) Plasmid 62(3):191-200.
Masani and Parveez (2008) Electron. J. Biotechnol. Vol.11, No.3
Yunus et al (2008) J. Oil Palm Res. (2):37-55.

Gene Construct For Transformation



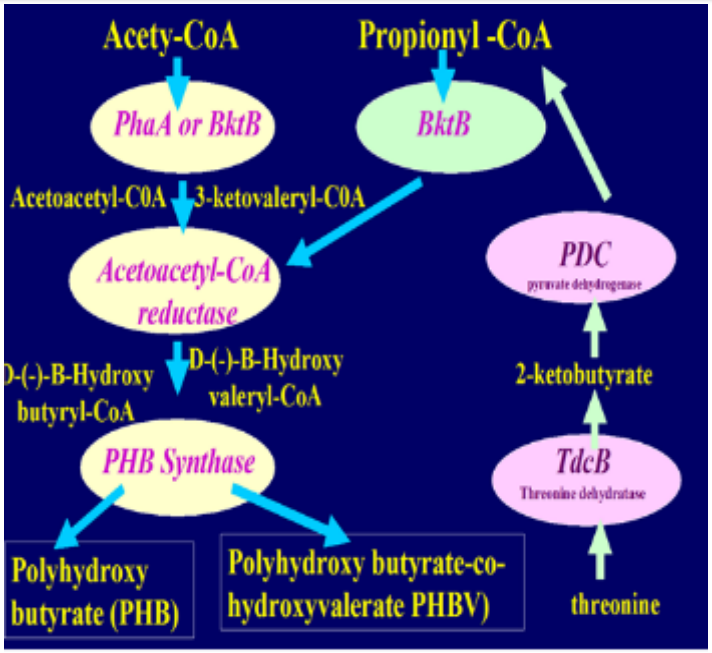




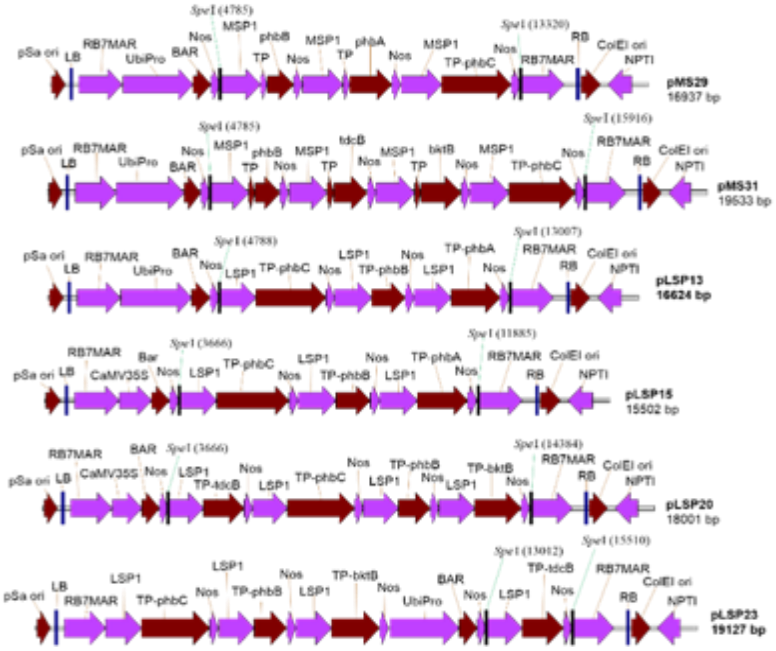


Masani, et (2018). Biocatal. Agric. Biotechnol. 15: 335-347
Masani et al. (2009) Plasmid 62(3):191-200.
Masani and Parveez (2008) Electron. J. Biotechnol. Vol.11, No.3
Yunus et al (2008) J. Oil Palm Res. (2):37-55.

Gene Construct For Transformation



Pathway for the biosynthesis of PHB and PHBV



Gene constructs for targeted traits: completed
Constitutive, tissue specific promoters

Masani, et (2018). Biocatal. Agric. Biotechnol. 15: 335-347
Masani et al. (2009) Plasmid 62(3):191-200.
Masani and Parveez (2008) Electron. J. Biotechnol. Vol.11, No.3
Yunus et al (2008) J. Oil Palm Res. (2):37-55.

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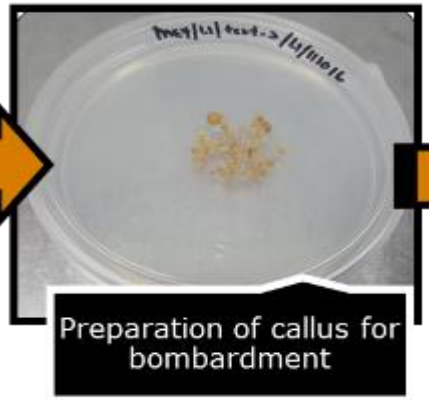
Preparation of gold-DNA mixture


Preparation of callus for bombardment

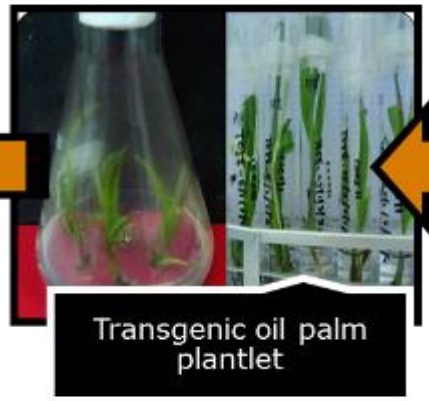

Bombardment

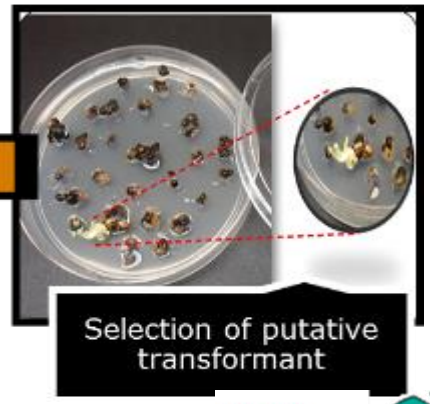

Subculture of bombarded callus on selection media

OIL PALM GENETIC TRANSFORMATION VIA PARTICLE BOMBARDMENT


Transgenic oil palm in screenhouse at MPOB Keratong


Transgenic oil palm in screenhouse at MPOB HQ

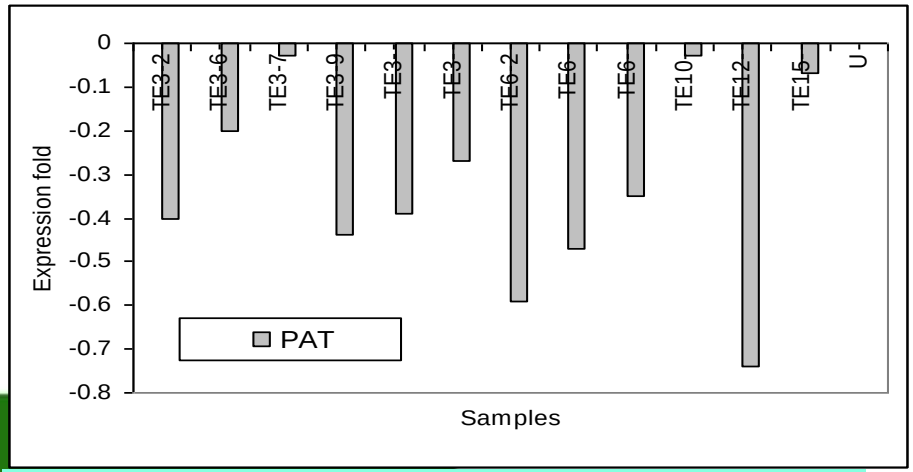

Transgenic oil palm plantlet

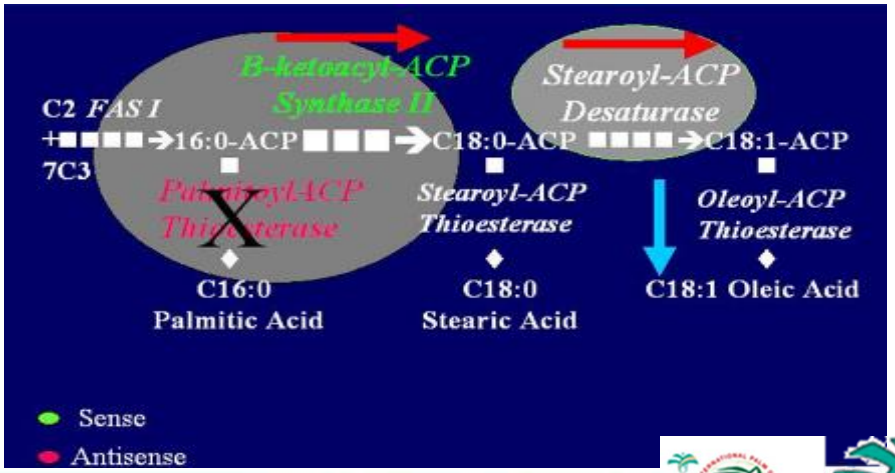

Selection of putative transformant

Parveez GKA (2008) Advanced Protocols for Oxidative Stress 1, Humana Press, 301-320
Parveez and Bahariah (2012) Methods and Protocols, Humana Press.163-175

High Oleic Transgenic Oil Palm

	Composition (%) of Fatty Acid IN Oil Palm Samples (Polyembryogenic)							
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	
	Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic	Total
Control	0.78	62.00	2.05	5.57	24.88	4.07	0.65	100.00
10 samples								
TE3-2	0.97	28.59	0.18	9.31	27.67	25.28	8.00	100.00
TE3-3	0.37	29.41	0.36	10.38	25.36	25.84	8.28	100.00
TE3-7	0.48	30.17	0.22	8.53	29.46	24.34	6.80	100.00
TE3-9	0.75	33.34	0.00	13.69	22.77	23.09	6.36	100.00
TE3-16	0.31	31.05	0.35	9.24	22.05	29.05	7.95	100.00
TE3-18	0.75	34.59	0.18	7.51	18.19	31.52	7.26	100.00
TE4-6	0.80	38.90	0.00	19.75	16.93	19.91	3.71	100.00
TE4-8	0.61	29.85	0.00	14.71	30.03	21.55	3.25	100.00





RT-PCR for palmitoyl ACP thioesterase gene

Parveez et al (2015) Plant Cell Rep. 34:533-543.

Transgenic Oil Palm Synthesizing PHB

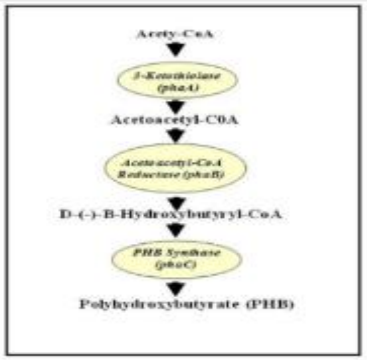


FIGURE 1 | Biosynthesis pathway of polyhydroxybutyrate (PHB) from acetyl-CoA. Adapted from Purkiss (2002).

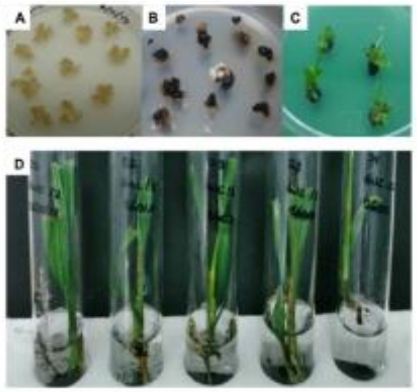


FIGURE 3 | Regeneration process of oil palm plantlets. (A) Embryogenic calli before bombardment; (B) Transformant calli surviving on selection media; (C) Shoot development on selection media; and (D) Transformed plantlets with roots.

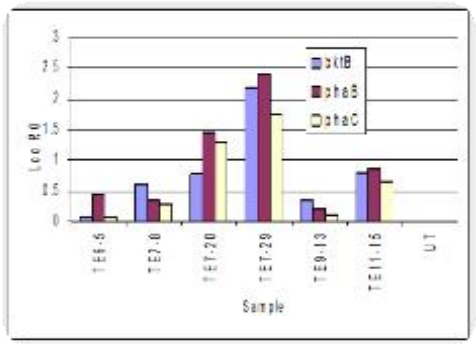
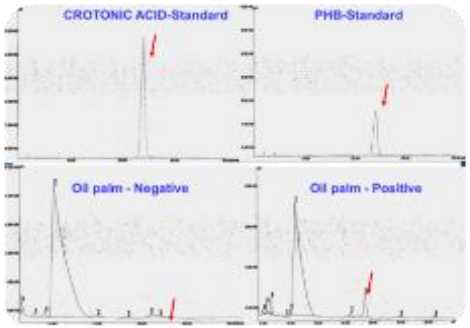


FIGURE 6 | Expression of *bktB*, *phaB*, and *phaC* genes in transgenic oil palm determined by real-time PCR analysis. UT represents the untransformed oil palm (calibrator).



Detection of PHB in the form of crotonic acid by HPLC.

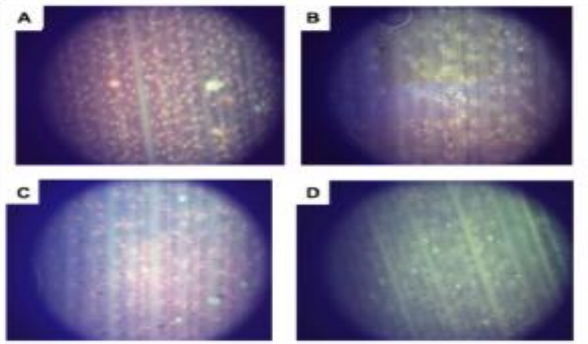
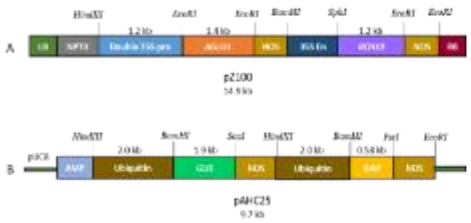


FIGURE 9 | Accumulation of PHB granules in leaf surface of transformants number TE7-27 (A), TE7-28 (B), and TE7-29 (C) stained with Nile blue A. No PHB granule was observed for untransformed oil palm leaf (D).

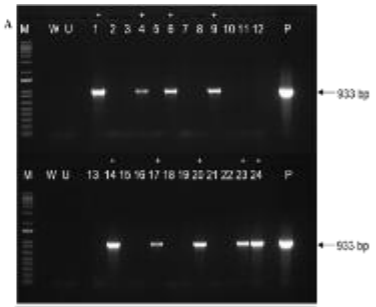
Mature transgenic palms (PHB)

Parveez et al. (2007). Conference on Plantation Commodities. Pp 93-102.
Parveez et al., 2008; J Oil Palm Res 2: 76-86.
Parveez et al. (2015). Front. Plant Sci. 6:598.

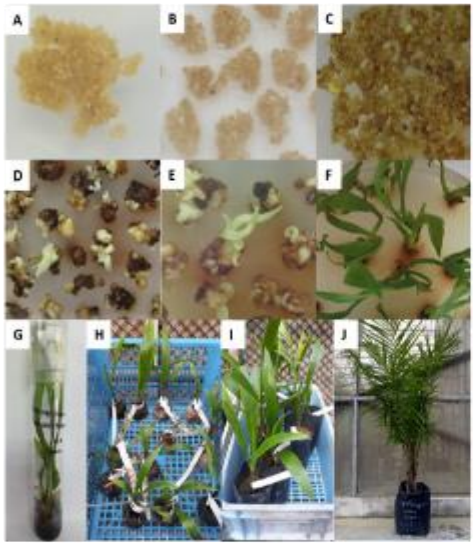
Ganoderma Resistant Transgenic Oil Palm



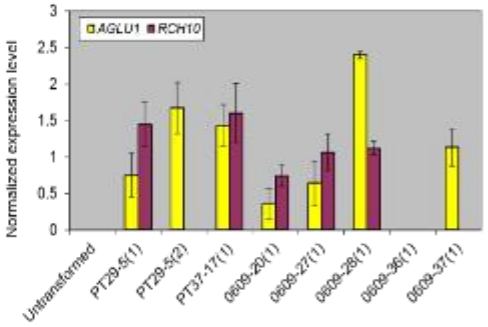
Schematic diagram of pZ100 (A) and pAHC25 (B) plasmids.



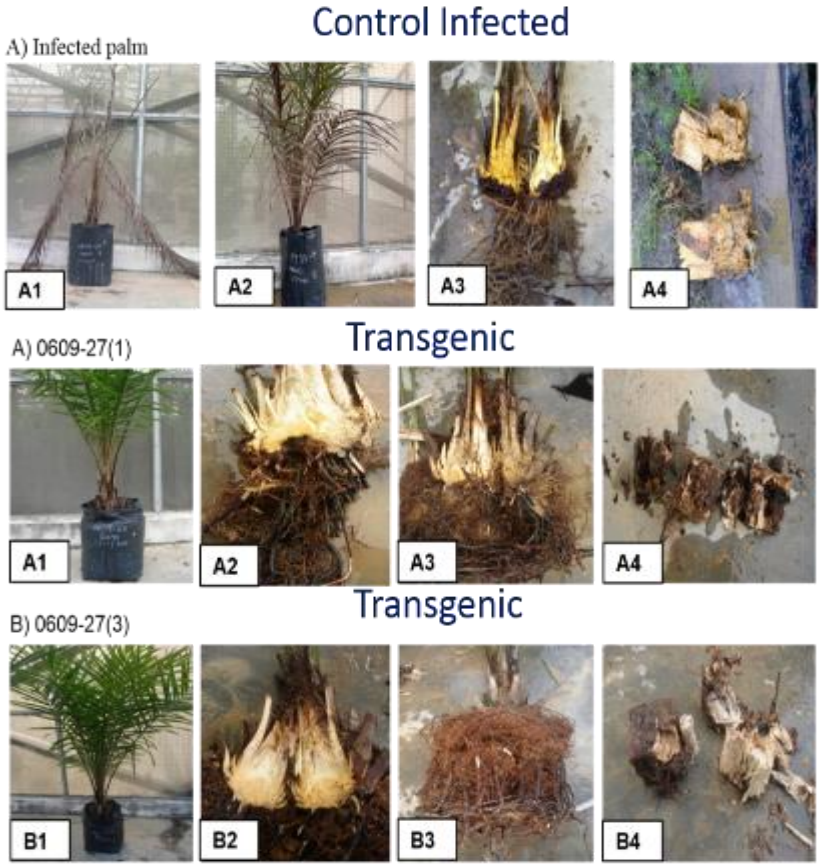
PCR amplification of AGLU1 (A) and RCH10 (B) genes



Selection and regeneration of Basta® resistant oil palm plants.



Relative expression level of AGLU1 and RCH10 genes in transgenic oil palms



Comparison of infected palm from Ganoderma-challenged bioassay

Hanin et al.(2020) Ind. Crops and Prod., 144.

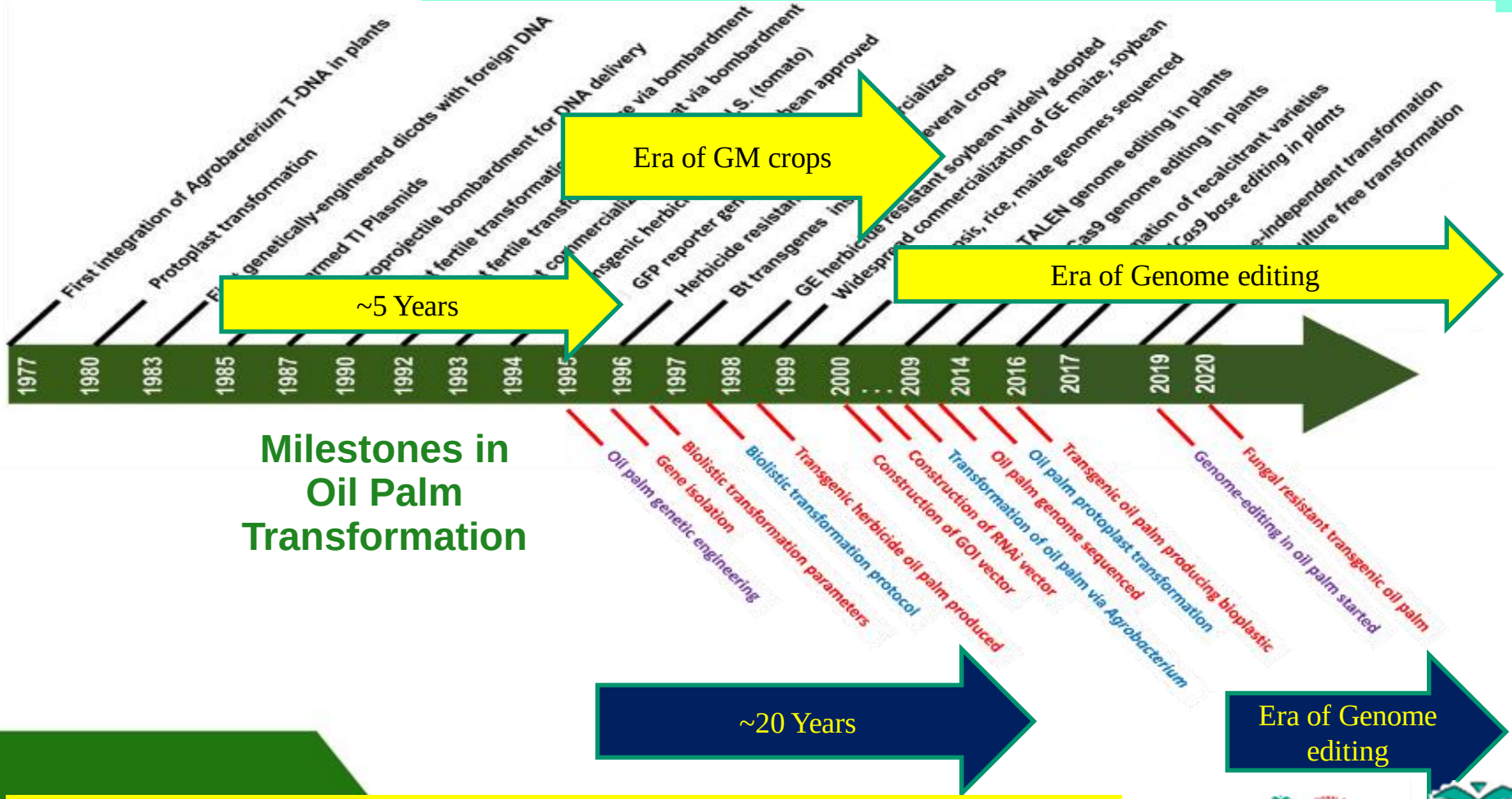
Status of Transgenic Oil Palm

	Constitutive	Tissue-specific
Oleic	√	√
Bioplastics	√	√
Stearic	√	√
Ricinoleic	√	√
Palmitoleic	√	√
Fungal	√	
Insect	√	
Lycopene		√
Ganoderma	√	

Parveez et al (2012) American Oil Chemists Society Press. 87-135.
Parveez et al (2011) Further Advances Oil Palm Research (2000-2010) pp.141-201.
Parveez et al (2010) In Biocatalysis and Molecular Engineering John Wiley & Sons. 67-81
Parveez et al (2015) Plant Cell Rep. 34:533-543.
Masani et al (2018) Biocatal. Agric. Biotechnol., 15: 335-347.

Milestones in Annual Crops Transformation

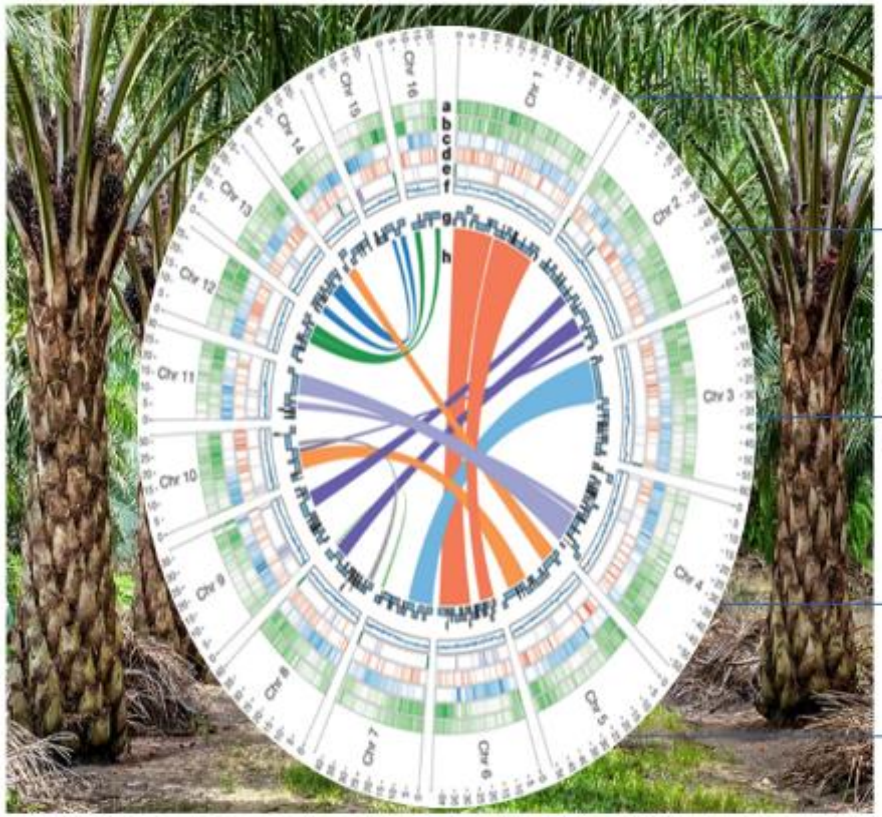
Milestones in Plant Transformation



Modified Fredy Altpeter et al. Plant Cell 2016;28:1510-1520
Parveez et 2000; Parveez, 2003; Parveez et 2012; Parveez et al.,2015;
Masura et al; 2017; Masani et al;2018

Opportunities CRISPR
Genome Editing Technology For Oil Palm

In OIL PALM, the need to produce crops




Fatty acid composition (High oleic)


Low lipase


Height (Dwarf)


Diseases/Pests resistance


Fruit color (Virescens)

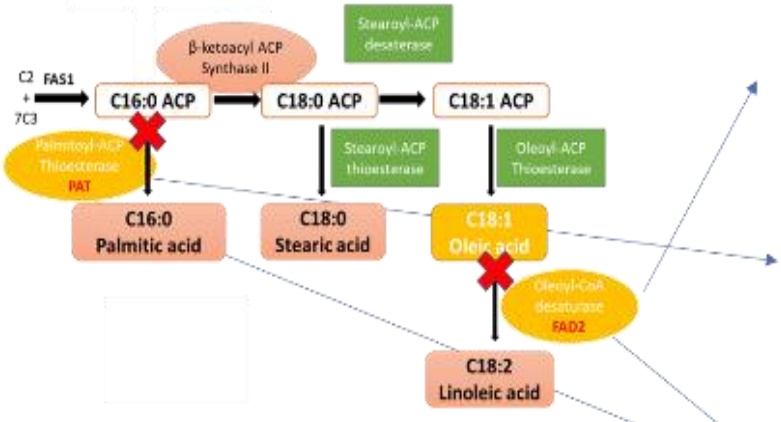
Benefits for consumers, farmers and industry:.

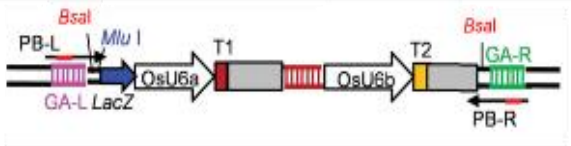
- Increases versatility of the crop in industrial applications.
- Diversify Malaysian palm oil exports to every market destination, eg. Temperate countries.
- Increase ease of FFB harvesting due to the dwarfness
- Increase economic lifespan due to slow increment
- Lower the labor intensity (fewer workers needed)
- To facilitate mechanization of the harvest
- Increase labour productivity
- Maximise land use
- Improve yields and Increased FFB production
- Improve palm oil sustainability

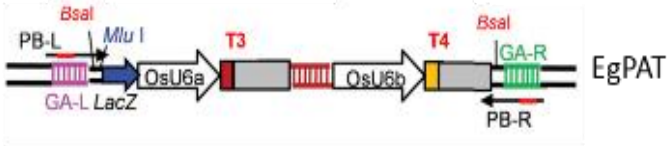
Oil palm genome sequences was published in 2013 (1.8 GB)

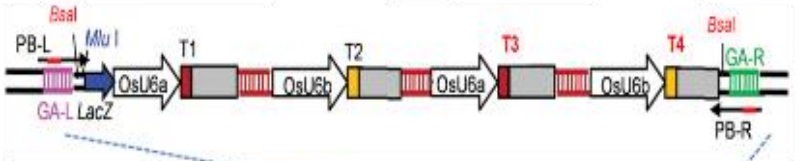
Parveez (2021) Oils and Fats International Congress
Masani et al. (2021) The Planter, Vol. 97, No. 1141

Genome Editing of Oil Palm (High Oleic Acid)



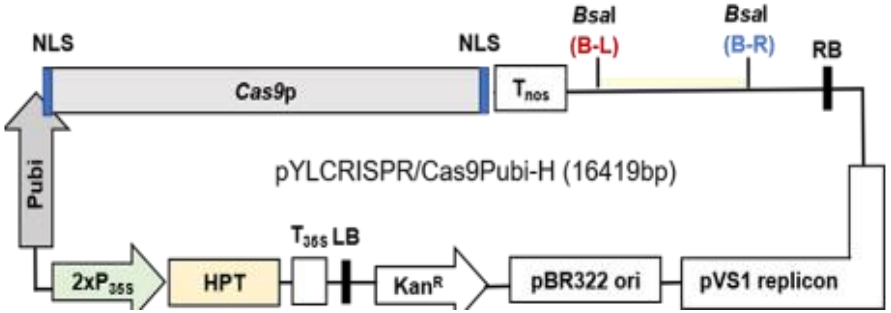






Selection of sgRNA Target Sequence

EgFAD2	EgPAT
• Omega-6 fatty acid desaturase, endoplasmic reticulum isozyme 2-like.	• Palmitoyl-acyl carrier protein thioesterase, chloroplastic
• EgFAD2-1	• Also known EgFATB-3
• Chromosome 8	• Chromosome 3 (T3)
• Two target gRNA (T1, T2)	• Chromosome 7 (T4)



Bahariah et al (2021) Genomics and Gene Editing Congress
Bahariah et al (2021) J. Genet. Eng. Biotechnol. 19(1):86.

Genome Editing of Oil Palm (Virescens)

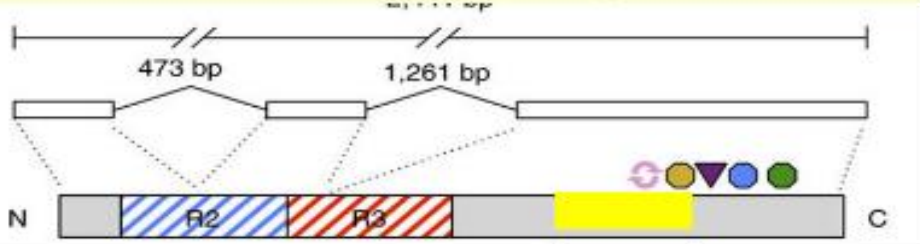
VIRESCENS (*Vir*) NIGRESCENS (*Nir*)

Nir

Unripe Intermediate Ripe

Vir

Gene: R2R3-MYB Transcription factor



WILD TYPE

VIR

Edit to initiate a premature stop codon

Singh et al. (2013) Nature. 2013 Aug 15;500(7462):335-339
Singh et al (2013) Nature. 2013 Aug 15;500(7462):340-344

PRESENTATION OUTLINE

1. Introduction
2. Oil Palm Transformation Methods
3. Selection agent for transformation
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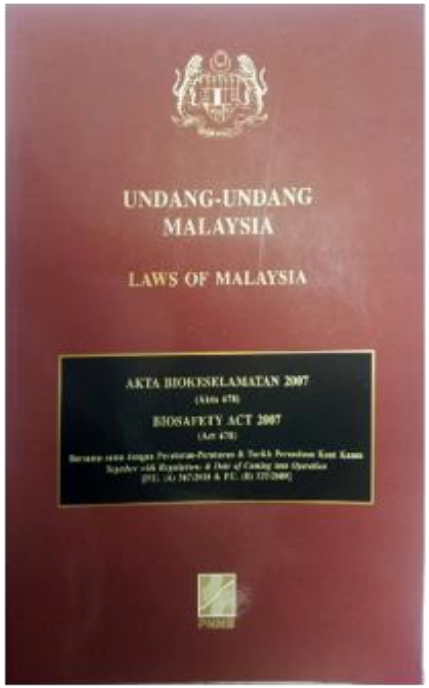
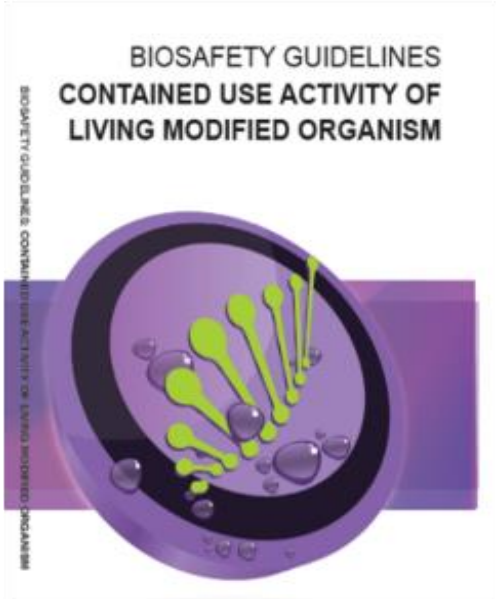
Biosafety Act 2007 (ACT 678)

- Any new GMOs/LMOs intended for cultivation, contained use, field trials, and consumption must be approved by the National Biosafety Board (NBB) on the advice of the Genetic Modification Advisory Committee (GMAC). Applicants are required to submit their applications to the Department of Biosafety

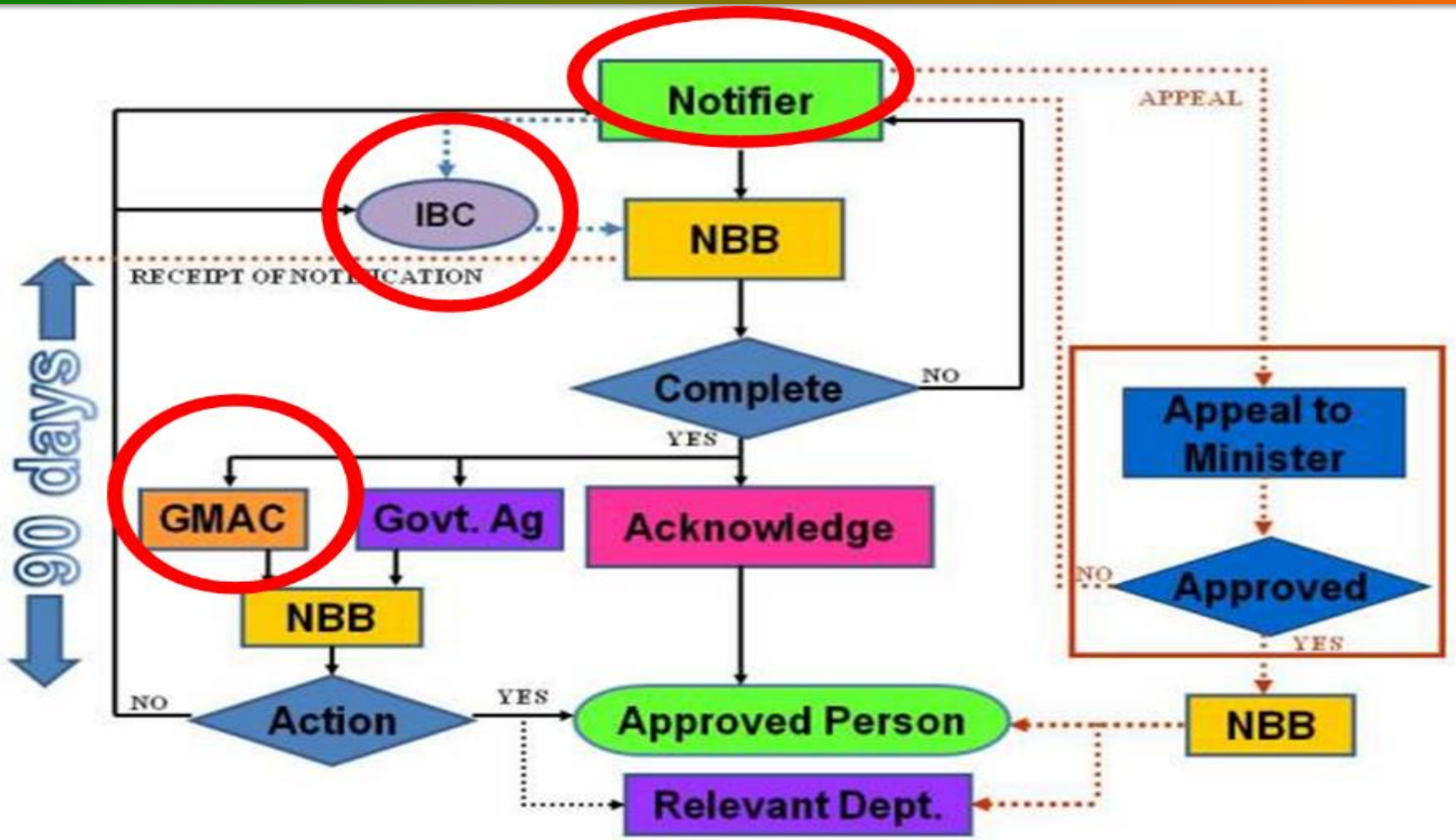




JBK biosafety Kit

http://www.biosafety.gov.my/

Notification Process



Transgenic Oil Palm Plantlets In The Biosafety Nursery

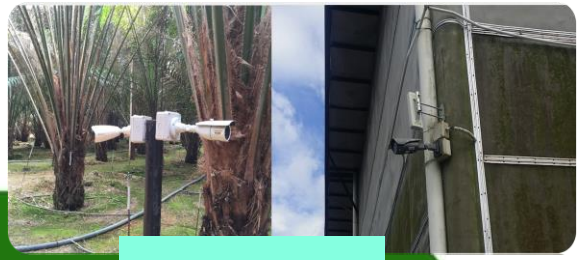
Specification: Biosafety Act 2007

- Constructed for evaluation of transgenes
- Stainless steel mesh size 30
- Automatic water irrigation system

Mature Transgenic Oil Palm In The Biosafety Screenhouse

Specification: Biosafety Act 2007

- Constructed for large scale evaluation of transgenic oil palms
- Size 100m x 120m (1.2 ha)
- Concrete structure 1m above and below ground
- Outer layer stainless steel mesh size 30
- Inner layer nylon mesh size 50



CCTV

Biosafety Assessments Of Transgenic Oil Palm

Transgenic male flower a in bag

Pollen and fruits are allowed to mature in closed bags.

Only shows resistance to herbicide glufosinate ammonium (Basta) and not to other herbicides such as glyphosate (Spark), paraquat (Gramoxone), 2,4D and pyridyloxyl-phenoxy propionic ester (Fusilade).

- Grass were allowed to grow on the soil.
- No bar gene fragments amplified from the grass samples
- No bar gene fragment amplified from the bacterial samples isolated from soils

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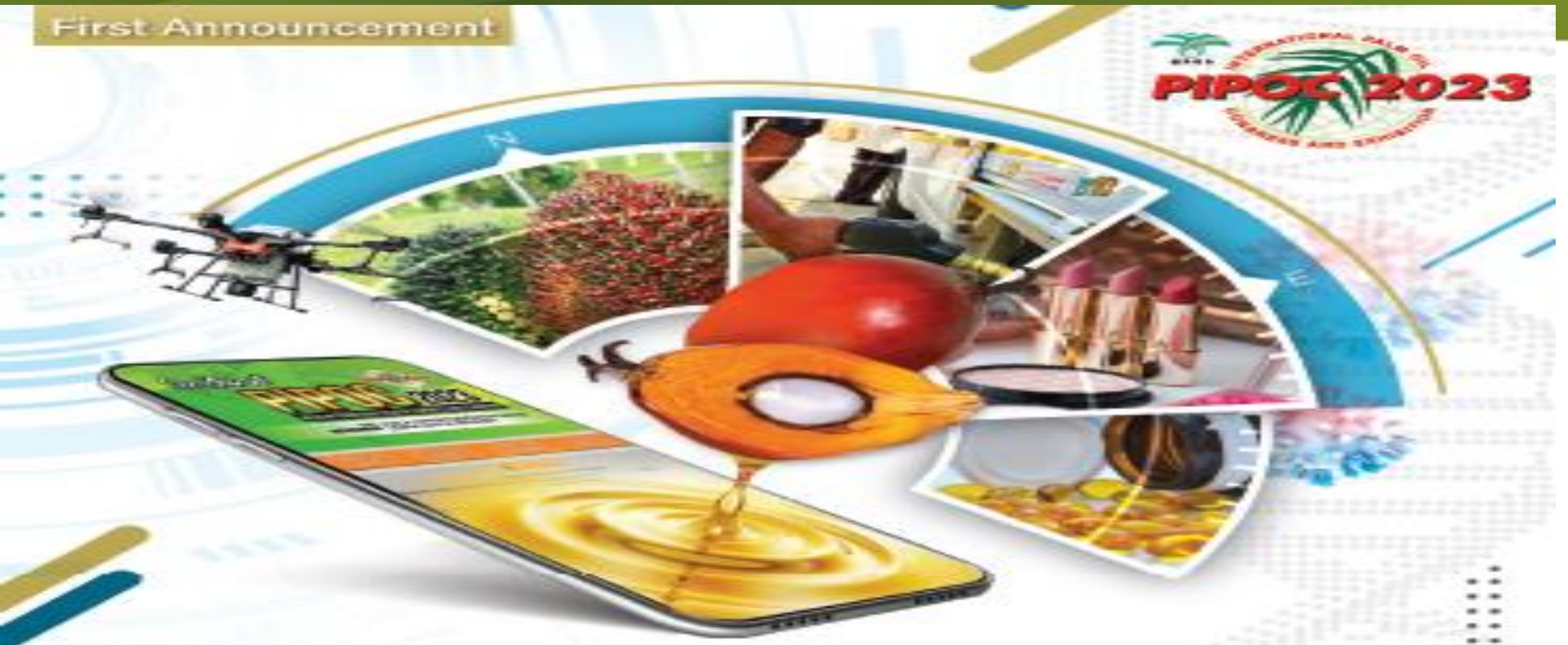
Conclusion

- **Improvements** of **transformation methods** are being carried– looking for the most effective: **escape free and high efficiency**
- **Target tissue** : the most effective and easy to obtain in large quantity and handle
- **Selection agent**: most effective, easy and escape free
- **Fertile transgenic oil palms** produced and grown in biosafety screenhouse-strictly regulated by Biosafety Act
- **Genomic editing** in oil palm initiated

XX OIL PALM International Conference

THE TRANSFORMATIVE POWER OF OIL PALM

First Announcement



PIPOC 2023

MPOB International Palm Oil Congress and Exhibition

Navigating Uncertainties Building Resilience

7-9 NOVEMBER 2023
Kuala Lumpur Convention Centre

Thank you

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