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OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 400 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 300 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

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OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

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Probing molecular and cellular mechanisms in tissue culture samples of commercial crops using Proteomic approach

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Plant tissue culture

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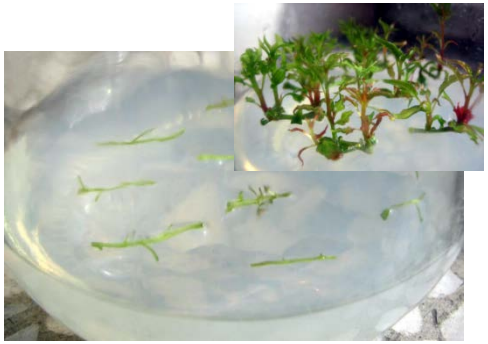
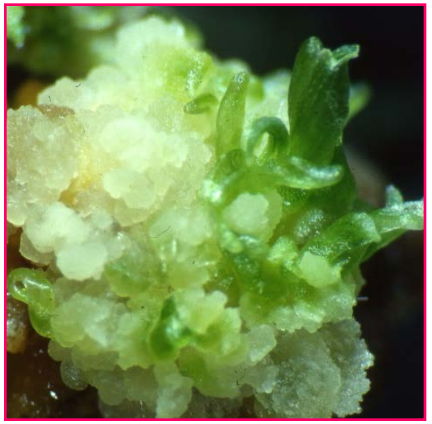
- A technique for mass micropropagation of clonal plants under sterile conditions on a nutrient medium of known composition in a laboratory
  - Shoot culture or nodal culture
  - Organogenesis
  - Somatic embryogenesis

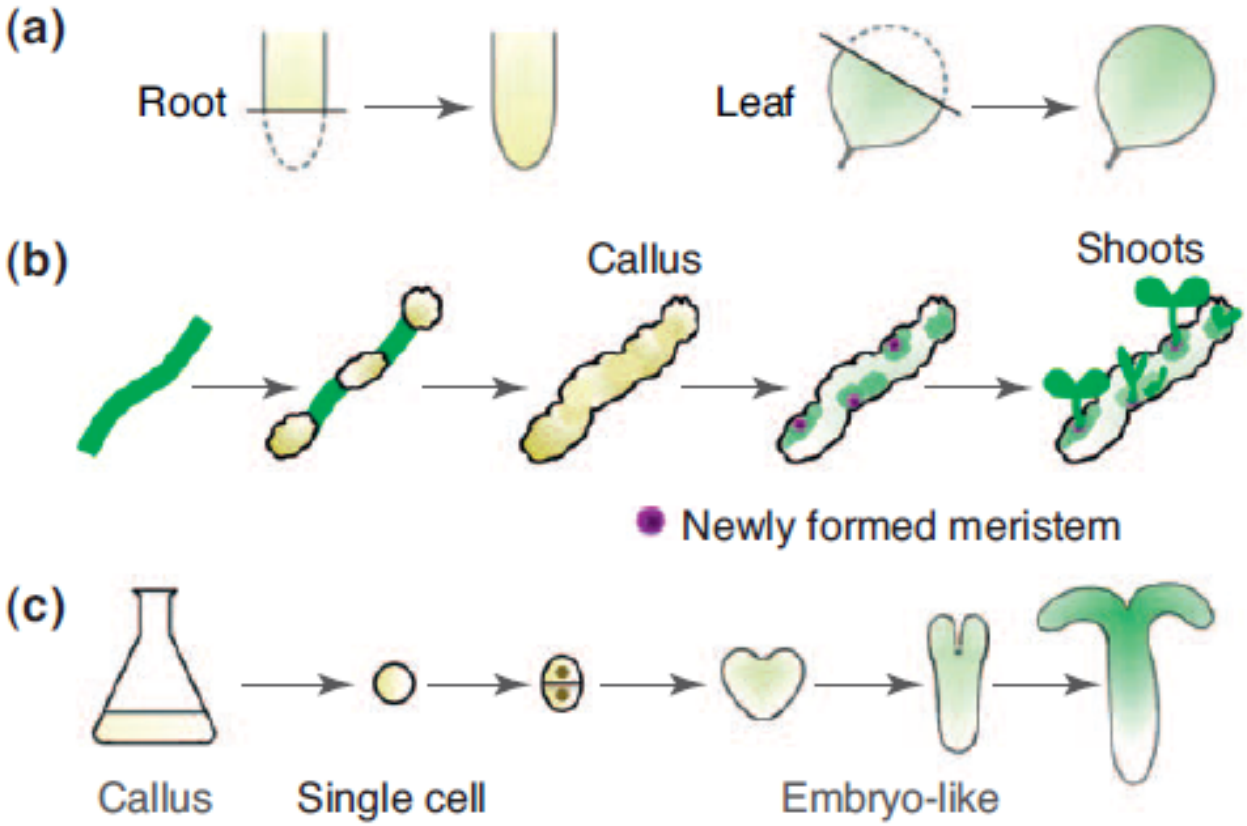




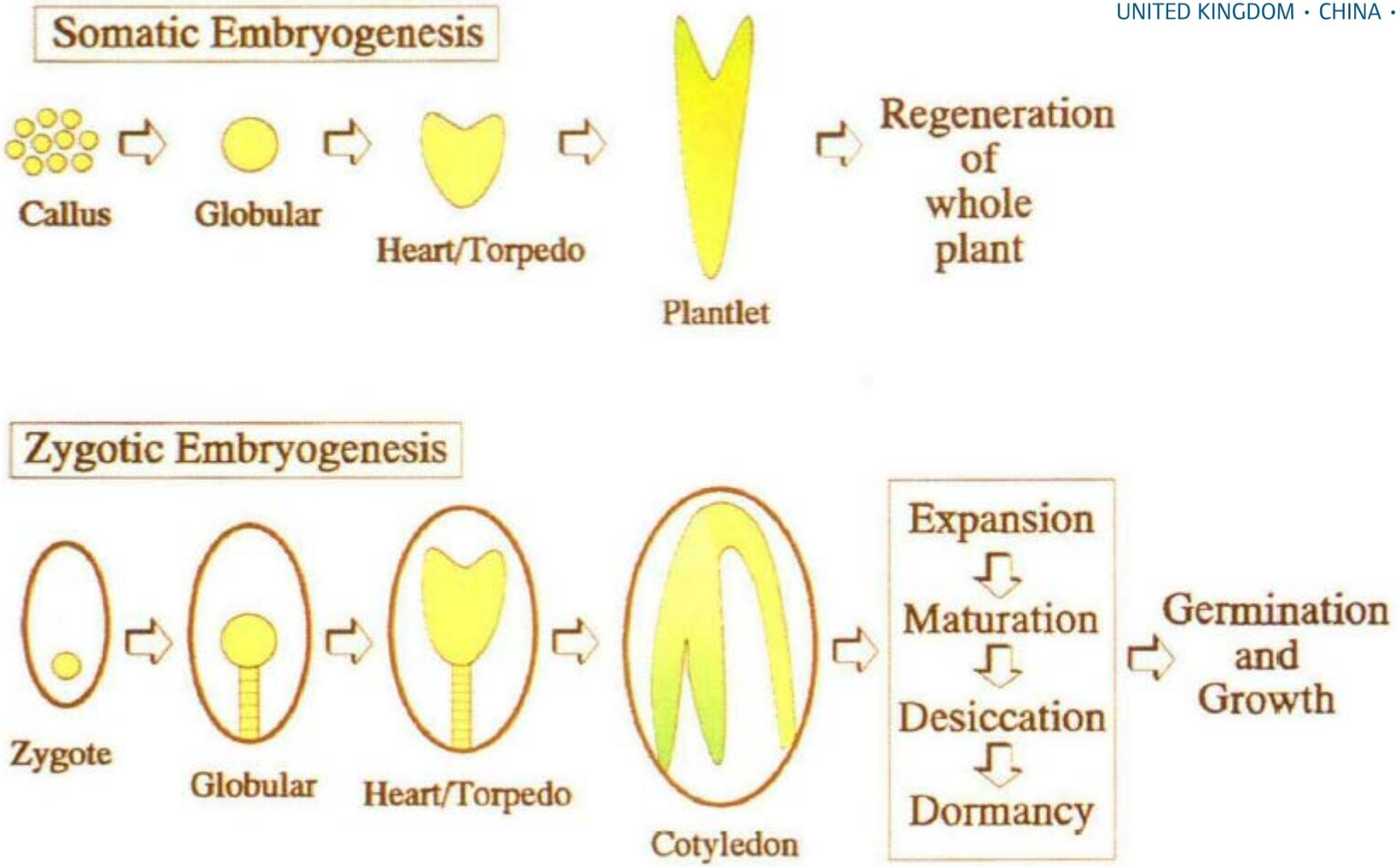
Somatic embryogenesis

- Formation of embryos from somatic cells without involvement of gamete fusion
- Unique to plants - totipotency
- Applications
  - biological studies
  - mass propagation of clonal planting materials
- Highly productive (e.g. up to 1 million plants may be regenerated from a 1 cm^2 leaf explant).

Regeneration in plants (Arabidopsis)

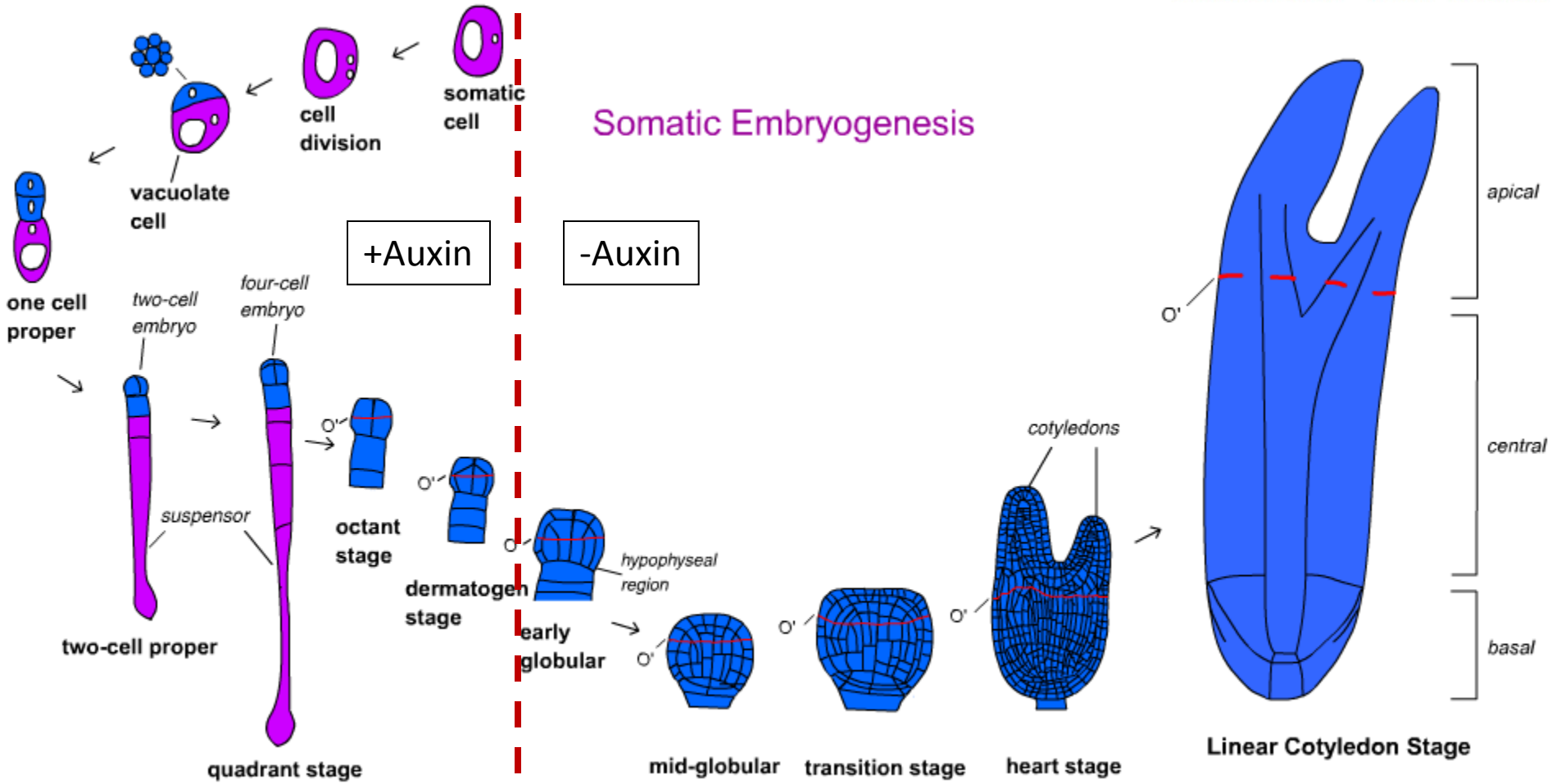


Sugimoto *et al* (2011)



Zimmerman (1993)

Developmental phases of somatic embryos



Challenges for SE applications

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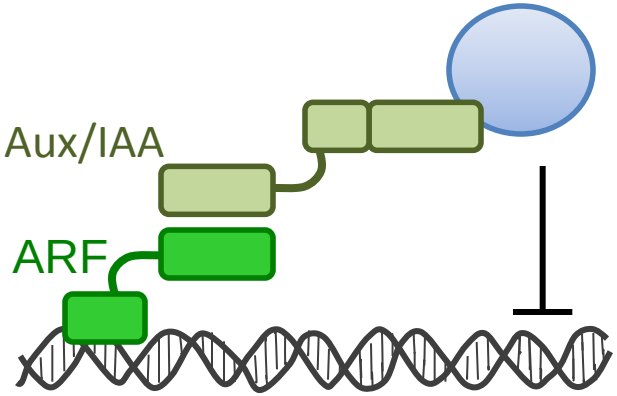
- Low callusing rate especially formation of embrogenic callus
- Low conversion rate from embryogenic callus to somatic embryos

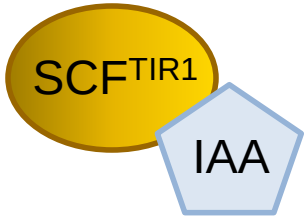
Examples of genes involved in SE

- Somatic embryogenesis receptor kinase (SERK)
- Arabidopsis leafy cotyledon (LEC)
- Baby Boom (BBM)
- Agamous-like 15 (AGL15)
- Somatic embryo related factor1 (MtSERF1)
- Glutathione-S-Transferase (GST)
- WUSCHEL (WUS)

The Auxin signaling pathway - Ubiquitin mediated protein degradation



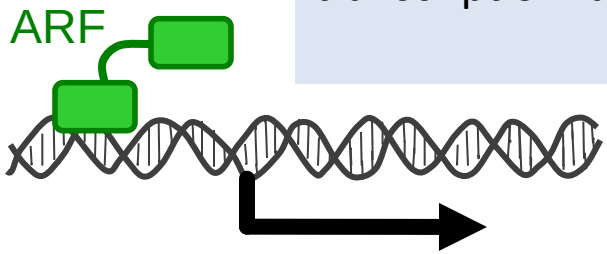
2. Aux/IAA ubiquitinated and degraded by 26S proteasome



IAA

1. Auxin binds to SCF^{TIR1} and Aux/IAA

3. Degradation of repressor permits transcriptional activation by ARF transcription factors



Proteomic analysis

- To investigate proteins associated with callusing and subsequently embryogenesis in plant tissue culture
- 2-DE coupled with mass spectrometer

2DE + MS/MS

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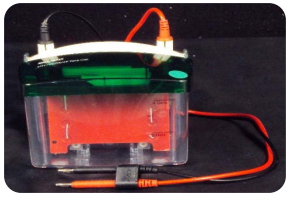
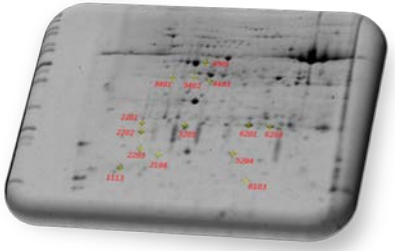
tissue samples

Protein extraction (TCA/acetone)

2D gel electrophoresis

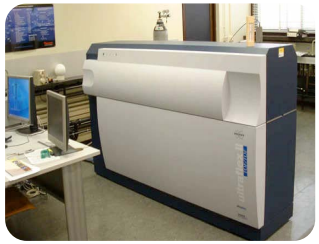


PDQuest software analysis



Excised Protein spots

LC MS/MS or MALDI TOF/TOF

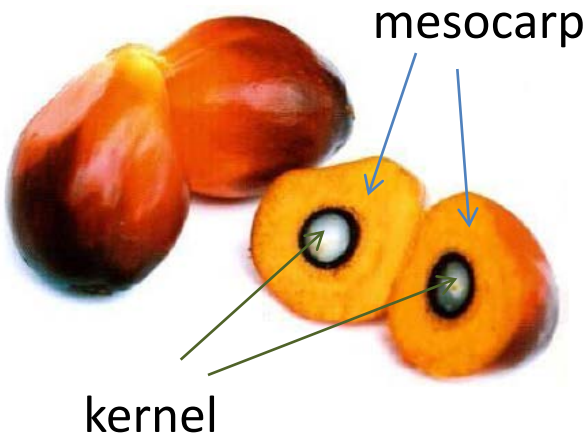


Quantification of protein expression & verification

Case Studies on Commercial Crops

- Oil Palm
- Vanilla Orchids

The Oil Palm(*Elaeis guineensis*)



Starts bearing fruits after 30 months of planting and be productive for the next 20 to 30 years

Tissue Culture of Oil Palm

- Single meristem shoot
- Clonal multiplication of elite materials
- 20 to 30% increment in oil yield

An overview of Tissue culture process of Oil Palm

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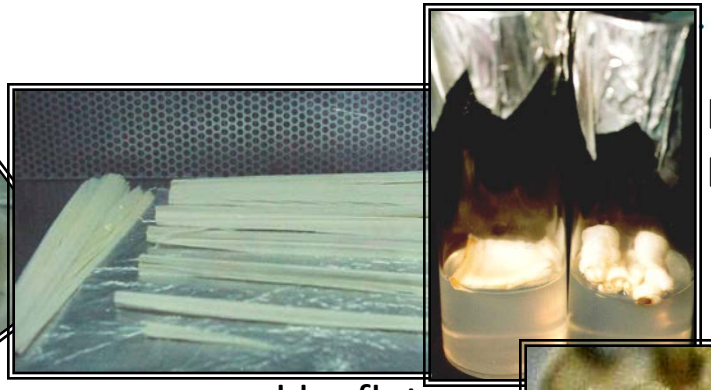
Young unopened leaflets



Leaf Explants

Calli

Embryogenic calli

Differentiation

TO LIQUID CULTURE

Shoot regeneration

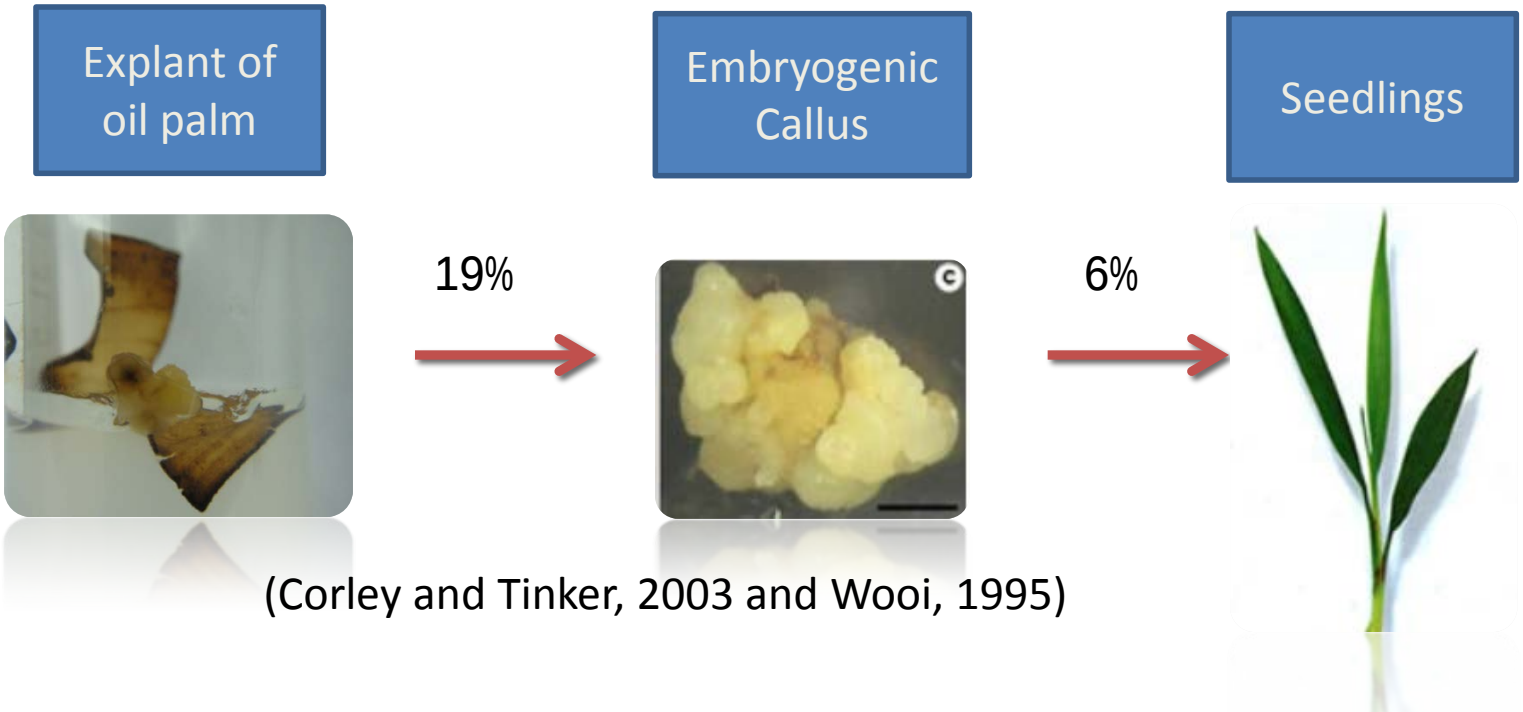
Small plantlets

Ramet

Pictures supplied by AAR lab.

Challenges in oil palm tissue culture

- The callusing and embryogenesis rates from proliferating callus culture were **low**.



(Corley and Tinker, 2003 and Wooi, 1995)

Plant Materials

- Leaf samples from high and low proliferation palms

Protein analysis and Identification

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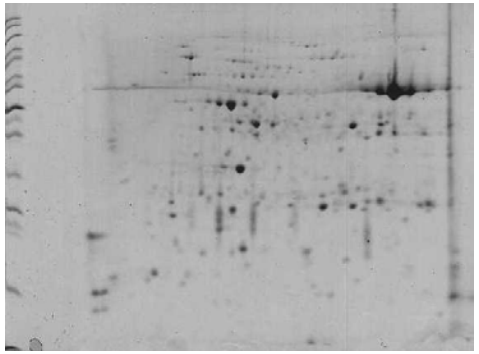
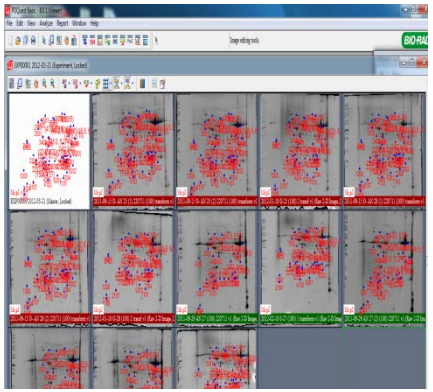
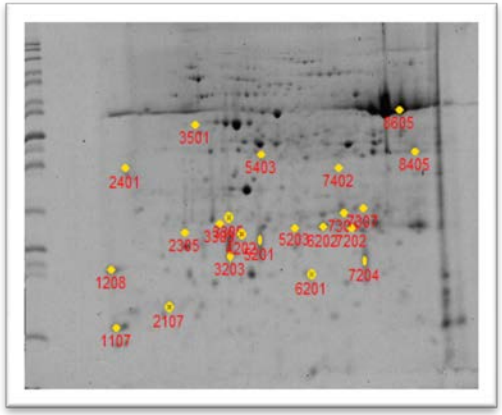


Gel image



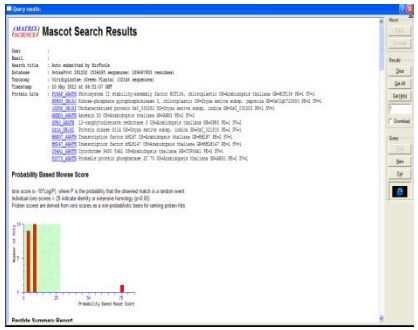
PDQuest analysis software, version 8.0.1



Different significant spots

spot cutter pen

MALDI TOF/TOF analysis



MASCOT search engine

Comparison of leaves with low and high proliferation rate of callusing

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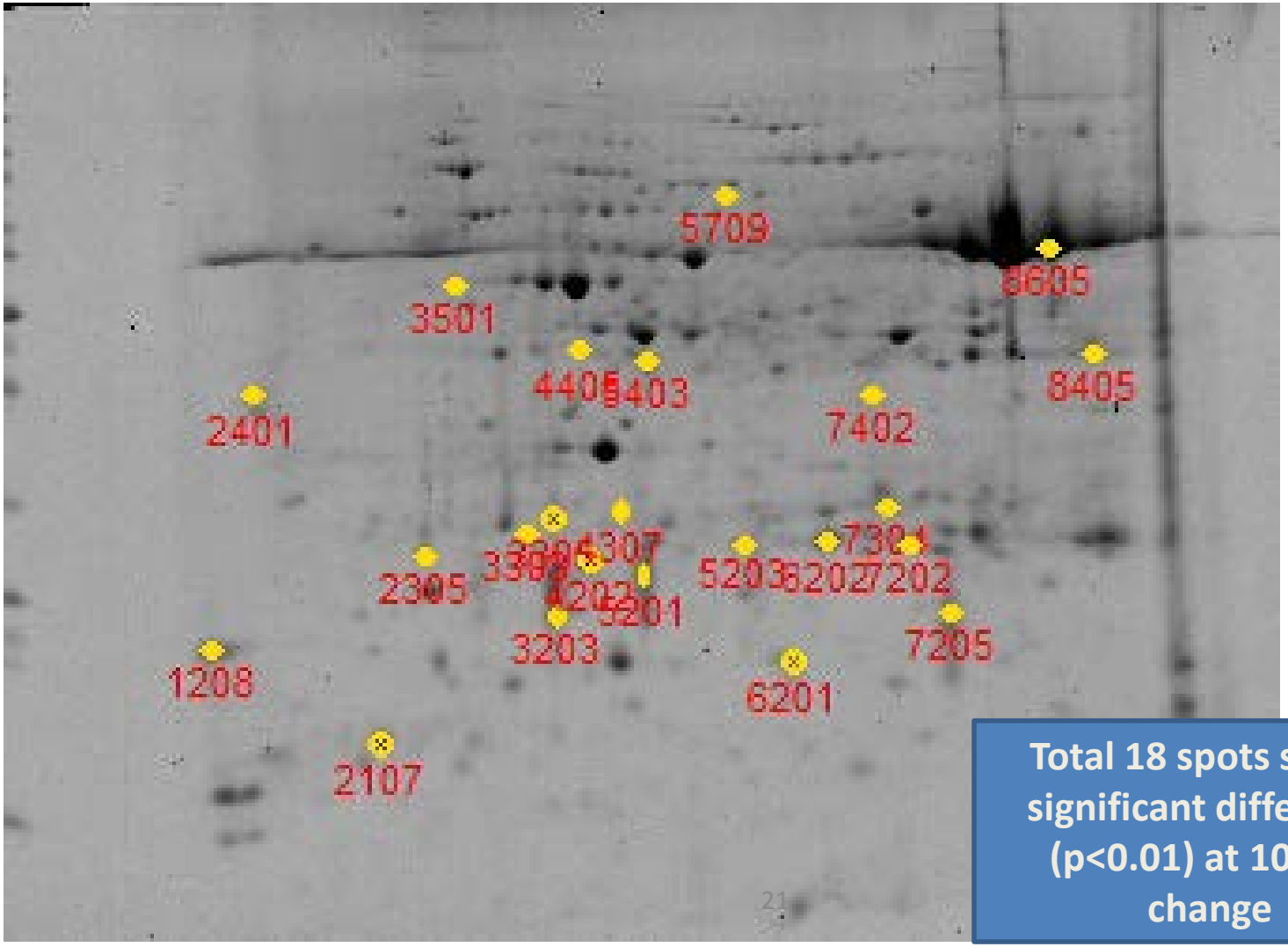

Total 18 spots shows significant difference ($p<0.01$) at 10 fold change

Summary of PDQuest analysis

- Spots that showed significantly difference in high and low proliferation rate samples were identified using MALDI TOF/TOF analysis

Protein gel	Number of spots
Higher abundance in high proliferation samples	14 (11 identified, 2 no hits, 1 spot too faint)
Present only in high	3 (no hits)
Lower abundance in high	1 (identified)

22

Protein Identification

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	Spot No	Protein name	Biological process	Molecular function	Cellular location	Reference organism	MW	pI	MOWSE
High abundance in high proliferation Rate samples	SSP 2107	RuBisCO large subunit-binding protein subunit alpha	Stress response	Binds RuBisCo small and large subunits and implicated in the assembly of the enzyme oligomer	Plastid, Chloroplast	Chlamydomonas reinhardtii	61999	5.57	65
	SSP 2305	Chlorophyll a-b binding protein of LHCII type III	Photosynthesis	Light receptor, captures and delivers excitation energy to photosystem	Plastid, Chloroplast membrane, Thylakoid	Hordeum vulgare	28798	4.99	51
	SSP 3305	Trisephosphate isomerase	-	Catalyzes interconversion of dihydroxyacetone phosphate and D-glyceraldehyde-3-phosphate. Isomerase	Substrate binding site	Gossypium hirsutum	51648	8.46	105
	SSP 4202	Oxygen-evolving enhancer protein 2	Photosynthesis	Regulation of Photosystem II	Plastid, Chloroplast thylakoid membrane	Solanum tuberosum	28158	8.27	76
	SSP 5403	Photosystem II stability/assembly factor HCF136	Photosynthesis	Essential for photosystem II (PS II) biogenesis, required assembly of an early intermediate in PSII assembly and Chlorophyll a binding.	Chloroplast membrane, Plastid, Thylakoid	Arabidopsis thaliana	44133	6.79	42

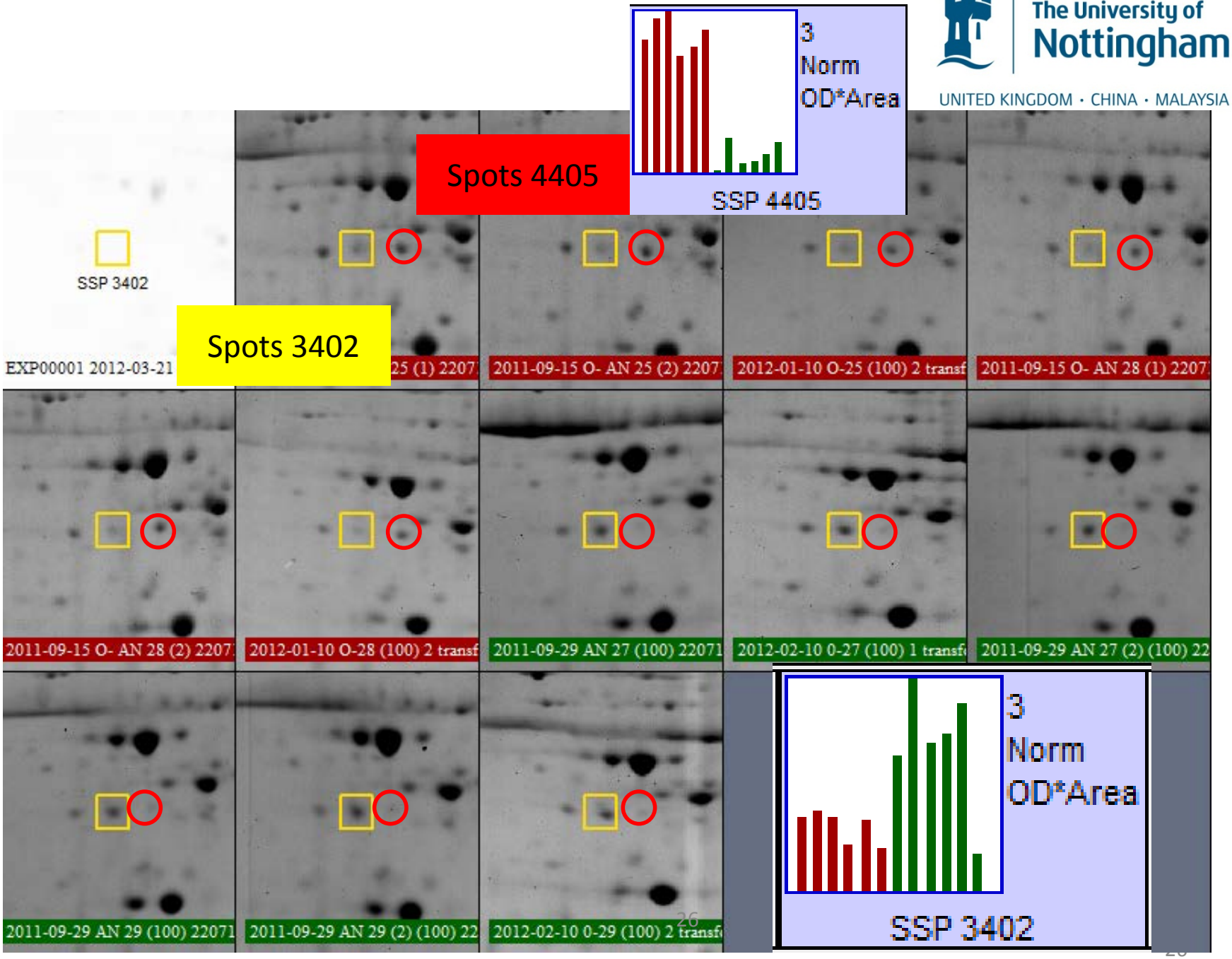
23

Cont..

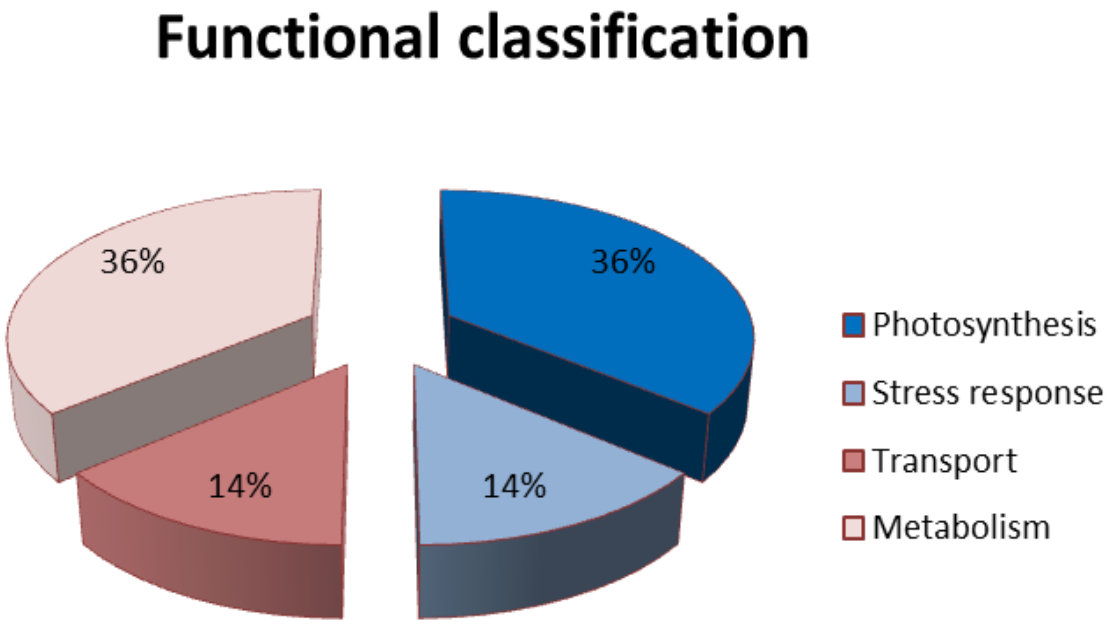
Spot No	Protein name	process	Molecular function	location	organism	MW	p/	MOWSE
SSP 6201	Cytochrome B6-F complex iron sulphur subunit 2	Electron transport	Iron sulphur protein, Rieske domain involved in electron transfer, metal ion binding, ubiquinol-cytochrome-c reductase activity.	Integral to membrane, thylakoid membrane	Sonneratia ovate	60147	8.60	135
SSP 6202	Oxygen-evolving enhancer protein 2	Photosynthesis	Regulation of Photosystem II	Plastid, thylakoid, chloroplast membrane	Fritillaria agrestis	28265	8.31	75
SSP 7202	No hit	-	-	-	-	-	-	-
SSP 7304	L-ascorbate peroxidase 2, cytosolic	Stress response, hydrogen peroxidase	Play a key role in hydrogen peroxidase removal	Cytoplasm	Oryza sativa japnonica	27215	5.21	149
SSP 7402	No hit	-	-	-	-	-	-	-
SSP 8405	Fructose- biphosphate aldolase 1	Glycolysis	Fructose-bisphosphate aldolase activity	Thylakoid lumen, apoplast, chloroplast envelope	Arabidopsis thaliana	43075	6.18	45
SSP 8605	Ribulose bisphosphate carboxylase large chain	Carbon dioxide fixation, photorespiration, photosynthesis, Calvin cycle	Primary event in carbon dioxide fixation, oxidative fragmentation in photorespiration	Plastid, Chloroplast	Acacia farnesiana	50860	6.22	220

24

	Spot No	Protein name	Biological process	Molecular function	Cellular location	Reference organism	MW	pI	MOWSE
	SSP 4405	Sedohepulose-1,7-bisphophatase	Calvin cycle, carbohydrate metabolism, Reductive pentose-phosphate cycle	Hydrolase, metal ion binding	Plastid, Chloroplast	Spinacia oleracea S17P_SPIOL	42568	5.87	106
Low abundance in high proliferation rate samples	SSP 3402	Sedoheptulose-1,7-bisphosphatase	Carbohydrate metabolism; Calvin cycle	Light activation through pH changes, Mg^{2+} levels and light-modulated reduction of essential disulphide groups via ferredoxin-thioredoxin f system.	Plastid, Chloroplast	Triticum aestivum S17P_WHEAT	42547	6.04	188
Present only in high proliferation rate samples	SSP 7307	No hit							
	SSP 7101	No hit							
	SSP 4404	No hit	-	-	-	-	-	-	-


Spots 4405
3
Norm
OD*Area
SSP 4405
SSP 3402
Spots 3402
EXP00001 2012-03-21
2011-09-15 O- AN 25 (2) 2207
2012-01-10 O-25 (100) 2 transf
2011-09-15 O- AN 28 (1) 2207
2011-09-15 O- AN 28 (2) 2207
2012-01-10 O-28 (100) 2 transf
2011-09-29 AN 27 (100) 22071
2012-02-10 0-27 (100) 1 transf
2011-09-29 AN 27 (2) (100) 22
2011-09-29 AN 29 (100) 22071
2011-09-29 AN 29 (2) (100) 22
2012-02-10 0-29 (100) 2 transf
3
Norm
OD*Area
SSP 3402

Protein Identification



Functional classification for the proteins that differently expressed

27

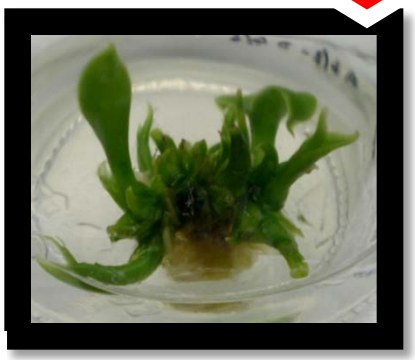
An Overview of Tissue Culture Process on *Vanilla planifolia*

Vanilla planifolia

Tissue sample

Nodal segments

Callus



Initiation of Shoots/Roots

Multiplication of Shoots

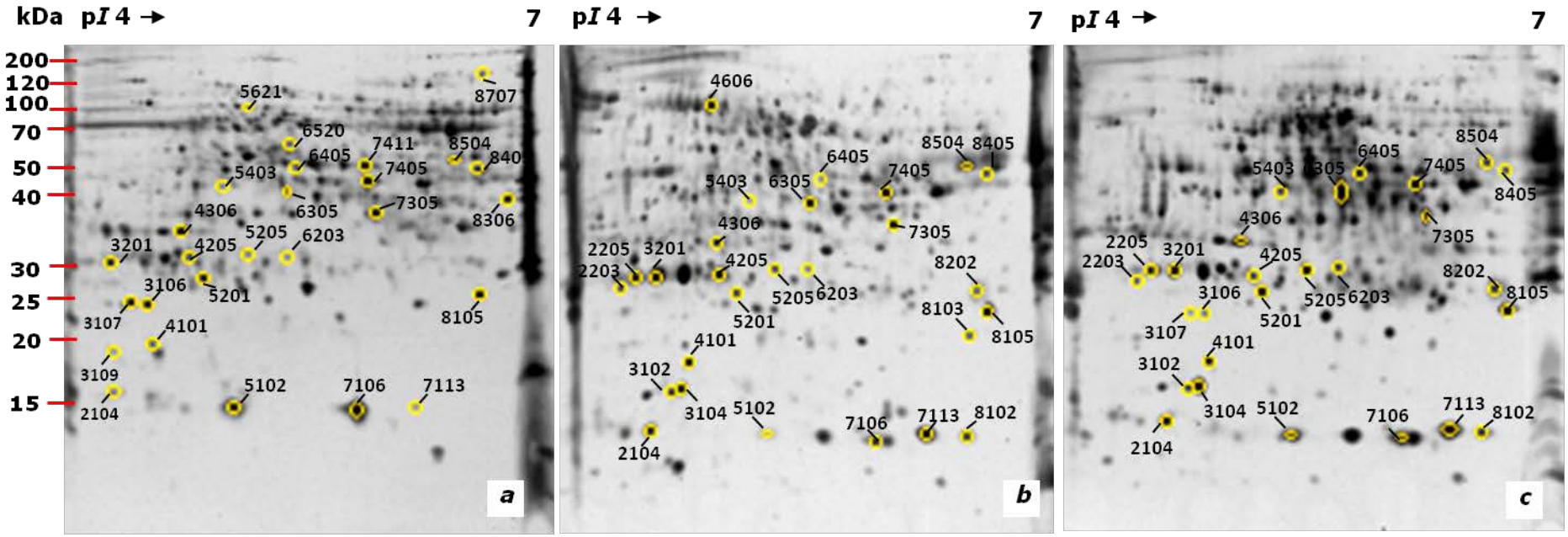
Rooting

Hardening

Nodal sample: non-callusing

Callusing : 15 days

Callusing : 45 days



Spot	Identification	Taxonomy	Accession number[b]	Mascot scores	Matched peptide
Defense and stress response					
4306	Peroxidase	Glycine max	Q9ZNZ5	47	8
3107	Cysteine protease	Prunus armeniaca	O50002	58	14
5205	L-ascorbate peroxidase 2, cytosolic	Oryza sativa	APX2_ORYSJ	154	18
5621	Heat shock protein 81-1	Oryza sativa	HSP81_ORYSI	116	61
8103	Os09g0367700 protein	Oryza sativa	Q0J294	48	9
7106	Pathogenesis-related protein 1	Asparagus officinalis	PR1_ASPOF	51	12
6305	Probable aldo-keto reductase 2	Arabidopsis thaliana	ALKR2_ARATH	74	13
5403	Probable aldo-keto reductase 4	Arabidopsis thaliana	ALKR4_ARATH	43	27
Carbohydrate and energy metabolism					
3106	Triosephosphate isomerase	Coptis japonica	TPIS_COPJA	29	15
6405	Malate dehydrogenase, cytoplasmic	Zea mays	MDHC_MAIZE	33	46
7305	Glyceraldehyde 3-phosphate dehydrogenase	Mikania micrantha	G8XWY8	63	25
Protein synthesis					
3201	Elongation factor 1-delta 1	Oryza sativa	EF1D1_ORYSJ	88	18
Transport					
3104	AT1G66240 protein	Arabidopsis thaliana	C0Z3B8	82	12
Nucleic acid metabolism					
8105	Putative uncharacterized protein Sb01g005010	Sorghum bicolor	C5WX48	47	18

Spot	Identification	Taxonomy	Accession number	Mascot scores	Matched peptide
Organ specific protein					
4606	Brain protein 44-like	Oryza sativa	Q6Z565	47	8
2203	Putative uncharacterized protein	Zea mays	B4FIN4	49	8
Protein catabolism					
5201	NPL4-like protein	Oryza sativa	NPL4_ORYSJ	32	9
Transcription					
2205	WRKY transcription factor 1	Arabidopsis thaliana	WRKY1_ARATH	30	10
Iron storage					
4205	Ferritin	Oryza sativa	Q94KA2	56	10
Photosynthesis					
6520	Ribulose-1,5-bisphosphate carboxylase/oxygenase	Vanilla cf. planifolia Chase O-170	O78667	59	33
Unknown					
7113	F28G4.3 protein	Arabidopsis thaliana	Q9LQK1	47	10
8102	Putative uncharacterized protein	Vitis vinifera	A5B9B8	46	13
8707	Predicted protein	Chlamydomonas reinhardtii	A8IBC7	50	24

Summary

- SE is an important process in plant tissue culture
- Proteins associated with callus formation can be elucidated with 2DE-MS
- In oil palms, isoform proteins that undergone post-translational modifications were detected
- In vanilla orchids, proteins specific to callus formation and proliferation were found to be associated with stress related proteins

Acknowledgements

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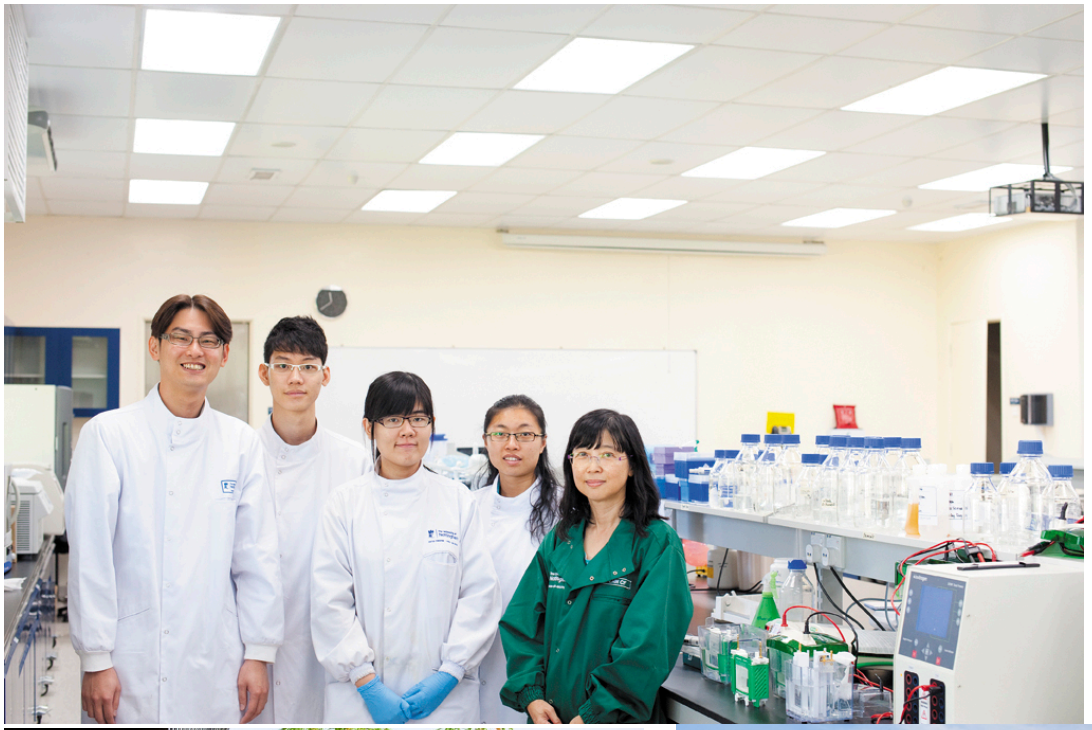

- Malaysia Palm Oil Board
- Advanced Agriecological Research Sdn Bhd

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- MPOB GSAS grant

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