Commercial-In-Confidence

Vital Vegetables®: 1 Extension
Final Report

1 January 2008 - 30 June 2008

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Complied by:
Crop & Food Research New Zealand and Department of Primary Industries Victoria

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Overview summary

The Australian and NZ vegetable industries are facing increased competition nationally and internationally. In particular, vegetables produced in relatively low cost economies such as China, India, Africa and South America are increasing in quality and quantity on world markets. To remain competitive Australia needs to differentiate its vegetable products in the market place. The Vital Vegetables (VV) project was initiated in 2003 to achieve this aim by identifying and commercialising vegetables that can attract a premium price based on their inherent health attributes. The current report refers to the six month period from 1 January 2008 to 30 June 2008. This large project is a trans-Tasman collaboration between DPI Victoria, Crop & Food Research in NZ, HAL and has the active involvement of both Ausveg and HortNZ, the peak grower groups in Australia and New Zealand respectively.

At the start of the project an extensive market research study was conducted to see if the public would buy these new, high health VV products. The results were very encouraging. Nearly 2 in 3 consumers interviewed indicated they would buy vegetables with proven health benefits. Furthermore, 84% of respondents preferred to consume healthy foods as fresh fruit and vegetables rather than in a processed form. However, a key finding was that consumers will not readily forgo other product attributes like freshness and flavour. Hence the research program is not only ensuring that Vital Vegetables have proven health benefits but that they maintain their freshness and they taste good.

During the project substantial scientific capabilities have been developed and secured in the key areas of phytonutrient analysis, bioefficacy, and the effects of agronomic and postharvest conditions on health compounds, freshness and flavour. In addition, the project worked closely with Clause Pacific Seeds, part of Groupe Limagraine, to screen their brassica seed collections for high phytonutrient varieties. As a result of these studies, the first VV product, a high-glucoraphanin broccoli variety called 'Booster' has been identified and a complete package of agronomic and postharvest handling protocols has been developed. Vital Vegetables has also conducted several bioefficacy studies on Booster which will serve to back up health claims made in the marketing campaign.

The project has also identified and studied several other high phytonutrient vegetable crops including a range of lettuces, brassicas, carrots, cauliflower and calebrini. It is hoped these new VV products will be developed to commercialization in the next Vital Vegetables (VV2) project.

A major focus in the past 6 months has been on establishing a robust route to market for VV products and widening access to large, national and international vegetable germplasm collections. These have been achieved with the formation of the VV Marketing and Germplasm Partners, respectively. The Marketing Partnership consists of six leading Australian fresh and processed food companies, who have made significant cash and in-kind contributions to the development of business and marketing plans for the VV brand. A similar partnership is also being formed in NZ. The Germplasm Partners consist of three leading international vegetable seed companies, who will become actively involved in VV2 by providing access to huge germplasm collections and by establishing targeted breeding programs.

With the significant support of the commercial partners, VV now aims to establish a wide range of high health vegetable products in the next 5 years. The range will include new varieties of whole fresh vegetables, salad mixes, stir fries and a range of processed products. All will attract premium prices and the added value will be shared down the marketing chain, including growers. Response from the major retailers has been very positive and it is hoped that this project will create a new, premium market category of Vital Vegetables that will help underpin the viability and sustainability of Australian and NZ vegetable growers.

KPI Report 1 January 2008 – 30 June 2008

KPIs	Achieved
Science publications, internal reports and presentations	17
Analytical protocols	3
Staff exchanges, meetings and visitors	3
Potential IP opportunities	6

Refereed publications

Toivonen P.M.A., Brummell D.A. 2008. Biochemical bases of appearance and texture change in fresh-cut fruit and vegetables. Postharvest Biology and Technology 48: 1-14.

Brummell D.A. 2007. How dynamic primary cell wall properties affect fruit tissue firmness, intercellular adhesion and the characteristics of processed fruit products. In: U Schmitt, AP Singh, PJ Harris (eds.), The Plant Cell Wall – Recent Advances and New Perspectives, Mitteilungen der Bundesforschungsanstalt für Forst und Holzwirtschaft, Hamburg, pp. 53-60.

McKenzie M.J., Hunter, D.A., Pathirana, R., Watson, L.M., Joyce, N, Rowan, D., Matich, A. and Brummell D.A. Wider applicability of the selenocysteine methyltransferase gene from selenium hyperaccumulators: accumulation of an organic anticancer selenium compound in a transgenic Solanaceous species. Manuscript under review by Transgenic Research.

Pathirana, R., Harris, J., McKenzie M.J. (2008) A comparison of microtubers and field-grown tubers of potato (Solanum tuberosum L.) for hexoses, sucrose and their ratios following postharvest cold-storage. Postharvest Biology and Technology 49 (1): 180-184.

Rochfort, S.J., Trenerry, V.C., Imsic, M., Panozzo, J., Jones, R.B. (2008) Class Targeted Metabolomics: ESI Ion Trap Screening Methods for Glucosinolates Based on MSn Fragmentation. Phytochemistry 69(8): 1671-9.

Conference presentations

Brummell D.A., Johnston S., Wong S., Schröder R. 2008. Ripening-related changes in xylose-containing hemicelluloses from tomato fruit. Plant Cell Walls: Diversity and Approaches to Understanding Their Function, 3rd New Zealand-Germany Workshop on Plant Cell Walls, University of Auckland, New Zealand. **Keynote address**

J. R. Eason, E. C. de Guzman, B. Page, P. West (2007) Controlled atmosphere (CA) treatment of broccoli after harvest delays senescence and induces the expression of novel *BoCAR* genes. *Proceedings of the Australasian Postharvest Conference*, 10-12 September 2007, Terrigal, NSW, Australia. Pp18. **Oral**

McKenzie, M. J., Brummell, D. A., Hunter, D. A., Pathirana, R., Watson, L. M., Joyce, N. I., Matich, A., Rowan, D. (2007). Identification of key enzymes that produce anti-cancer selenium compounds in plants (**Poster** 89). Conference Proceedings of the 17th Queenstown Molecular Biology Meeting.

Jones, R.B., Frisina, C., Tomkins, R.B., Lill, R., Heyes, J. (2007). Developing New High Health Vegetables – The Vital Vegetables Experience. *Proceedings of the Australasian Postharvest Conference*, 10-12 September 2007, Terrigal, NSW, Australia. **Poster # 20**

Winkler, S., Frisina, C., Tomkins, R.B., Jones, R.B. (2007). Changes to glucosinolate and flavonoid content of broccoli florets during controlled atmosphere storage. *Proceedings of the Australasian Postharvest Conference*, 10-12 September 2007, Terrigal, NSW, Australia. **Poster # 18**

Reports

West, P. J. (2008) Use of differential in gel electrophoresis (DIGE) for identifying proteins that change during cell death in *A. thaliana* cell culture tissue. *Crop & Food Research Confidential Report* No. 2111.

Lister et al. Cell-based assays for assessing the bioavailability of key compounds in Vital Veg vegetables.

Lister et al. Cell-based assays for assessing the bioefficacy of key compounds in Vital Veg vegetables.

Lister et al. Potential synergies between phytochemicals in Vital Veg elite vegetables.

Lister et al. Potential field based methods for the detection of phytochemicals in Vital Veg elite vegetable lines.

Lister et al. Cancer biomarkers relating to potential health claims for Vital Veg elite vegetables.

O'Donoghue, E.M. Investigation into the opportunities for generating antibodies to small molecules.

Jones, R (2008) Scientific evidence as a basis for Vital vegetables health claims. Crop Booster broccoli Version 1- 8 numbered copies. *Commercial-in-confidence Report*

Protocols

Prep-HPLC protocol for falcarinol has been developed. This will provide reference standard for analysis of falcarinol in carrots.

Optimal glucoraphanin protocol has been finalised, involving analysis of fresh material using either UPLC or HPLC. This will provide the basis for commercial laboratory testing for QA for high glucosinolate broccoli.

Novel model system for analysing senescence in broccoli. Harvested immature Arabidopsis inflorescences have been developed as a model for senescence events of harvested broccoli heads

Staff exchanges, meetings, visitors

Vital Vegetables science planning meeting, June 2008, in Melbourne. Meeting between VVRP, VVMP, and VVGP to discuss the programme vision for VV2, followed by a science planning meeting for key science staff.

IP opportunities

Optimal glucoraphanin protocol has been finalised, involving analysis of fresh material using either UPLC or HPLC. This will provide the basis for commercial laboratory testing for QA for high glucosinolate broccoli.

Lettuce screening trial of 70 lines has been completed at Daylesford (Vic) and Gatton (Qld) in collaboration with RZ. Lines with potential will be identified when analysis is completed in July 2008.

Carrot screening trial of 67 lines has been completed in collaboration with CP. Lines with potential will be identified when analysis is completed in July 2008.

Cauliflower screening trial of 53 lines has been completed in collaboration with CP. Lines with potential will be identified when analysis is completed.

Final screening of calebrini lines has been completed and a list of elite varieties will be prepared for commercial testing once analysis is completed in August 2008.

Molecular tools have been identified that may be eventually used as markers for glucosinolate/sulphorophane content in broccoli. An ESP and an ESM homologue from

Brassica oleracea have been identified. Further sequencing will be undertaken to determine how many separate genes exist in broccoli, then expression patterns will be examined (northern analysis) to identify genes that may influence glucosinolate /sulforophane content.

Molecular tools for controlling senescence in broccoli. We have made an RNAi construct for silencing the broccoli ortholog of AtNAP, a senescence-controlling NAC transcription factor. We have also made a silencing construct for an unknown gene that is localised to chloroplasts and one of the most strongly senescence upregulated genes in the Arabidopsis genome. Broccoli plants with altered protease inhibitor (BoPI) have modified cysteine protease activity and altered senescence phenotype.

Milestone Report January-June 2008

Sub-program 1: Project Governance, Management & Commercialisation

Key Project 1.1 VVMP & VVRP relationship

Activities

1. Collaborative relationship between VVMP and VVRP formalized and maintained: DPIV/CFR/VVMP

Key Project 1.2 VV Product commercialization and support

Activities

1. Definition and communication of health benefits of VV products: DPIV/CFR/VVMP

Milestones

Months	Milestone	Responsibility
6	Establish collaborative agreement between VVRP and VVMP	DPIV, CFR, VVMP
6	First tier commercial partner, investors established, and agreement on the key species, commercial activities and timelines for those species obtained	VV Commercialisation Manager
6	Clarity on the claims that can be made for Vital Vegetables within the Food Regulations achieved.	DPIV, CFR
6	Decision made on the application of the Phytonutrient Index to Vital Vegetables as a communication tool for consumers	DPIV, CFR, VVMP
6	Identify most suitable germplasm partner for carrot and red lettuce screening and access germplasm.	DPIV, CFR, VVMP
6	Design and plan consumer testing and launch for Booster broccoli	DPIV, CFR, VVMP
6	Design market research for new products to be developed as part of VV2	DPIV, CFR, VVMP

Status:

Outcomes for the agreements with VVMP & VVGP have been agreed to and contracts have been drafted and sent to VVMP for their response. The VVMP Australia first tier commercial partners have been established which include: Perfection Fresh, Fresh Select, Costa's Exchange, Simplot, Salad Fresh Huston Farms and they are well advanced in formation of a company. VVMP partners in NZ have been endorsed by VVGG and include MG Marketing, Turners & Growers, J Sutherland Produce and Simplot. VVMP NZ is progressing towards formation of a company.

Crop species have been agreed to with commercial partners and crop scheduled will be prepared which integrate the activities of research, genetics and marketing partners into a timeline to market. A market value offer matrix is being prepared which will integrate the research and marketing activities for each crop/product. The market value matrix will address the marketing and claims for each crop product and align them with regulatory requirements.

The Phytonutrient index will be discussed as part of the brand development and trade mark usage manual, however, a final decision on this has not been made.

Genetics partners have been agree for carrots, lettuce and capsicum with onions being the only outstanding crop at present.

The marketing partners have been conducting consumer testing of Booster and are working towards a possible launch in October 2008 of Booster.

As part of the preparation of the international development strategy Coregeo have been engaged to oversee this process which will include market research to inform which countries the program will target. The Marketing partners are providing market intelligence on the market segments and crop to be developed in VV2.

Sub-Program 2: Analysis Protocols

Key Project 2.1 Analytical standards

Activities

1. Identify standards required and source commercially: DPI/CFR
2. For unavailable standards, identify optimum preparation method and implement: DPI

Milestones

Months	Milestone	Responsibility
6	Identify and order key standards needed (e.g., carotenoids, flavonoids, falcarinol, phylloquinone) from commercial sources; list of unavailable standards prepared	DPI
6	Commence prep-HPLC methods of key standards unavailable commercially	DPI & CFR

Status:

All standards have been sourced commercially, with the exception of falcarinol. Prep-HPLC protocol for falcarinol has been developed.

Key Project 2.2 Lab-based multi-phytochemical analysis systems

Activities

1. Develop new rapid phytochemical or bioefficacy analysis protocols: DPI

Milestones

Months	Milestone	Responsibility
6	Identify optimal lab-based GR protocol from commercial Booster trials	DPI & CFR

Status:
Optimal GR protocol for Booster has been finalised, involving analysis of fresh material using either UPLC or HPLC. A short list of commercial labs capable of conducting the analyses has been put together and will be presented to the VVMP, who will then choose the best candidate. Sampling procedures for Booster growers have also been developed.

Key Project 2.3 Field based phytochemical detection

Activities

1. Undertake a literature review on in-field phytochemical analysis systems and identify most promising technologies: DPI & CFR

2. If feasible, assess suitability of analysis protocols for flavonols, anthocyanins, carotenoids, glucosinolates/ isothiocyanates: DPI & CFR

Milestones

Months	Milestone	Responsibility
6	Complete literature review and identify most promising protocols for field-based phytochemical detection	DPI 50% & CFR 50%

Status:
The lit review has been completed and a short-list of most promising field-based protocols has been completed. These are: NIR for glucosinolates and flavonoids, Raman spectroscopy for carotenoids, and antibody 'dipsticks' for glucosinolates. These methods will be tested as part of VV2.

At CFR we have investigated the opportunities for generating antibodies to small molecules, and the hardware required for this. This has been written into a short report that can be found on VV website. . A literature review has also been completed and written into a formal report: "Potential field based methods for the detection of phytochemicals in Vital Veg elite vegetable lines". This work will form part of a new project in VV2 (Key Project 2.3).

Sub-Programme 3: Agronomy Protocols

Aim: Develop a range of phytochemical and mineral-specific protocols accounting for the effects of specific climate parameters on phytochemical/mineral content, crop yield, shelf life and taste. Models

and protocols will be developed based on work on model crops that can, in the future, be applied to a wider range of vegetables.

Key Project 3.1 Germplasm Screening

Activities

1. Plan and implement germplasm field trials for carrot and red lettuce using germplasm accessed in SP-1.

Milestones

Months	Milestone	Responsibility
6	Plan and set up year 1 trial on field-grown red lettuce and carrot	DPI 80% & CFR 20%

Status:

Lettuce field trial (70 lines) completed at Daylesford (Vic) and Gatton (Qld) in collaboration with RZ; harvested March 08 & May 08, respectively & analysis completed July 08 indicated several promising varieties that will be further investigated in VV2. Carrot screening trial (67 lines) in collaboration with CP, harvested in May 08 and analysed indicated a low degree of variation in total carotenoids. Cauli screening trial (53 lines) harvested March 08 and analysis completed indicated three lines with promise based on GB content; calebrini trial planned and planted; harvesting will commence July 08.

Key Project 3.2 Boosting beneficial phytochemical content and optimizing quality: effect of specific environmental conditions

Activities

1. Plan and implement Australian Booster commercial trials in collaboration with VVMP.

Milestones

Months	Milestone	Responsibility
6	Conduct, harvest and analyse Booster samples from Aust. Commercial trials	DPI

Status:
Booster trials (x 25) completed and analysed – trials have been extended indefinitely. Results have shown an increase in GR after late March, and less variability in autumn/winter than was experienced in summer/autumn. Trials in Vic bolted in June due to cold conditions, trialling has therefore moved to Qld. Competitor samples now being taken every 2 weeks at retail level. SF samples are now being routinely taken on each sample (Booster and competitor) to ensure sufficient data is available to back Booster content claim. Don Brash will meet with VVMP NZ members at the HortNZ conference later in July to plan the NZ Booster trials for 08-09.

Key Project 3.3 Sulphur uptake

Activities

1. Conduct glasshouse and field-based studies to identify genetic and physiological mechanisms determining responses to N and S nutrition of onion: CFR (FRST).

Milestones

Months	Milestone	Responsibility
6	Compare accumulation of ATPs transcripts and miRNA in onion in genotypes with contrasting S accumulation phenotypes in a glasshouse experiment with and without S deprivation.	CFR (FRST)

Status:
Establishment of the glasshouse experiment has been delayed, so this milestone will not be completed by 30 June 2008. The focus has been on development of molecular tools and analytical methods, which is progressing well.

Key Project 3.4 Boosting beneficial mineral nutrient content and optimizing quality: modelling and protocols

Activities

1. Development of excess nutrient supply models to produce nutrient-dense vegetables: CFR (FRST).

2. Development of protocol for effective selenium supplementation in field-grown broccoli: CFR (FRST).

3. Assess the uptake of selenium and analyse the partitioning of sulphur and selenium into target compounds in brassicas: CFR (FRST).

Milestones

Months	Milestone Activities 1 & 2	Responsibility
6	Evaluation of current models.	CFR (FRST)

Status:
Completed. In addition the first glasshouse experiment has been completed, with data analysis currently underway.

Months	Milestone Activity 3	Responsibility
6	Establish Booster in glasshouse conditions.	CFR (FRST)

Status:
Complete. Booster seed was imported into New Zealand and plants are now growing in the greenhouse. The germination rate of Booster appears to be higher than cv. Triathlon and the plants appear to be growing more vigorously.

Sub-Programme 4: Postharvest Protocols

Aim Develop phytochemical-specific protocols that optimise phytochemical retention through the marketing chain. Protocols will be developed based on work on model crops that can then be applied to a wide range of vegetables that also contain the target phytochemical. Sea freight protocols, based on knowledge of responses to controlled atmospheres, humidity and textural change after harvest, will also be developed with the aim of extending freshness during export.

Key Project 4.1 Through-chain integrity of functional constituents and quality characteristics

Activities

1. Develop and provide protocols that identify the optimum domestic and export marketing chains to maintain anthocyanin, carotenoid and phylloquinone content: DPI.

2. Develop and provide a sea freight protocol for preservation of texture in capsicums: CFR (FRST).

3. Develop and provide protocols that identify the optimum harvest stage in capsicum to optimise carotenoid content: DPI.

4. Identify key enzymes that are associated with cell wall modification and loss of crisp texture during storage: CFR (FRST).

Milestones

Months	Milestone	Responsibility
6	Identify domestic and export chain conditions and plan trials.	DPI 50% & CFR (FRST) 50%

Status:
Activity 1:
Domestic chain trials on lettuce and carrot completed and analysed; export chain trial on carrot also completed. Carrot analysis of both domestic and export chains was discontinued due to rapid

degradation of total carotenoids after freeze drying and grinding; analysis henceforth will be conducted on fresh material. These trials will be repeated as part of VV2. Results from lettuce domestic chain handling indicate no significant change in total phenolics, anthocyanins or antioxidant capacity (FRAP) after 3 days at 4C followed by 9 days at 7oC. This indicates that phytonutrients do not decline in red lettuce over a 12 day period, providing the cool chain is maintained.,.

Activity 2:

The first capsicum storage trials have been completed and the data is being analysed. We noted that the condition of the fruit going into the trial is a critical factor in the storage and shelf life of capsicums.

Activity 3:

Not completed – will be conducted as part of VV2 using Hazera ACE capsicum lines.

Activity 4:

The molecular component of this work has identified 5 expansin genes in capsicum. Expression analysis of the genes will be carried out in the first quarter of 2009. One keynote address and two refereed papers have been produced from cell wall research.

Key Project 4.2 Texture and dietary fibre

Activities

1. Develop rapid protocols for assessment of cell wall (dietary fibre) content and composition in red lettuce and Asian brassicas: CFR.

2. Identify the impacts of postharvest storage regimes on texture, cell wall content and dietary fibre quality: CFR.

Milestones

Months	Milestone	Responsibility
6	Assess analysis protocol for cell wall/dietary fibre quantification for application to red lettuce.	CFR (FRST)

Status:

Completed. We freeze-dried red and green lettuce from 6 different supermarkets in Palmerston North, and assessed whether a total dietary fibre assay could be used appropriately with these samples. Of the samples obtained, three were frilly red, one was a softer bronzy oak, and two were frilly green. The amount of dry matter varied according to supplier for the reds, but there was no real difference between red v green. Total fibre analysis was very simple to adapt from the method used for other crops. Lettuce ranged from 1.67 to 2.10 g fibre/100 g fresh weight. There was almost no difference in fibre recovery on a dry weight basis for any of the samples, suggesting that the small differences found on a fresh weight basis are simply due to a variation in leaf water content.

Key Project 4.3 Processing

Activities

1. Plan and commence commercial trials on salad and stir fry mixes with VVMP members: DPI & CFR

2. Complete research on overcoming the effects of cold temperature storage on starch degradation and subsequent processing quality: CFR (FRST).

Milestones

Months	Milestone Activity 1	Responsibility
6	Commercial trials on salad and stir fry mixes investigating ingredient selection, mixes, taste and shelf life commenced.	DPI & CFR
Months	Milestone Activity 2	Responsibility
6	Invertase inhibitor isoforms characterised in various potato cultivars using antibodies.	CFR (FRST)

Status:

First round screens completed on mixes grown in autumn. This will need to be repeated in winter, spring & summer (as part of VV2) to ensure a full picture of year round supply is obtained. Major competitor mixes have also been sampled and analysis is pending. These results, coupled with the RZ lettuce screening conducted in 2.4 will give a complete picture of the Australian lettuce market with regards to competitor antioxidant levels and potential VV opportunities. Invertase inhibitor isoforms have been

characterised for a range of potato cultivars with varying resistance to cold induced sweetening. Data is currently being analysed to identify relationships between inhibitor expression pattern and cold induced sweetening.

Key Project 4.4 Harvested vegetable responses to controlled atmosphere storage

Activities

1. Develop sea freight storage protocols for broccoli: CFR (FRST).

Milestones

Months	Milestone	Responsibility
6	Desk top survey to identify CA storage systems available commercially. Desk top survey to identify packaging materials for MAP in long term storage.	CFR (FRST)

Status:
Commercially available CA storage systems range from huge cool stores, smaller door seals to simple bag systems. We will investigate the use of a versatile bag system in our trials in 2009. A full summation of our molecular CA research was presented to end users at a conference in 2007 (Australasian Postharvest Conference, Terrigal).

Sub-Programme 5: Bioefficacy and Health Claim Data

Aim Develop new bioefficacy tests to enable effective health and marketing claims.

Key Project 5.1 Bioefficacy and bioavailability of phytochemical combinations

Activities

1. Select combinations that are in VV products: CFR & DPI.
2. Identify appropriate biomarkers and test phytochemical combinations: CFR & DPI.

Milestones

Months	Milestone	Responsibility
6	Literature review of cell-based assays for bioavailability/efficacy testing.	CFR
6	Literature review of potential synergies between phytochemicals.	DPI & CFR

Status:
Three literature reviews have been completed and written into formal reports:

1) Cell-based assays for assessing the bioavailability of key compounds in Vital Veg vegetables
2) Cell-based assays for assessing the bioefficacy of key compounds in Vital Veg vegetables
3) Potential synergies between phytochemicals in Vital Veg elite vegetables

These will provide some good starting points for further development of assays in VV2.

Key Project 5.2 Biomarkers

Activities

1. Develop new specific biomarkers that relate to the health claims being made for VV products: CFR & DPI.
2. Develop new analysis systems to allow health claims based on bioefficacy rather than solely on phytochemical content: CFR & DPI.

Milestones

Months	Milestone	Responsibility

6	Literature review for cancer biomarkers.	CFR

Status:
A literature review has been completed and written into a formal report: Cancer biomarkers relating to potential health claims for Vital Veg elite vegetables

Key Project 5.3 Gut health

Activities

1. Develop and conduct a rat feeding experiment to assess the effect of vegetable fibres on gut health: CFR.

2. Investigate the potential to identify novel non-invasive biomarkers indicative of gut health: CFR.

Milestones

Months	Milestone	Responsibility
6	No milestones due for this for period	CFR

Key Project 5.4 Feeding trials - high-selenium broccoli

Activities

1. Develop and conduct a mouse feeding experiment to assess the effect of glucosinolate and selenium from brassica sources on cancer prevention: CFR.

Milestones

Months	Milestone	Responsibility
6	Finalise animal part of experimental design and submit ethics approval.	CFR

Status:
Experimental design was completed as part of the "Supraselenium Food" proposal submitted to FRST at the beginning of 2008. If successful this bid would extend the research of Key Project 5.4, therefore the decision was made to wait on this outcome before ethics approval was sought.

Sub-Programme 6: Biochemical Pathways

Aim Understanding key control points affecting the production of health-providing constituents and the deterioration of quality in harvested vegetables. Identification of the key pathways and controlling factors to provide effective markers for germplasm screening and new breeding opportunities to maximize health content and resistance to deterioration.

Key Project 6.1 Sulphur metabolism

Activities

1. Evaluate and enhance molecular marker methods for selecting onion pungency/ bioactivity and seek new targets by identifying structural and regulatory genes for S-methyl cysteine sulfoxide (SMCO) biosynthesis in *Brassica*: CFR (FRST).

Milestones

Months	Milestone	Responsibility
6	Compile set of phenotyped onion samples and DNA samples to conduct disequilibrium study of association between ATPS/SiR and pungency in breeding materials and cultivars.	CFR (FRST)

Status:
Completed

Key Project 6.2 Genes governing bioavailability of glucosinolates

1. Outline the association of ESP and ESM gene expression in broccoli that can govern the bioavailability of glucosinolates: CFR.

Milestones

Months	Milestone	Responsibility
6	Design primers and amplify ESP and ESM genes from *Brassica oleracea*	CFR

Status:
Completed. We have amplified a ESP and a ESM homologue from *Brassica oleracea* using PCR. The gene sequences have been used to screen our broccoli cDNA library, and multiple genes of BoESP (epithiospecifier protein) and BoESM (also known as MyAP – myrosinase-associated proteins) have been isolated. This work is part of VV2. Further sequencing will be undertaken to determine how many separate genes exist in broccoli, then expression patterns will be examined (northern analysis) to identify genes that may influence glucosinolate /sulforophane content, and ultimately may be used as markers for this trait.

Key Project 6.3 Selenium uptake and metabolism

Activities

1. Assess whether manipulation of a single gene can convert a non-selenium accumulating crop plant into one with increased levels of the anti-cancer compound Se-methylselenocysteine, and investigate molecular regulation of incorporation: CFR (FRST).

Milestones

Months	Milestone	Responsibility
6	Molecular characterisation of transgenic tomato lines completed.	CFR (FRST)

Status:
Completed. Several lines of two transgenic tomato populations, one with upregulated selenocysteine methyltransferase (SMT) gene expression (35S::AbSMT) and one with upregulated SMT gene and ATP-sulfurylase gene expression (Ubq::ATPS/35S::AbSMT), have been fully characterised. Selenium feeding of selected lines has been completed and samples are currently being analysed for total selenium and methyl selenocysteine content.

Key Project 6.4 Control of pigment production for colour and health

Activities

1. Profile anthocyanins in onion: CFR (FRST).

2. Identify regulatory genes controlling pigment biosynthesis: CFR (FRST).

Milestones

Months	Milestone	Responsibility
6	A range of germplasm samples obtained for chemical analysis, genetic analysis and testing in transient gene function assays.	CFR (FRST)

Status:
DFR has been screened as a potential co-segregating gene for colour in onions, but it does not look to be a major component as yet (no SNPs identified). Several MYBs have been isolated from onion, and among the novel sequences is a candidate for the anthocyanin regulator. Using transient assays with heterologous anthocyanin regulatory genes we have been able to induce anthocyanin production in onion seedling tissues.
John McCallum and Colin Eady have obtained a range of germplasm sources. Sampling of these has started and a large range of primers has been ordered to test for association of genes to traits in the germplasm.

Key Project 6.5 Regulation of senescence after harvest

Activities

1. Identify gene promoters and transcription factors critical to the immediate response to harvest: CFR (FRST).

2. Identify molecular regulators with a wholesale effect of plant senescence through identification of early protein changes, the role of proteases in cell death, and identification of components of Bax inhibitor cell death pathway in harvest-induced senescence: CFR (FRST).

Milestones

Months	Milestone	Responsibility
6	Specific harvest treatment times and conditions determined for microarray, RNA isolation begun and first construct for delaying postharvest senescence made. Purify MBP-tagged recombinant BoBI-1 and BoUSP proteins for antibody production in NZ white rabbits at Massey University	CFR (FRST) CFR (FRST)

Status:
Completed. We tested whether harvested immature *Arabidopsis* inflorescences could be used to model senescence events of harvested broccoli heads. We showed that the sepals of the harvested *Arabidopsis* inflorescences yellowed when placed in the dark and showed using Real Time PCR and northern analysis that gene changes occurring in harvested Arabidopsis inflorescences are similar to that occurring in harvested broccoli heads.

We have made an RNAi construct for silencing the broccoli ortholog of AtNAP, a senescence-controlling NAC transcription factor. We have also made a silencing construct for an unknown gene that is localised to chloroplasts and one of the most strongly senescence upregulated genes in the *Arabidopsis* genome.

We have previously isolated proteins that putatively bind to the Bax Inhibitor protein (a cell-death related protein). Gene expression of all binding partners indicates they are expressed at the same time as the Bax Inhibitor gene and we have produced polyclonal antibodies to both Bax Inhibitor and BoUSP (Universal Stress Protein, first binder to be isolated. Four other genes have been cloning into expression vectors for future functional analysis.

Broccoli plants with altered protease activity have been grown and analysed. We have confirmed the activity of a protease inhibitor (BoPI) modifies specific cysteine protease activity (profiling experiments), although how the protein functions to inhibit cysteine protease action has yet to be determined. Plants with altered BoPI have characteristic early-death phenotypes, and a second trial to examine whether freshness is maintained will be done next year.

Key Project 6.6 New tools for accelerated plant improvement

Activities

1. Develop a strategy for linking molecular information and bioefficacy studies to assist in the development of new breeding targets: CFR.

2. Improve accessibility of molecular and biochemical tools in vegetable breeding programmes: CFR.

Milestones

Months	Milestone	Responsibility
6	Desktop evaluation of screening concept completed	CFR (Erin)

Status:
Completed. A desktop evaluation of strategies that link molecular and nutritional/health components, and assessment of the benefit of this as an approach have been completed. My opinion on this option is that VV should consider the judicious use of metabolic fingerprinting, or more quantitative (but more narrowly focused) metabolic profiling to provide a faster and more effective means of identifying germplasm with multiple health-enhancements, for new crops that have not yet been a VV focus. We should consult with experts in the metabolomics and genomics fields and there appears to be a useful opportunity to use facilities such as Metabolomics Australia, based at the University of Melbourne

(www.metabolomics.net.au; interim CEO Prof Tony Bacic, manager Dr. Ute Rosenner-Tuneli), and to involve the VV germplasm providers (who may have marker assisted selection capabilities and gene maps), if not in Australia/New Zealand, then in their parent companies) to link information on the metabolome of a chosen vegetable to a means of breeding higher-health vegetables.

A project that lines up a suite of bioavailability/effectiveness parameters and non-targeted metabolomic fingerprint with genetic information into QTL, in the same way as phenotypic linking of more standard properties (e.g., quality-related, visual or agronomic properties) would be a powerful way to generate new breeding opportunities for developing vegetables with enhanced or multi-layered health properties. Such an approach would fit with the VVGP and VVMP end-goals, as well as the overall aspirations of the VV programme, as well as being up-to-the-minute science.

This direction was discussed at the VV planning meeting in June and a metabolomics investigation is now a part of SP2.