Australian Government

Cotton Research and Development Corporation

FINAL REPORT 2010

If you are participating in the presentations this year, please provide a written report and a copy of your final report presentation by 31 October.
If not, please provide a written report by 30 September.

Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: **CSP0004**

Project Title: Development of cottonseed oils with improved nutritional and functional properties

Project Commencement Date: 01/10/2008 **Project Completion Date:** 1/11/2009

CRDC Program: 5 Breeding and Biotechnology

Part 2 – Contact Details

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Background

1. Outline the background to the project.

As a by-product of more valuable cotton fibre, cottonseed oil (CSO) in its current form is widely used in various food applications around the world. In order to maintain and expand the market use of cottonseed oil, CRDC and CSIRO has co-invested in the genetic modification of cottonseed oils in previous years and this has led to the development of MonoCott, the world's first nutritionally improved and value-added cottonseed oil. The distinct feature of the novel germplasm is its radically altered seed oil composition containing a five-fold increase in oxidative stable and heart healthy oleic acid, and concomitant major reductions in nutritionally undesirable fatty acids, including palmitic and cyclopropenoid fatty acids. MonoCott is the ultimate outcome of several previous CRDC/CSIRO projects on genetic modification of cottonseed oil. The radical re-design of fatty acid composition which has been successfully modified to address the consumer concerns over the saturated and trans fatty acids should enable cottonseed oil compete more effectively with other rival vegetable oils, such as canola and soybean oils .

This project concerns OGTR approved seed increase under field conditions including the evaluation of MonoCott for its physiological and agronomic performance under typical Australian cotton farming conditions. The information and data collected through this project is pivotal for potential commercialisation of this valuable germplasm. The seeds increased through the trial can also provide necessary raw materials for functional evaluations by various industries including the food, feed and biodiesel industries.

Objectives

2. List the project objectives and the extent to which these have been achieved.

This project had two main objectives. Firstly, it was conducted to validate the performance of the new quality trait under field conditions and to confirm that it has no negative impacts on crop performance. This is essential information to demonstrate that the improved seed oil trait can be integrated into cotton varieties without any negative consequence on cotton production. Secondly, the trial was used to generate sufficient seeds to enable evaluation of performance in functionally improved food, feed and biodiesel applications. These evaluations will provide critical information for the relevant industries to determine their interest in utilising MonoCott germplasm based on its advantage over other oils, and making decisions for further R&D investment as well as the commercial exploitation.

Methods

3. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

- Select and verify homozygous MonoCott cotton line and multiply sufficient seeds in PC2 glasshouse growth facilities for planting at the field trial site.
- Work with the numerous key players including OGTR for approval of limited release in designated field trial site; with field scientists and land owners for carrying out the planning, planting, collecting data in field, harvesting, ginning and post-harvest monitoring following OGTR regulations. The MonoCott line DCS9-34 and pollen trap plants (Sicot71) were planted on November 6, 2008, on the "Appletrees" site owned by Auscott Ltd. It is located approximately 5 km north of Australian Cotton Research Institute (ACRI). The size of the GMO planting was 1.15 hectares. A small

experiment consisting of 4 replicates of 40 m^2 plots enabled the comparison of the GMO and the cultivar Coker 315 from which it was derived. The GMO was surrounded by a 20 metre wide buffer zone. Plant vigour and morphology were closely observed and recorded by CSIRO scientists throughout the entire growth period. The GMO and surrounding buffer zone were harvested on the 14 May 2009. All the seed cotton of the GMO was harvested separately and transported to ACRI and ginned at the ACRI ginning facility between 18-21 May. A total of 1.4 tonnes of MonoCott seeds were collected and subsequently transported to CSIRO Plant Industry Black Mountain (ACT) Laboratories where they were stored in a lock-up PC2 seed storage facility. All the other plant materials derived from the trial were destroyed in accordance with OGTR requirements.

- Work with cotton breeders in data collecting and analysing a range of important traits, including fibre quality, fibre yield, oil yield and quality. Traditional agronomy and plant physiology approaches were used by field scientists for a range of measurements. HVI technology commonly used by cotton industry was used to evaluate fibre quality.
- Extract and provide oil to food scientists for analysis of oxidative stability. Small volume of oil was extracted using Soxhlet extractor from the MonoCott mature seeds and provided to Dr Chakra Wijesundera in Food Science Australia, Werribee, Victoria, for oxidative stability analysis by measuring breakdown compounds produced under accelerated oxidative conditions.
- Store MonoCott seeds in PC2 facility and prepare for further analysis including pilot scale food flavour analysis and animal feeding trials with approval of OGTR and Animal Ethics Committee.

Results

4. Detail and discuss the results for each objective including the statistical analysis of results.

The sample plots were harvested separately and hand ginned. Comparisons were made among three entries, including the GMO (MonoCott), its parent variety (Coker315) and an elite Australian variety, Sicot71, for a range of yield and quality parameters. Fibre quality was analysed using the HVI system. Although Sicot71, as a current elite variety, showed superior yields of both seed cotton and fibre, there was no significant difference between MonoCott and its parent Coker315 in all the measurements (Figure 1). This clearly indicates that the genetic modification of fatty acid composition has no significant impact on plant growth, morphology and yield and quality.

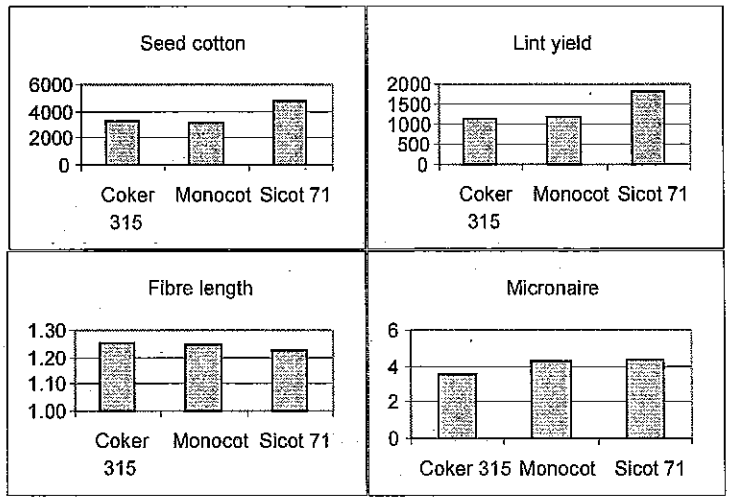

Figure 1: Performance of MonoCott, Coker315 and Sicot71 in DIR085 field trial in Narrabri in 2008/09 season. Parameters examined are yield of seed cotton (kg/ha), fibre yield (kg/ha), fibre length (cm) and micronaire.

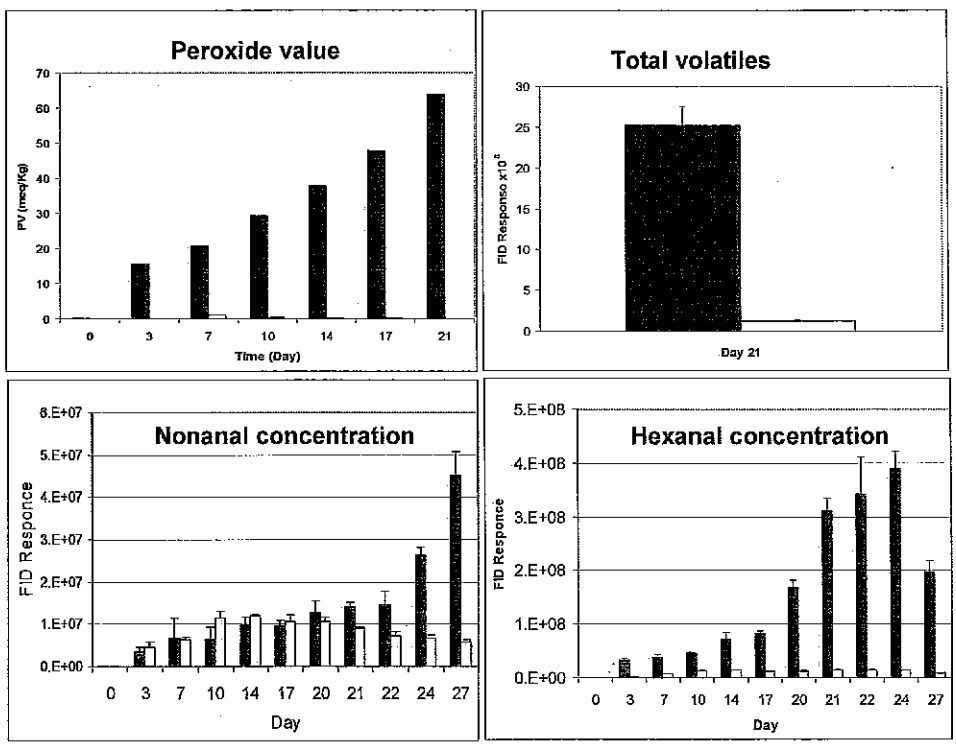

Figure 2: Oxidative stability analysis of MonoCott oil (yellow bars), in comparison to its untransformed parent Coker315 (red bars). Peroxide values, concentrations of total volatiles, and individual volatiles, such as nonanal derived from autoxidation of oleic acid and hexanal derived from linoleic acid, were measured using mass spectrometry.

About 50 ml of seed oil was extracted from MonoCott (GMO) and Coker315 (Control) using Soxhlet system with organic solvent in a CSIRO PC2 facility. The oils were then subjected to oxidative stability analysis under accelerated oxidation conditions at Food Science Australia, Werribee, Victoria.

As shown in Figure 2, MonoCott cottonseed oil has extremely low peroxide value indicating a very low susceptibility to autoxidation for the full time period of testing up to 21 days. This is in sharp contrast with its untransformed parent variety Coker315, which had significant and increasing PV values over the same period. Likewise, the concentrations of volatile oxidative products, including hexanal (derived from breakdown of linoleic acid) and nonanal (derived from breakdown of oleic acid), were minimal in the MonoCott line compared to Coker315. All these results are highly encouraging for the further development of MonoCott because of its extraordinarily high oxidative stability.

Outcomes

5. Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

The output of this short project includes the evaluation data of field performance of MonoCott cotton with significantly improved oil qualities, and the seed material made available for further industrial evaluations for value added applications. MonoCott cotton is a world-first nutritionally improved cottonseed oil that combines high monounsaturates with low saturates and low CPFAs. MonoCott oil is characterised by high oxidative stability, low processing costs, and greatly improved health appeal to consumers. It is expected that the its commercialisation could reposition cottonseed oil as a premium product in the vegetable oil market, enabling it to compete against the expected increasing encroachment of other high-oleic oils into that market, and potentially to enter the retail bottled oil market for the first time. The lowering of the antinutritional cyclopropenoic fatty acids provides an improvement in feed value by enabling higher incorporation of cottonseed in livestock rations. The oil composition changes also improve the versatility of cottonseed oil by opening up the possibility of its use in the emerging biodiesel fuel industry. Currently cottonseed oil is not well-suited to biodiesel because of its high levels of saturates and polyunsaturates.

In addition to the commercial aspects, the data collected through the field trial has significant scientific values as there is no precedent knowledge about the simultaneous RNAi down-regulation of the three enzymes, i.e FAD2, CPFAS and FatB, involved in oil biosynthesis in any plant. The physiological and agronomic performance of the selected GM cotton plants with radically altered fatty acid composition in seed oil will enrich our understanding of the coordinated accumulation of various fatty acids in cottonseed oil.

6. Please describe any:-
   a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);

   The data derived from the current field trial and oil stability test of MonoCott cotton is not included in the patent application. However, they are significant in the decisions for further commercial development of this novel germplasm. The provisional international patent of MonoCott has been filed in July 2009. The patent for the methods of genetic modification of cottonseed oil to achieve high-oleic trait has been issued in the US (US 6,974,898 B2) and Australia (WO0179499). It is also currently under examination in other countries.

   b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and

   There is no other development through this project.

c) required changes to the Intellectual Property register.

There is no need for changes in IP register at present.

Conclusion

7. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

The successful completion of MonoCott cotton field trial is a significant advancement towards the development of nutritionally improved cottonseed oil. The current project has generated some valuable data on the field performance of MonoCott and generated seed source for various application testings. The transgenic event has no detectable negative impact on the plant physiology, plant growth, the yield and quality of both fibre and seed. The initial testing of oxidative stability of seed oil generated through this field trial has already demonstrated impressive improvement of oxidative stability. The 1.5 ton of seeds generated from this field trial are sufficient for further analyses in the food application area including a pilot scale food flavour test and margarine production. Seeds will also be allocated for additional field trial assessments at possibly multiple sites, pending for the decisions on further commercial development.

CRDC and CSIRO conducted a business case study on MonoCott cottonseed oil which indicates that there is a clear economic and social benefit in adopting MonoCott cotton germplasm by Australian and international cotton industry. The current world production of cottonseed is about 36 million metric tonnes per annum, while Australia has a production capacity of one million tonnes. The domestic cottonseed oil supply in Australia is currently limited by Cargill's crushing capacity at Narrabri. Therefore, production of MonoCott oil is likely to be restricted to 40,000 tonnes, coming from 250,000 tonnes of seed crushed, which is around 20-30% of the Australian cotton crop. For the stockfeed industry, MonoCott seed offers the advantage of substantially reduced CPFAs which are toxic and antinutritional to animals. Currently more than half of cottonseed produced in Australia and the US are used as stockfeed. Adopting MonoCott cotton could therefore deliver significant benefits to animal rations. MonoCott oil is also expected to have superior functionality when used as feedstock for biodiesel, as it contains significantly less oxidatively unstable polyunsaturates and solid fat components.

Extension Opportunities

8. Detail a plan for the activities or other steps that may be taken:
   (a) to further develop or to exploit the project technology.

The current project has been successful in achieving its objectives including field evaluation of the MonoCott cotton plant and seed increase for further evaluations in various industrial application areas. Additional field evaluations at multiple sites would be necessary prior to pursuing direct commercial planting. It is also envisaged that the inclusion of an additional trait, reduced level or free of gossypol could ensure the commercial success of such a nutritionally and functionally enhanced product. The complete elimination or substantial reduction of the toxic gossypol, would not only save the extra cost of removal during oil processing, but also broaden the application of whole cottonseed and cottonseed meal as more palatable and nutritionally enhanced livestock and aquatic feed.

MonoCott trait is controlled by a single transgene and it would be transferred to elite cotton varieties through backcrossing assisted with genetic/biochemical selection on the MonoCott trait. It is anticipated that within Australia this would happen through the current commercial

pathways for CSIRO-bred cotton varieties. Discussions are underway with potential global commercialisation partners.

(b) for the future presentation and dissemination of the project outcomes.

We have already presented the research outcome at the Australia Oil Federation (AOF) meeting. We are currently preparing manuscripts for publication in peer reviewed journals.

(c) for future research.

The MonoCott trait provides an excellent platform for further development of value-adding and multi-functional genetic material. For example, the high-oleic and low-CPFA traits could be combined with high saturate germplasm to create cocoa butter substitute. Further research could also be directed to develop a super high oleic germplasm which is highly sought after by the oleochemical industry.

8. A. List the publications arising from the research project and/or a publication plan.
(NB: Where possible, please provide a copy of any publication/s)

Liu Q, Wijesundera C, Singh S and Green A. A genetically modified cottonseed oil with enhanced oleic acid content and significantly reduced palmitic and cyclopropenoid fatty acids. World Congress on Oils and Fats &28th ISF Congress 27-30 September 2009, Sydney, Australia.

B. Have you developed any online resources and what is the website address?

No.

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

Cottonseed is a valued by-product of cotton fibre production and ranks as one of the most important oilseed worldwide. Cottonseed oil is widely used in food service sector because of its neutral flavour and its ability to be partially hydrogenated into a hard-stock for margarine production. However, the relatively high level of saturated fatty acids and trans fatty acids (TFA) produced during hydrogenation are nutritionally undesirable because of their LDL-cholesterol raising properties. In addition, the presence of small amounts of cyclopropenoid fatty acids has severely limited the levels of incorporation of cottonseed into animal feeds.

With substantial research investment by CRDC/CSIRO, we have successfully achieved a radical redesigning of the fatty acid composition of cottonseed oil, removing the nutritionally undesirable components at the same time as improving the functional properties of the oil. This was achieved by down-regulating specific genes involved in the fatty acid synthesis in cottonseed using advanced RNAi-mediated gene silencing technologies. The new cottonseed oil, termed MonoCott, is high in oleic acid, low in palmitic acid and low in CPFAs.

The current project enabled us to successfully conduct an OGTR approved field trial during the 2008-09 cotton growing season in Narrabri, NSW. This demonstrated that there is no significant variation between MonoCott and its parent, Coker315, in a range of agronomic parameters measured, including fibre yield and quality, and seed yield. Analysis of oil composition of seeds confirmed the high-oleic fatty acid composition of MonoCott was maintained under field conditions, and resulted in extremely high oxidative stability, indicated the suitability of MonoCott cottonseed oil as a high-stability food oil. Seeds obtained from this trial provide a valuable material for further more detailed study of oil stability, flavour and functionality, as well as performance of the seed and seed meal in animal feeding trials. In addition to the commercial aspects, the data collected through the field trial has significant scientific merit as it has enriched our understanding of the coordinated accumulation of various fatty acids in cottonseed oil, in drastically altered form, without significant impact on cotton physiological and agronomic traits.

MonoCott has substantial commercial potential and can deliver significant social, economic and environmental benefits. MonoCott oil is expected to have extended shelf life and excellent oxidative stability, and therefore will be suitable for direct use as high performance frying oil in the food service sector, without the need for hydrogenation. This is highly appealing to increasingly health-conscious consumers because it will contain no trans fatty acid and much lower level of saturated fatty acid compared to the conventional cottonseed oil. It is also more suitable for broad applications in animal feed industry as it contains significantly less antinutritional CPFAs. It could also be more widely used as feedstock for biodiesel production as it has superior oxidative stability and better cold flow properties. Without these quality improvements, cottonseed oil can be expected to lose market share or suffer price decline compared to quality-enhanced forms of other vegetable oils that are now entering the marketplace.

SCHEDULE 2 - IP REGISTER

Project Code	Material Description	IP Category	Ownership	Nature of IP	Conditions of Use	Freedom to Operate	Confidentiality Issues	Commercial Significance	Risks Identified
(CRDC Code)	(Information, article, report, equipment, computer model incl date created)	(Background, Third Party, Project Technology IP or Scholarship IP. Separate background & Project IP)	(Name(s) of Party inventor; student organisation or researcher which or who owns the IP.)	(copyright, trade secret, patent(provisional standard or innovation -list filing date), exclusive/non-exclusive licence(s). If shared IP, detail bass. Renewal dates, Jurisdictions covered by protection. Nation, internation-list countries)	subject permit, licence, research agreement: revocable/Irrevocable; Local/Worldwide; Free/Royalty; Unconditional/Conditional use in Projects; Only CRDC; all non-commercialistion uses; commercialisation any background conditions)	(Access granted by owner of IP: Yes/No/Pending)			
CSP0004	US patent issued, 6974898	Previous project IP	CSIRO	Standard patent, granted 2005	use in project	Yes	External release	world wide applications	
	Australian Patent No. 2001250163	Previous project IP	CSIRO	Standard patent, granted 2007	use in project	yes	External release	world wide applications	
	US patent issued, 7619105	Previous project IP	CSIRO	Standard patent, granted 2009	use in project	yes	External release	world wide applications	
	European Patent application No. EP1282709	Previous project IP	CSIRO	Filed 17/4/01	use in project	Yes	External release	world wide applications	
	PCT application	previous project IP	CSIRO	filed 22/07/2009	use in project		confidential	worldwide application	
	Kanamycin resistance: Chimeric genes suitable for expression in plant cells. US patent: US 6174724.	Third party IP	Monsanto	Standard patent		use will need licence	already in pubic domain	world wide application	
	Cotton transformation: Treatment of cotton. U.S. Pat. No. 4,796,334;		Monsanto (formerly Agracetus)	Standard patent		use will need licence	already in pubic domain	world wide application	
	FAD2 gene sequence: Genes for microsomal delta-12 fatty acid desaturases and related enzymes from plants. WO 1994/011516 A1		DuPont	Standard patent		use will need licence	already in pubic domain	world wide application	
	FatB gene sequence: Plant acyl-ACP thioesterase sequences. WO 1995/013390 A3		Calgene, Inc. Davis, CA	Standard patent		use will need licence	already in pubic domain	world wide application	
	CPA-FAS gene sequence: Plant cyclopropane fatty acid synthase genes, proteins and uses thereof. WO03060079.		Michigan State University, USA	Standard patent		use will need licence	already in pubic domain	world wide application	

	SCSV promoter: Circovirus transcription regulatory sequences and related constructs -useful in plants, esp. leguminous plants, for the modulation of gene expression. World Patent Number AU9606932-A1.		CSIRO	Standard patent		use will need licence	already in pubic domain	world wide application	
	RNAi gene silencing technology: "Methods and means for obtaining modified phenotypes", Australian patent 760041. Other patents and applications based on WO99/53050.		CSIRO	Standard patent		use will need licence	already in pubic domain	world wide application	

Signed by: _____ Date: _____

((Name, Position, Organisation))