

## Summary

## Summary

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### Client report summary:

<b>Key:</b>	CONT-47267-CRFRP-AGR C10X1603-CR-5
<b>Project:</b>	Forages with Elevated Photosynthesis and Growth
<b>Contract ID:</b>	C10X1603
<b>Investment process:</b>	CRFRP 2016 Contestable Research Fund - Research Programmes
<b>Organisation:</b>	AGR AgResearch Limited
<b>IMS assigned to:</b>	s9(2)(a)
<b>Reporting period:</b>	01/07/2020 to 30/06/2021
<b>Contract total value:</b>	\$11,500,000.00
<b>Team:</b>	

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## Progress Reporting

### Annual Update

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#### 2020-21 Annual Update

#### **Outcome Benefits to New Zealand**

##### **Science Progress**

High Metabolisable Energy (HME) ryegrass is being developed as a future option for grazing pastoral farmers in New Zealand, Australia and in temperate climates internationally, to help reduce environmental impacts of grazing ruminants while increasing farm efficiency and productivity.

The programme is designed to develop new genetically modified (GM) varieties of ryegrass with increased lipid levels, to deliver increased energy for livestock. Over the last five years we have progressed our understanding of the mechanisms for enhanced photosynthesis and the physiological and biochemical changes including water use and nitrogen use efficiency. This led to new patent application on sustaining photosynthesis to create value for investors by increasing patent life. We have developed a unique world leading genetically modified plant breeding capability run entirely within PC2 containment with support from our seed industry partners. This programme underpins the pastoral industry funded field trial analysis of HME ryegrass. The plant breeding capability has supported the field trial analysis by developing seed in elite founder plants expressing the HME trait. These have been assessed in USA based field trials from 2017-2021.

The initial HME ryegrass lines were developed prior to 2016 using the gene gun transformation technology. This had the unintended effect of yielding lines with multiple copies of the HME transgenes, which created challenges for breeding. Despite these challenges, the HME ryegrass lines were tested in the field between 2017 and 2019. During 2018 and 2019 we developed a superior Agrobacterium based transformation system and 100+ Agrobacterium-transformed HME ryegrass events were produced and screened for desirable characteristics (*e.g.* increased plant fat, single copy, single locus T-DNA insertion events). We used Whole Genome Sequence analysis to map the exact location of the transgene insertion in 18 events and confirm the intact status of the T-DNA insertion. Plants with the HME phenotype entered the breeding pipeline and were crossed into elite germplasm provided by seed company partners.

##### **Translation from lab to field**

The 2019 Field Trial in the USA demonstrated that the increases in fatty acids, gross energy and plant growth measured in a PC2 containment facility translated into the field. Field trials in 2020 and 2021 focused on the Agrobacterium-derived material and evaluated the performance of hemizygous and homozygous progeny of a line intended for animal nutrition trials. Plant fatty acid content and gross energy were consistent with controlled environment generated data, confirming the suitability of this line for animal nutrition trials.

##### **Commercial Applications**

We use a co-innovation approach towards delivering HME Ryegrass as a commercial elite variety with significantly enhanced qualities. Elite germplasm has been provided by seed industry partners and used in the breeding programme. Advice on requirements for the planned Animal Nutrition Trial is also provided by the relevant Technical Advisory Group, whose membership includes s9(2)(b)(ii) as representatives of one of the key customers and end users.

##### **New product on the market**

The Programme's strategy is to have new high value HME Ryegrass varieties available for sale by 2028. It requires several reproductive cycles to identify suitable lines for breeding, then another series of cycles to produce enough seed to go to market (each cycle takes 1 year).

The new HME Ryegrass is predicted to deliver higher levels of lipid in the main forage species used on NZ farms. At present, evidence of benefit comes from nutrition models, supplementary feeding experiments and *in vitro* experiments. This will enable potential productivity efficiency gains (increased meat and milk from lower forage inputs), improved nitrogen partitioning in the animal leading to reduced nitrogen excretion and subsequent leaching, and reduced methane emissions of 10-17% (via lipid inhibition of methane production). The 2023 animal trial will assess methane inhibition and estimate nitrogen partitioning in the animal.

### **Increasing recognition of new industry advisory group members**

In the past the Programme has delivered a number of media pieces that have raised awareness of the technology, and its potential to impact positively on productivity and environmental change. Part of the Programme has included liaising with Farmer Groups (Objective 5), and this has found continued recognition and desire to introduce the new technology. Discussing outcomes and impacts with stakeholders is a managed process to ensure that the messaging is synchronised with the new knowledge being generated. The farmer engagement has helped to understand adoption behaviours of pastoral farmers and this can help the commercialisation pathway.

### **Supporting development and commercialisation of new research areas**

Aspects of the research have identified new applications for this technology, which will be protected by new patent applications. This not only extends the commercial life-time of the HME Ryegrass technology, but also opens the potential to develop the new technology towards new applications.

### **Commercialisation of technology in other crops**

AgResearch works closely with its US partner ZeaKal, Inc. ([www.zeakal.com](http://www.zeakal.com)). Zeakal has progressed commercialisation in soybean, the largest crop in the USA (38M ha). Proof of concept for enhanced photosynthesis and benefit to soybean yield and composition has been achieved and the product is undergoing the regulatory approval process. s9(2)(b)(ii)

### **Implementation Pathway**

#### **Timely data delivery**

Over the last reporting period the Programme has made steady progress and met its agreed milestones. The earlier than expected shift from using HME lines generated by gene gun-mediated transformation, to lines generated by *Agrobacterium*-mediated transformation significantly advanced the project's timetable. Progress was even further enhanced when the programme developed the Rapid Homozygous Breeding plan to cross *Agrobacterium* generated lines with elite commercial varieties. This means that by the end of the project the research team is expecting to have data that will support the delivery of significantly improved material, with greater genetic stability, which can be used to achieve the **2 year post-contract (2023)** outcomes.

#### **Enhanced innovation pipeline**

Early results clearly showed that while the initial gene gun-mediated method could produce lines with the desired trait (*i.e.* higher lipid levels), the lines were often genetically complex and unstable. The subsequent in-house development of the novel *Agrobacterium*-mediated transformation method helped to ensure that the Team was able to generate more derivations of suitable material that would be used in the production of commercial-ready lines.

#### **Technology Transfer**

Experts within the project's partner seed breeding organisations have reviewed the Rapid Homozygous Breeding plan and consider it to be a new gold standard approach to producing seed under controlled (glasshouse *cf.* field) conditions.

The novel *Agrobacterium*-mediated transformation method is also being utilised by the Team to support the University of Otago's MBIE-funded programme - *Generating non-heading ryegrass* (CONT-61385-ENDSI-UOO).

### **New Commercial Partnerships**

The current commercial partners continue to provide valuable guidance around delivery, particularly in the design of the series of open field trials run over the past four years in the US. AgResearch and its partners are developing a commercialisation strategy to progress this technology post the end of this contract. This has included engagement with s9(2)(b)(ii) and other parties to identify research and delivery priorities. We are investigating options for commercialisation in New Zealand and Australia. This is likely to include new partners.

### **Research Science and Technology Benefits to New Zealand**

#### **Embedding Vision Mātauranga**

The Research Team has consulted with AgResearch's Māori Strategy & Engagement team to try to identify how aspects of Vision Mātauranga and the HME Ryegrass programme's technology and goals may mesh together and build on one another. There is agreement that while there may not be areas in common in the research, there does need to be an awareness and dialogue around how genetically modified organisms (GMOs) may - and in some circumstances may not - align with Māori values. As part of developing a wider dialogue around GMOs in NZ, the HME Ryegrass work was represented during discussions at a recent wānanga (Māori, Genetics, and Genomics | Wānanga Tuatoru, Cambridge; July 2021).

#### **Proof of concept**

Feeding of HME Ryegrass to livestock and measuring: Energy intake; Methane emissions; Nitrogen partitioning; Productivity; etc., is aimed to provide definitive proof of concept. This is planned to occur in 2023/24, when the Team is expecting to have generated enough seed of the optimum HME Ryegrass line to plant and manage enough pasture to feed approximately 16 cows for several weeks. The seed currently needs to be produced in NZ under containment, and this is limited by the availability of suitable containment glasshouse space.

#### **Other Information**

##### **Capability Development**

s9(2)(a)

##### **Science Outputs**

s9(2)(a)

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## **Publicly Available Information**

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High Metabolisable Energy (HME) Ryegrass is being developed as an option for farmers in New Zealand, and temperate climates overseas. HME has the potential to increase farm productivity while reducing livestock's environmental impacts, for example nitrogen leaching and methane emissions. Many of the environmental impacts occur because the proportion of protein in NZ's forage plants is far in excess of the energy available to livestock. This means that there is an excess of nitrogen from plant proteins, which is excreted in urine and subsequently lost from the farm through nitrate leaching. In addition, the greenhouse gas methane is produced by methanogenic microbes in the rumen (stomach) of livestock as they digest the forage.

To improve the nutritional balance and increase the overall amount of energy available to livestock on each hectare of pasture the Team has produced genetically modified (GM) plants that has specialised microscopic oil micro-organelles in the leaves of ryegrass. This extra oil, while only being a small proportion (2.3%) of the plant's dry matter, delivers up to a 10% increase in the amount of energy available to an animal eating the plant. This means that an animal can eat less grass to obtain the energy it needs, then along with that - lower intake means less excess protein/nitrogen in the urine.

This project was started almost 20 years ago and since then the amount of methane produced by NZ's livestock has come under national and international scrutiny due to its impact on increasing global warming. However, along with reducing nitrogen losses, HME Ryegrass also has the potential to reduce methane production. Studies have shown that livestock diets with higher amounts of oils in their diets produce lower amounts of methane. By matching the level of oil in our plants against the results from those other studies, it appears that HME Ryegrass has the potential to reduce methane emissions by 10-17%. This may not be the 'silver bullet' but when combined with other products in development it can become part of the bigger solution to global warming and climate change.

Serendipitously we also found that under good growing conditions HME Ryegrass has enhanced photosynthesis and growth. All plants have exquisite control mechanisms that allow them to effectively 'snack' on light as needed, where one of these control mechanisms is the rate of carbohydrate/sugar formation going on in the plant. By using the carbon-based molecules, typically used to produce carbohydrates, to produce additional oils instead, it appears that the plant overcompensates, capturing over 20% more carbon dioxide to and converting it into more plant biomass and energy.

While it was possible to apply to undertake field trials with the GM HME Ryegrass in NZ, it was decided that the programme would be able to generate the information it required within a shorter timeframe by conducting trials in the mid-west of the USA. In 2020 we demonstrated that key characteristics of heterozygotes (one copy of the HME Ryegrass transgenes) were similar when measured in PC2 containment growth chambers and glasshouses or the field. This year we have taken the breeding one step further and have developed homozygotes (two copies of the HME ryegrass transgenes) and again shown that the even greater increase in oil in homozygous plants (compared to heterozygous plants) translated from lab to field.

## Key Achievements

Sequence	Key achievements

1	<p><b>Novel mechanism for enhanced photosynthesis</b></p> <p>Plants have exquisite feedback mechanisms to regulate photosynthesis based on the carbohydrate status of the plant. We identified that the HME technology enables the plant to overcome this negative feed-back and both elevate and sustain photosynthesis. This enables plants to capture up to 24% more carbon dioxide, converting this into greater levels of plant energy and in favourable conditions also translate this into accelerated growth. The technology is applicable to all plant species, both C3 and C4 photosynthetic crops. A new patent protecting this invention was filed in 2019. This will extend protection for the technology by an additional 10 years beyond the life of the Cysteine Oleosin patent (expires in 2029) if granted. Commercial applications in soybean, hemp and corn are underway in the USA under a licence to ZeaKal Inc. s9(2)(b)(ii)</p> <p>This demonstrates that a novel technology developed in New Zealand for forages can provide international benefits in multiple crops and help farmers world-wide to maintain productivity while reducing environmental impacts including greenhouse emissions.</p>
2	<p><b>World class GM trait integration capability</b></p> <p>The protocols developed by the programme to Integrate the HME trait into the commercial ryegrass lines was recognised by one of NZ's leading ryegrass breeders as a world-leading methodology. The design enabled homozygous lines to be produced in elite founder plants approximately one year ahead of schedule, and the first homozygous plants of a promising line are currently (August 2021) undergoing field trial analyses in the USA.</p>
3	<p><b>Increase in Plant energy</b></p> <p>One key result from the analyses of the homozygous HME lines that were grown in the glasshouse is the 1 kJ/g DW increase in energy. This is approximately equivalent to a 10% increase in energy and is a step-change in ryegrass nutrition - it has been calculated that to achieve the same increase using traditional breeding methods would have taken over 40 years.</p>
4	<p><b>Altered nitrogen use and water use efficiency</b></p> <p>We discovered HME ryegrass has altered nitrogen use efficiency and can tolerate increase levels of reduced nitrogen (ammonium and urea). HME ryegrass also has altered water use efficiency and transpires at greater rates in ideal conditions compared to conventional ryegrass. This is associated with enhanced photosynthesis and growth. Under water limiting conditions HME ryegrass faces no penalty compared to conventional ryegrass.</p>

5	<p>s9(2)(i) [REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p>
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## Project Deliverable Status

Click on the deliverable to enter a status

Sequence	Short title	Type	Due Date	Status	Reason	Action
1	Carbon Dioxide Recycling in HME Ryegrass	Impact statement	31/03/2021	Achieved		
1.1	Infra-Red Gas Analysis	Research aim	31/03/2021	Achieved		
1.1.1	IRGA analysis of Ryegrass	Critical step	31/10/2018	Achieved		
1.1.2	IRGA analysis of rice	Critical step	31/03/2021	Achieved	<p><b>VARIATION (2020):</b> <i>This was due to the COVID lockdown; we were unable to perform IRGA analysis of the HME rice plants. We were able to collect seed. This will be repeated in the future. We were able to complete the IRGA analysis by March 2021.</i></p> <p><b>RESULTS:</b> No difference in photosynthetic performance was observed between non-HME and HME rice.</p>	
1.2	Isotope partitioning of metabolic pathways	Research aim	30/09/2020	Achieved		
1.2.1	Isotope partitioning in model species	Critical step	23/12/2019	Achieved		
1.2.2	Isotope partitioning in forage species	Critical step	30/09/2020	Achieved		
2	Nitrate Utilization in HME Ryegrass and other species	Impact statement	30/09/2020	Achieved		

2.1	Nitrate utilization in C3 plant species	Research aim	30/09/2020	Achieved		
2.1.1	Nitrogen utilization in model species	Critical step	30/09/2019	Achieved		
2.1.2	Nitrate utilization in forage species	Critical step	30/09/2019	Achieved		
2.1.3	Appropriate Fertilizer Composition	Critical step	24/12/2019	Achieved		
2.1.4	Effects on rhizobium symbiosis	Critical step	30/09/2020	Achieved		
3	Nitrogen and water use efficiency in HME plant species	Impact statement	30/09/2021	Achieved		
3.1	Nitrogen use efficiency	Research aim	30/09/2019	Achieved		
3.1.1	Assess stomatal conductance in grass species	Critical step	28/09/2018	Achieved		
3.1.2	Measurement of NUE	Critical step	30/09/2019	Achieved		
3.2	Water use efficiency	Research aim	30/09/2021	Achieved		
3.2.1	WUE in Ryegrass	Critical step	29/06/2018	Achieved		

3.2.2	WUE in model grass species	Critical step	30/09/2021	Achieved	<p><b>VARIATION (2020):</b> <i>The first set HME rice developed for this aim was not suitable as expression had overshot the ideal expression window (reported last year). We developed HME rice that was more suitable and during the early part of 2020 the COVID-19 lockdown prevented us from performing any detailed experimentation, although we were able to harvest seed. We can repeat this with a goal to perform WUE experiment on HME rice by 30 Sept 2021.</i></p> <p><b>RESULTS:</b> HME rice was used as the model grass species. No difference in WUE between non-HME and HME rice was observed.</p>
3.2.3	WUE in commercial ready ryegrass	Critical step	30/09/2020	Achieved	<p><b>RESULTS:</b></p> <p>Initial assessments of WUE in perennial ryegrass were conducted using <b>spaced pots</b> replicating the establishment growth phase of perennial ryegrass. During this phase, water deficient HME maintained 16% higher WUE, compared to WT controls, delivering significantly more biomass.</p> <p>In the subsequent assessment of HME WUE in <b>established swards</b>, the WUE for water-deficient HME ryegrass did not statistically differ to that of WT controls and no biomass advantage was observed.</p>
4	Creating genetic material and knowledge for overseas field trial assessment of HME forages	Impact statement	31/12/2021	Achieved	
4.1	Ryegrass HME Trait Fixing	Research aim	31/05/2018	Achieved	
4.1.1	T1 Generation	Critical step	31/05/2017	Achieved	
4.1.2	T2 Generation	Critical step	22/12/2017	Achieved	

4.1.3	T3 Generation	Critical step	31/05/2018	Achieved		
4.2	Commercial Ready HME Ryegrass trait fixation	Research aim	31/12/2021	On track		
4.2.1	T1 Generation	Critical step	30/06/2019	Achieved		
4.2.2	T2 Generation	Critical step	31/03/2021	Achieved	<p><b>VARIATION (2020):</b> <i>1/3 waves of T2 seed production are complete. The final waves were completed by March 2021. We have a very robust and successful breeding plan. Part of the delay was due to the new PC2+ glasshouse taking 1 year longer to complete than planned.</i></p> <p><b>RESULTS:</b> Completed production of T2 generations and selected Events progressed to producing T3 generation.</p>	
4.2.3	T3 Generation	Critical step	31/12/2021	On track		
4.3	In vitro digestion and GHG assays	Research aim	30/09/2020	Achieved		
4.3.1	Analysis of first generation Ryegrass	Critical step	29/06/2018	Achieved		
5	Increasing farmer awareness and understanding of HME forages	Impact statement	30/09/2021	Achieved		
5.1	Farmer focus groups	Research aim	30/09/2021	Achieved		
5.1.1	Farmer focused groups	Critical step	31/12/2019	Achieved		

5.1.2	Establish wider industry linkages	Critical step	01/12/2020	Achieved	<p>As part of larger AgResearch-led pan-CRI conversations on GMOs in NZ, the programme is now having direct discussions with s9(2)(b)(ii) about the potential of the HME technology.</p> <p>We have established a positive and ongoing relationship with Manawatu Iwi as part of the consultation process.</p>
5.1.3	Design of a farmer-led, Farmer Awareness and Understanding Raising Programme	Critical step	31/12/2019	Achieved	
5.1.4	Stakeholder Feedback	Critical step	01/10/2020	Achieved	<p><b>RESULTS: Report: Gatekeepers and New Zealand agri food exports.</b></p> <p>Survey to understand:</p> <ul style="list-style-type: none"> <li>• Whether there is significant gatekeeping behaviour in New Zealand export supply chains.</li> <li>• Where in the supply chains the gatekeeping occurs.</li> </ul> <p>Whether GM technology might be expected to trigger gatekeeping behaviour.</p>
5.1.5	Monitoring and evaluation of the Farmer Awareness and Understanding Raising Programme	Critical step	30/09/2021	Achieved	<p><b>Results</b></p> <p>The outcomes are now being used by s9(2)(a) to develop the science plan post contract (2022-2025). This allows a farm systems approach for progressing the science.</p>

**Click on the deliverable to enter a status**

**Short title**

Carbon Dioxide Recycling in HME Ryegrass

**Due Date**

31/03/2021

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**Action**

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**Click on the deliverable to enter a status**

**Short title**

Infra-Red Gas Analysis

**Due Date**

31/03/2021

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**Action**

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**Click on the deliverable to enter a status**

**Short title**

IRGA analysis of Ryegrass

**Due Date**

31/10/2018

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**Action**

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**Click on the deliverable to enter a status**

**Short title**

IRGA analysis of rice

**Due Date**

31/03/2021

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**VARIATION (2020):** *This was due to the COVID lockdown; we were unable to perform IRGA analysis of the HME rice plants. We were able to collect seed. This will be repeated in the future. We were able to complete the IRGA analysis by March 2021.*

**RESULTS:** No difference in photosynthetic performance was observed between non-HME and HME rice.

**Action**

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**Click on the deliverable to enter a status**

**Short title**

Isotope partitioning of metabolic pathways

**Due Date**

30/09/2020

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**Action**

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**Click on the deliverable to enter a status**

**Short title**

Isotope partitioning in model species

**Due Date**

23/12/2019

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**Action**

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**Click on the deliverable to enter a status****Short title**

Isotope partitioning in forage species

**Due Date**

30/09/2020

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

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**Click on the deliverable to enter a status****Short title**

Nitrate Utilization in HME Ryegrass and other species

**Due Date**

30/09/2020

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

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**Click on the deliverable to enter a status****Short title**

Nitrate utilization in C3 plant species

**Due Date**

30/09/2020

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

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**Click on the deliverable to enter a status****Short title**

Nitrogen utilization in model species

**Due Date**

30/09/2019

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

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**Click on the deliverable to enter a status****Short title**

Nitrate utilization in forage species

**Due Date**

30/09/2019

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

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**Click on the deliverable to enter a status****Short title**

Appropriate Fertilizer Composition

**Due Date**

24/12/2019

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

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**Click on the deliverable to enter a status****Short title**

Effects on rhizobium symbiosis

**Due Date**

30/09/2020

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

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**Click on the deliverable to enter a status****Short title**

Nitrogen and water use efficiency in HME plant species

**Due Date**

30/09/2021

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

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**Click on the deliverable to enter a status****Short title**

Nitrogen use efficiency

**Due Date**

30/09/2019

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

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**Click on the deliverable to enter a status****Short title**

Assess stomatal conductance in grass species

**Due Date**

28/09/2018

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

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**Click on the deliverable to enter a status****Short title**

Measurement of NUE

**Due Date**

30/09/2019

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

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**Click on the deliverable to enter a status****Short title**

Water use efficiency

**Due Date**

30/09/2021

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

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**Click on the deliverable to enter a status**

**Short title**

WUE in Ryegrass

**Due Date**

29/06/2018

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**Action**

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**Click on the deliverable to enter a status****Short title**

WUE in model grass species

**Due Date**

30/09/2021

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**VARIATION (2020):** *The first set HME rice developed for this aim was not suitable as expression had overshot the ideal expression window (reported last year). We developed HME rice that was more suitable and during the early part of 2020 the COVID-19 lockdown prevented us from performing any detailed experimentation, although we were able to harvest seed. We can repeat this with a goal to perform WUE experiment on HME rice by 30 Sept 2021.*

**RESULTS:** HME rice was used as the model grass species. No difference in WUE between non-HME and HME rice was observed.

**Action**

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**Click on the deliverable to enter a status****Short title**

WUE in commercial ready ryegrass

**Due Date**

30/09/2020

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****RESULTS:**

Initial assessments of WUE in perennial ryegrass were conducted using **spaced pots** replicating the establishment growth phase of perennial ryegrass. During this phase, water deficient HME maintained 16% higher WUE, compared to WT controls, delivering significantly more biomass.

In the subsequent assessment of HME WUE in **established swards**, the WUE for water-deficient HME ryegrass did not statistically differ to that of WT controls and no biomass advantage was observed.

**Action**

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**Click on the deliverable to enter a status****Short title**

Creating genetic material and knowledge for overseas field trial assessment of HME forages

**Due Date**

31/12/2021

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

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**Click on the deliverable to enter a status****Short title**

Ryegrass HME Trait Fixing

**Due Date**

31/05/2018

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

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**Click on the deliverable to enter a status**

**Short title**

T1 Generation

**Due Date**

31/05/2017

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**Action**

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**Click on the deliverable to enter a status**

**Short title**

T2 Generation

**Due Date**

22/12/2017

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**Action**

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**Click on the deliverable to enter a status****Short title**

T3 Generation

**Due Date**

31/05/2018

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

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**Click on the deliverable to enter a status****Short title**

Commercial Ready HME Ryegrass trait fixation

**Due Date**

31/12/2021

**Achievement measure**

No achievement measure available

**Status**

On track

**Reason****Action**

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**Click on the deliverable to enter a status**

**Short title**

T1 Generation

**Due Date**

30/06/2019

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**Action**

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**Click on the deliverable to enter a status****Short title**

T2 Generation

**Due Date**

31/03/2021

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**VARIATION (2020):** *1/3 waves of T2 seed production are complete. The final waves were completed by March 2021. We have a very robust and successful breeding plan. Part of the delay was due to the new PC2+ glasshouse taking 1 year longer to complete than planned.*

**RESULTS:** Completed production of T2 generations and selected Events progressed to producing T3 generation.

**Action**

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**Click on the deliverable to enter a status**

**Short title**

T3 Generation

**Due Date**

31/12/2021

**Achievement measure**

No achievement measure available

**Status**

On track

**Reason**

**Action**

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**Click on the deliverable to enter a status**

**Short title**

In vitro digestion and GHG assays

**Due Date**

30/09/2020

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**Action**

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**Click on the deliverable to enter a status****Short title**

Analysis of first generation Ryegrass

**Due Date**

29/06/2018

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

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**Click on the deliverable to enter a status****Short title**

Increasing farmer awareness and understanding of HME forages

**Due Date**

30/09/2021

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Action**

---

**Click on the deliverable to enter a status**

**Short title**

Farmer focus groups

**Due Date**

30/09/2021

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**Action**

---

**Click on the deliverable to enter a status**

**Short title**

Farmer focused groups

**Due Date**

31/12/2019

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**Action**

---

**Click on the deliverable to enter a status****Short title**

Establish wider industry linkages

**Due Date**

01/12/2020

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

As part of larger AgResearch-led pan-CRI conversations on GMOs in NZ, the programme is now having direct discussions with s9(2)(b)(ii) about the potential of the HME technology.

We have established a positive and ongoing relationship with Manawatu Iwi as part of the consultation process.

**Action**

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**Click on the deliverable to enter a status**

**Short title**

Design of a farmer-led, Farmer Awareness and Understanding Raising Programme

**Due Date**

31/12/2019

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**Action**

---

**Click on the deliverable to enter a status**

**Short title**

Stakeholder Feedback

**Due Date**

01/10/2020

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason**

**RESULTS: Report: Gatekeepers and New Zealand agri|food exports.**

Survey to understand:

- Whether there is significant gatekeeping behaviour in New Zealand export supply chains.
- Where in the supply chains the gatekeeping occurs.

Whether GM technology might be expected to trigger gatekeeping behaviour.

**Action**

---

**Click on the deliverable to enter a status****Short title**

Monitoring and evaluation of the Farmer Awareness and Understanding Raising Programme

**Due Date**

30/09/2021

**Achievement measure**

No achievement measure available

**Status**

Achieved

**Reason****Results**

The outcomes are now being used by s9(2)(a) to develop the science plan post contract (2022-2025). This allows a farm systems approach for progressing the science.

**Action**

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## Project Deliverable Status (cont)

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**End user relationship:**

On track

**End user relationship  
comment:**

Expanding industry linkages including processors.

**Key personnel:**

On track

**Key personnel comment:****Research progress:**

On track

**Research progress  
comment:****Has any Change Event occurred in the Reporting Year?**

Yes

**If YES when was MBIE advised?**

Contract variation approved by MBIE January 2021 – extension of end date.

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## Work Programme Conditions

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## Outputs

## Knowledge Transfer

ModifiedDate	Knowledge transfer type	Number of Events	Knowledge transfer comments (optional)
30/08/2021	Workshops and hui	12	<ul style="list-style-type: none"> <li>• Presentation to s9(2)(b)(ii) - 10 August 2021.</li> <li>• Presentation to s9(2)(b)(ii) - 09 August 2021.</li> <li>• Presentation at Cellular Agriculture Symposium, The Factory - 04 August 2021.</li> <li>• Presentation to EPA and MfE - 04 August 2021.</li> <li>• Māori, Genetics, and Genomics - Wānanga Tuatoru, Cambridge, 28 29 July 2021.</li> <li>• Presentation to s9(2)(b)(ii) - 20 July 2021.</li> <li>• Presentation to Taihape farmers - 27 May 2021.</li> <li>• Presentation to Waituna West farmers - 11 May 2021.</li> <li>• Presentation to Rural Professionals - 04 November 2020.</li> <li>• Presentation to North Island farmers - 22 September 2020</li> <li>• Discussion with s9(2)(a), s9(2)(b)(ii) (17 Nov, 2020; 5 Feb and 22 Jun 2021)</li> </ul>



**Knowledge transfer type**

Workshops and hui

**Number of Events**

12

**Knowledge transfer comments (optional)**

- Presentation to s9(2)(b)(ii) - 10 August 2021.
- Presentation to s9(2)(b)(ii) - 09 August 2021.
- Presentation at Cellular Agriculture Symposium, The Factory - 04 August 2021.
- Presentation to EPA and MfE - 04 August 2021.
- Māori, Genetics, and Genomics - Wānanga Tuatoru, Cambridge, 28-29 July 2021.
- Presentation to s9(2)(b)(ii) - 20 July 2021.
- Presentation to Taihape farmers - 27 May 2021.
- Presentation to Waituna West farmers - 11 May 2021.
- Presentation to Rural Professionals - 04 November 2020.
- Presentation to North Island farmers - 22 September 2020
- Discussion with s9(2)(a), s9(2)(b)(ii) (17 Nov, 2020; 5 Feb and 22 Jun 2021)

**Non-peer Reviewed Published Articles**

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**Number of non-peer reviewed published articles**

0

**Non-peer reviewed published articles comments (optional)**

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**New Products, Processes and Services**

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**Number of new products**

0

**Number of new processes**

0

**Number of new services**

0

**New products, processes and services (optional)**

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**Science Quality**

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**Peer-reviewed journal articles in the year they are accepted for publication**

2

**Number of books or chapters**

0

<b>Number of published conference proceedings</b>	0
<b>Awards for science achievement (not open internationally)</b>	0
<b>Awards for science achievement (open internationally)</b>	0
<b>Keynote presentations (not open internationally)</b>	0
<b>Keynote presentations (open internationally)</b>	0
<b>Number of masters or doctoral theses</b>	1

### Science quality comments (optional)

#### Peer Reviewed Scientific Publications In Press

Beechy-Gradwell et al., (2020) Pasture grasses with high energy density and yield potential under simulated grazing. *The Plant Journal*. In Press

Somrutai Winichayakul, Richard Macknight, Robyn Lee, Hong Xue, Tracey Crowther, Philip Anderson, Shona Brock, Zac Beechey-Gradwell, Luke Cooney, Gregory Bryan, and Nick Roberts (2021) Physiological and Transcriptional Insights into the Sequential Responses to Storing Carbon as Leaf Lipid Sink under Different Irradiances. *Plant Physiology*. In Press

#### Peer Reviewed Scientific Publications During the term of this Contract

Beechy-Gradwell, Z., Winichayakul, S., Roberts, N., (2018) High lipid perennial ryegrass growth under variable nitrogen, water and carbon dioxide supply. *Proc. NZ Grasslands Assoc.* 80:219-224.

Beechy-Gradwell, Z., Cooney, L., Winichayakul, S., Andrews, M., Hea, S-Y., Crowther, T., and Roberts N., (2020) Storing carbon in leaf sinks enhances perennial ryegrass carbon capture especially under high N and elevated CO<sub>2</sub>. *J. Experimental Botany* 71:2351-2361.

Cooney, L., Beechy-Gradwell, Z., Winichayakul, S., Richardson, K., Crowther, T., Anderson, P., Scott, R., Bryan, G., Roberts, N. (2020) DGAT+CO expression alters leaf carbon allocation in *Lolium perenne* and increases growth and photosynthesis. *The Plant Journal*.

Winichayakul, S., Beechy-Gradwell, Z., Muetzel, S., Molano, G., Crowther, T., Lewis, S., Xue, H., Burke, J., Bryan, G., and Roberts, N., (2020) In vitro gas production and rumen fermentation profile of fresh and ensiled genetically modified high-metabolizable energy ryegrass. *J. Dairy Sci* 103 (3):2405-2418.

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## Provisional Patent and PVR Applications

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<b>Number of Patent or Plant Variety Right (PVR) applications</b>	0
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**Provisional patent and PVR applications comments (optional)**

Currently drafting provisional patents around:

- N-use efficiency.
- **s9(2)(i)** [REDACTED].
- Carbon sequestration.

**Patent and PVR grants****Number of Patents or Plant Variety Rights (PVRs) that have been granted.**

92

**Name the countries in which you have been granted Patents or PVRs.**

The Team's relevant patent portfolio covers three broad areas of plant improvement. The **CYSTEINE OLEOSIN** and **DGAT1** patents protect the HME technology and they are licensed to ZeaKal for row crops, biofuels and algae. They have generated substantial licensing and investment revenue to AgResearch (~\$10M). The **PEAPOD** patents are a future technology and this is being progressed using SSIF funding.

Patents granted (92) since 2009 and jurisdictions:

Photosynthesis/HME

Cysteine Oleosins:	26
Reducing WSC:	1
Increased CO <sub>2</sub> and root oil:	6

DGATs

DGAT N/C Chimera:	19
DGAT1 Modified N-term:	9
DGAT Zm-long:	6
Diarginine EDGAT:	1

PeaPod

Peapod in monocots:	12
Peapod in any plant:	12

Jurisdictions

Indonesia; Iraq; India; Canada; Paraguay; Philippines; Japan; Chile; Mexico; NZ; South Africa; Germany; Spain; France; China; AU; Brazil; Brazil; EPO; US; Argentina; India; Malaysia; Thailand; Uruguay; Venezuela

## Revenue and Contracting

## Co-funding and Subcontracting

Reporting financial year: 2020 (This report covers the period 01/07/20 - 30/06/21)

Select type	Organisation	Listed in the contract	Type	Cash or In-kind	Listed amount (NZD excl GST)	Actual amount (NZD excl. GST)	Comment
Co-Funding	s9(2)(b)(ii)	Yes	Direct	Cash	s9(2)(b)(ii)	s9(2)(b)(ii)	
Co-Funding	s9(2)(b)(ii)	Yes	Direct	Cash	s9(2)(b)(ii)	s9(2)(b)(ii)	
Co-Funding	s9(2)(b)(ii)	Yes	Direct	Cash	s9(2)(b)(ii)	s9(2)(b)(ii)	

Reporting financial year: 2020 (This report covers the period 01/07/20 - 30/06/21)

**Organisation**

s9(2)(b)(ii)

**Select type**

Co-Funding

**Listed in the contract**

Yes

**Listed amount (NZD excl GST)**

s9(2)(b)(ii) (Exc GST)

**Type**

Direct

**Cash or In-kind**

Cash

**Actual amount (NZD excl. GST)**

s9(2)(b)(ii)

(Exc GST)

**Percentage of listed funding  
achieved:**

100%

**Comment**

---

**Reporting financial year: 2020 (This report covers the period 01/07/20 - 30/06/21)**

**Organisation**

s9(2)(b)(ii)

**Select type**

Co-Funding

**Listed in the contract**

Yes

**Listed amount (NZD excl GST)**

s9(2)(b)(ii) (Exc GS )

**Type**

Direct

**Cash or In-kind**

Cash

**Actual amount (NZD excl. GST)**

s9(2)(b)(ii)

(Exc GS )

**Percentage of listed funding  
achieved:**

100%

**Comment**

---

**Reporting financial year: 2020 (This report covers the period 01/07/20 - 30/06/21)**

**Organisation****s9(2)(b)(ii)****Select type**

Co-Funding

**Listed in the contract**

Yes

**Listed amount (NZD excl GST)****s9(2)(b)(ii)** (Exc GS )**Type**

Direct

**Cash or In-kind**

Cash

**Actual amount (NZD excl. GST)****s9(2)(b)(ii)**

(Exc GS )

**Percentage of listed funding  
achieved:**

100%

**Comment**

## Formal Collaborations

### Collaborations by Country

Country	Level	Number of collaborations	Comment
United States of America (the)	Strong	4	
Australia	Strong	1	



## Capability Building

### Students

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**Number of students obtaining Masterate qualifications** 0

**Number of students obtaining Doctoral qualifications** 1

**Number of students obtaining Post-Doctoral qualifications** 0

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### Secondments to or from end users

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**Number of secondments as FTEs from an end user organisation** 0

**Number of secondments as FTEs to an end user organisation** 0

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# End User Relationships

## End user details

Organisation	Briefly describe how you are working with this organisation	Contact person	Contact phone	Contact email
s9(2)(b)(ii)	[Redacted]	s9(2)(a) [Redacted]	[Redacted]	[Redacted]
s9(2)(b)(i)	[Redacted]	s9(2)(a) [Redacted]	[Redacted]	[Redacted]
s9(2)(b)(ii)	[Redacted]	s9(2)(a) [Redacted]	[Redacted]	[Redacted]
s9(2)(b)(ii)	[Redacted]	s9(2)(a) [Redacted]	[Redacted]	[Redacted]

## Spinouts and Startups

### Spinouts and Startups (super-users only)

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#### **Spinouts and startups**

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## COVID-19 Information

### COVID-19 Information

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**Was the contract impacted by COVID-19 in this reporting period?**

No

**If your contract was impacted by COVID-19 in the reporting period**

0

**Do you anticipate future impact to this contract from COVID-19?**

No

**Which area(s) were impacted by COVID-19 during this reporting period?**

**If other is selected, please explain**

Timing to complete some tasks was affected but these were all completed.

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## Declaration

## Declaration

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**The Contractor declares that:**

I Agree

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