PICFPA	SAAPPA / SASPA / SAT	DFTS	Winetech
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	X		X

Indicate (X) client(s) to whom this final report is submitted.
Replace any of these with other relevant clients if required.

FINAL REPORT
FOR 2009-2011

PROGRAMME & PROJECT LEADER INFORMATION

	Programme leader	Project leader
Title, initials, surname	Prof MA Vivier	Prof MA Vivier
Present position	Professor	Professor
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PROJECT INFORMATION

Project number	IWBT-P 08/14-2009
Project title	Transformation and Regeneration platform
Project Keywords	Tissue culture, transformation, regeneration

Industry programme	**CFPA**	
	Deciduous	X
	DFTS	
	Winetech	X
	Other	

Fruit kind(s)	Table and wine grapes
Start date (dd/mm/yyyy)	01/01/2009
End date (dd/mm/yyyy)	01/12/2011

FINAL REPORT

(Completion of points 1-5 is compulsory)

1. Executive summary

The Transformation and Regeneration platform provided the grapevine community of South Africa a centre to conduct grapevine transformations and regenerations. In addition it provided support to the grapevine research programme of the IWBT, the teaching efforts of the Department of Viticulture and Oenology, as well as to research groups in Genetics and the Institute for Plant Biotechnology.

Genetic transformations were continuously performed with different constructs to cultivars Sultana, Red Globe, Merlot and R110 as rootstock. The transgenic plants, cultures and cell-lines that have been established were sustained. The constructs included several antifungal genes, carotenoid genes, ascorbate/tartate metabolism genes as well as anti-viral constructs. Solid-plate cultures, suspension cultures, cryopreservation stock material, as well as *in vitro* and *ex-vitro* plant material, as well as the optimised methods and skilled people performing the work form part of the platform.

2. Problem identification and objectives

The ability to handle grapevine materials in tissue culture is key to many viticultural experiments, specifically in molecular biology, plant propagation and plant material quality studies. Equally important is the ability to perform transformation and regeneration experiments. Based on the successes achieved in previous projects with these methods, the IWBT has established a platform facility for these purposes.
The aims of this platform are to maintain and expand the capacity to:

1. Handle a wide range of grapevine cultivars and rootstocks in tissue culture for various applications;
2. Initiate, select, proliferate and maintain somatic embryogenic cultures (for genetic transformation experiments and other applications such as virus-elimination of important germplasm);
3. Initiate and maintain liquid cell lines for transformation and regeneration experiments, cryopreservation of important germplasm, as well as molecular biology applications;
4. Cryopreserve somatic embryogenic cell lines for long-term storage of target material for transformation and regeneration experiments;
5. Perform genetic transformation and regeneration experiments in support of the fundamental, as well as biotechnology research projects conducted at the IWBT and by other grapevine researchers.

3. Workplan (materials & methods)

List trial sites, treatments, experimental layout and statistical detail, sampling detail, cold storage and examination stages and parameters.

All methods have either been developed or optimised in-house and were applied routinely to support the various aims.

4. Results and discussion

State results obtained and list any benefits to the industry. Include a short discussion if applicable to your results.

Milestone	Achievement
1. Handle a wide range of grapevine cultivars and rootstocks in tissue culture for various applications	All materials of the platform facility were maintained successfully. New material that was introduced during the reporting period is material

	of the Pinot Meunier dwarf mutant of grapevine that was obtained from Australia (Boss et al 2002) for research purposes, organ (grape berry) cultures, as well as material supporting the agro-infiltration efforts in collaboration with the group of Johan Burger, Genetics. Currently four wine grape cultivars, four table grape culivars and three rootstock cultivars are maintained for routine purposes.
2. Initiate, select, proliferate and maintain somatic embryogenic cultures	Somatic embryogenic cultures were successfully initiated from cultivars Red globe, Sultana, Merlot, Chardonnay and R110. These are routinely subjected to transformations and regenerations.
3. Initiate and maintain liquid cell lines	Embryogenic suspension cultures have been initiated and maintained for cultivars Red globe, Sultana, Merlot, Chardonnay and R110 to support the transformation program. Non-embryogenic organ-specific cultures (berry derived) have been developed in one of the Winetech research projects and this technique have become routine enough now to be taken up as one of the Platform's routine techniques.
4. Cryopreserve somatic embryogenic cell lines for long-term storage of target material for transformation and regeneration experiments	A method was optimised and published.
5. Perform genetic transformation and regeneration experiments	Sultana, Red Globe, Merlot and R110 were transformed on a routinely basis with different constructs.

Figure1 summarises the activities of the platform, whereas Figures 2 and 3 summarise the transgenic populations generated in the platform.

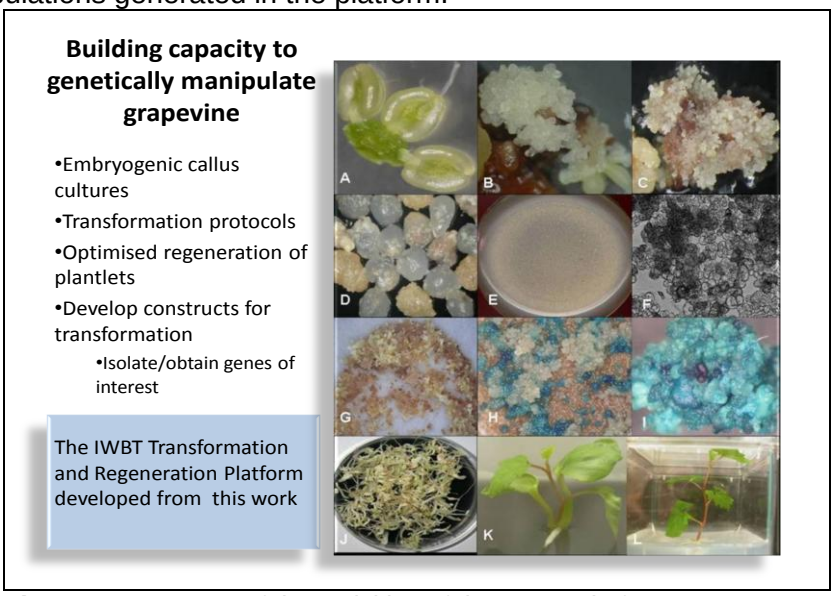

Figure 1. Summary of the activities of the IWBT platform

Towards disease resistance

Gene of interest	Cultivar	Target	Group
Yeast Chitinase	Sultana	Disease resistance	IWBT
Yeast Glucanase	Sultana	Disease resistance	IWBT
Barley Glucanase	Sultana	Disease resistance	IWBT
HSP-70	Merlot	Disease resistance	Genetics
HSP-mut	R110	Disease resistance	Genetics
Grapevine antifungal peptide	Sultana	Disease resistance	IWBT
Radish antifungal peptide	Sultana RedGlobe	Disease resistance	IWBT
Heliophilia antifungal peptide 1	Sultana RedGlobe	Disease resistance	IWBT
Heliophilia antifungal peptide 5	Sultana RedGlobe	Disease resistance	IWBT
Non-vinifera Grapevine PGIP A Non-vinifera Grapevine PGIP B	Sultana RedGlobe Merlot	Disease resistance	IWBT

Figure 2. Summary of transgenic populations generated towards the aim of disease resistance

Towards quality and environmental stress resistance

Gene of interest	Cultivar	Target	Group
Ascorbic acid gene 1	Sultana	Metabolic engineering	IPB
Ascorbic acid gene 2	Sultana	Metabolic engineering	IPB
Ascorbic acid gene 3	Sultana	Metabolic engineering	IPB
Grapevine terpene synthase 2*	Merlot	Quality/ Aroma	IWBT
Grapevine terpene synthase 3*	Merlot	Quality/ Aroma	IWBT
Grapevine NCED* gene (ABA)	Sultana	Drought stress/ripening	IWBT
Grapevine CCD1* (overexpressed)	Sultana	Quality/ Aroma	IWBT
Grapevine CCD1* (silenced)	Sultana	Quality/ Aroma	IWBT

*Grapevine carotenoid pathway (41 genes) has been studied: expression analysis, function and regulation

Figure 3. Summary of transgenic populations generated towards quality aspects

5. Accumulated outputs

List ALL the outputs from the start of the project.
The year of each output must also be indicated.

Technology development, products and patents

- 21 Transgenic grapevine populations were generated
- Optimisations to multiply and harden-off the Pinot Meunier dwarf mutants were completed (2009)
- Various methods to manipulate plant material for agro-infiltration was tested (2008-2010) (in collaboration with Dr Dirk Stephan and Prof J Burger, Genetics)
- Routine optimisations of steps involved in the transformation and regeneration technologies of grapevine were implemented.

- The development of an efficient system to establish, synchronise and maintain berry-specific non-embryogenic suspension cultures

Human resources development/training

1. Hildegard Witbooi: intern from CPUT from January-July 2009
2. Mukani Moyo, Kari van Rensburg and Zanele Noqobo, new MSc students were trained for six months in tissue culture technologies to provide them with the necessary skills for their projects.
3. 1 Junior Researcher
4. 1 Technician
5. 1 Technical Assistant

	Student level (BSc, MSc, PhD, Post doc)	Cost to project (R)
1.	Hildegard Witbooi (Diploma January-July 2009)	27,000
2.	Mukani Moyo (MSc 2009-2010)	9,162
3.	Kari van Rensburg (MSc 2011-2012)	3,400
4.	Zanele Noqobo (MSc 2011-2012)	22,800
5.		

Publications (popular, press releases, semi-scientific, scientific)

(1) Ramaschandra, S.G., C. Stander, D. Jacobson, B.K. Ndimba & M.A. Vivier, 2011. Proteomic analysis of grape berry cell cultures reveals that developmentally regulated ripening related processes can be studied using cultured cells. *PLoS ONE* 6(2), e14708.

(2) Vasanth, K. & M.A. Vivier, 2011. Improved cryopreservation procedure for long term storage of synchronised culture of grapevine. Biologia Plantarum 55(2): 365 – 369.

(3) Stander, C., K. Vasanth & M.A. Vivier, 2012. Anther and axillary bud derived somatic embryogenesis and plant regeneration of grapevine (*Vitis vinifera*) cultivars (in preparation).

Presentations/papers delivered

(1) Vasanth, K. & M.A. Vivier. 2008. Grapevine tissue culture and transformation. Fourth International Symposium on Acclimatization and Establishment of Micropropagated Plants, Bangalore, India. (8-12 Dec)

(2) Du Preez, J., D. Stephan, M. Blignaut, C. Stander, M.A. Vivier, D. Goszczynski & J.T. Burger. 2008. The characterisation of virus-based vectors for functional genomic studies in grapevine. Eighth International Symposium on Grapevine Physiology and Biotechnology, Adelaide, Australia. (23-28 Nov)

(3) Stephan, D., J. du Preez, C. Stander, M.A. Vivier, M. Muruganantham, M. Mawassi & J.T. Burger. 2009. Vacuum-agroinfiltration of different *V. vinifera* cultivars and application of VIGS in the cv. Sultana. 16th Meeting of the International Council for the Study of Virus and Virus-like diseases of the grapevine (ICVG), Dijon, France. (31 Aug - 4 Sept)

(4) Freeborough, M.-J., S. Malan, C. Stander, M.A. Vivier & J.T. Burger. 2008. Molecular analysis of transgenic grapevine plants containing a virus resistance construct. Thirty First Conference of the South African Society for Enology and Viticulture, Somerset West. (11-14 Nov)

(5) Stander, C. & M.A. Vivier. 2008. Grape berry suspension cultures as potential tools to model ripening-related processes. Cape Biotechnology Forum, Lord Charles Hotel, Somerset West. (30 Nov – 2 Dec)

(6) Stander C., Vasanth K., Korkie M. and Vivier M.A. 2009. *In vitro* culture systems to study and genetically improve grapevine (*Vitis vinifera*) cultivars. SASM 09/BIO-2-BIZ, International Convention Centre, Durban (20 - 23 September)

(7) Sharathchandra R.G., C. Stander, B. Ndimba and M.A. Vivier. 2010. Optimising a proteomics approach to evaluate grapevine berry suspension cultures. 15th Annual Australasian Proteomics Conference, Lorne, Australia

(8) Sharathchandra R.G., C. Stander, B. Ndimba and M.A. Vivier. 2010. Proteomic analysis of secreted proteins during grape berry ripening. Human Proteome Organisation's World Congress, Sydney, Australia

(9) Vivier, M.A. & P.R. Young. 2011. GMO grapevines: excellent study models and promising (future) products. Second International Society for Horticulture Science Genetically Modified Organisms in Horticulture (GMO 2011), Nelspruit. (11-15 September) (Keynote speaker - By invitation).

4. Total cost summary of project

	Year	CFPA	Deciduous	DFTS	Winetech	THRIP	Other	TOTAL
Total cost in real terms for year 1	2008		R 150,000		R 150,000	R 142,500		**R 442,500**
Total cost in real terms for year 2	2009		R 160,000		R 160,000	R 160,000		**R 480,000**
Total cost in real terms for year 3	2010		R 175,000		R 176,000	R 175,500		**R 526,500**
Total cost in real terms for year 4	2011		R 187,250		R 192,000	R 189,625		**R 568,875**
Total cost in real terms for year 5	2012		R 0		R 0	R 0		**R 0**
TOTAL			**R 672,250**		**R 678,000**	**R 667,625**		**R 2,017,875**