Summary

Summary

Client report summary:

Key:	CONT-47267-CRFRP-AGR C10X1603-CR-6
Project:	Forages with Elevated Photosynthesis and Growth
Contract ID:	C10X1603
Investment process:	CRFRP 2016 Contestable Research Fund - Research Programmes
Organisation:	AGR AgResearch Limited
IMS assigned to:	9(2)(a)
Reporting period:	01/07/2021 to 30/06/2022
Contract total value:	$11,500,000.00
Team:	

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Progress Reporting

Annual Update

2021-22 Annual Update

As this is the final report, this annual update will cover a brief history of the basis for this contract and the science that led up to the start. We will describe the significance of each of the five key impact areas and then how this research progressed. Finally, the outputs of the research and their significance will be discussed, including seven peer reviewed scientific publications and the major findings as well as areas yet to be published. Importantly this contract has had strong stakeholder support and we summarize the significance of this to the contract and to the stakeholders.

A Brief Science History

The genesis for this outstanding science originally came from research to increase the energy density of forages in the early 2000s. The team were developing a fatty acid expression and protection system in the model species Arabidopsis (Winichayakul *et al.,* 2013). They successfully doubled leaf fat levels and a serendipitous discovery opened the door to a wide range of applications in crops.

During analysis of the transgenic Arabidopsis, the team observed that the plants grew 50% faster than control plants. The team identified that these plants had elevated photosynthesis. This was subsequently demonstrated in forage perennial ryegrass. More recently (from research in this contract), the team discovered that the main mechanism for enhanced photosynthesis is a reduction in the negative feedback based on the carbohydrate status of the plant (Beechy-Gradwell *et al.*, 2020). Over the last two decades, there has been enormous investment internationally toward improving this complex process in plants. Before this discovery, while incremental improvements were achieved, other research efforts were limited by this negative feedback mechanism plants use to regulate photosynthesis.

The discovery of enhanced photosynthesis led to the recognition that this novel technology had applications in multiple crops. Over the last 14 years, the team developed new partnerships, progressed the science in other crops, and contributed to building novel commercialization models. Within a few years, this important technology is expected to benefit farmers and consumers in several countries (soybean in 2026).

During presentations to DairyNZ, PGG-Wrightsons Seeds, Agriseeds and Grasslanz Technology in 2015 a strategy to prepare a joint MBIE-Industry funded research programme was developed to progress the development of HME ryegrass to a stage where it became a commercialization programme. There were important fundamental questions on the plant response to nitrogen, its water use, the mechanism for enhanced photosynthesis and the challenges of breeding in PC2 containment to be answered. The need for HME ryegrass field trials and animal nutrition trials to establish the value proposition for New Zealand was recognized. As New Zealand had not adopted genetically modified crops the social license and farmer engagement was another important activity.

Initiation of the Research Programme for Contract C10X1603

The research is divided into five key areas: Carbon Dioxide Recycling, Nitrogen Utilisation; Nitrogen and Water Use Efficiency; Breeding in Containment; Increasing Farmer Awareness and understanding of HME Forages. The industry co-funding supported some of the five key areas and in addition helped support the USA based field trials. The plan was to progress to an animal nutrition trial in dairy cows but in 2021/2022 the strategy for this changed due to a new understanding that the USA climate was not suitable and the emerging opportunity of the Australian market.

Carbon Dioxide Recycling

High metabolizable energy (HME) ryegrass plants have increased levels of lipids stored in the green tissues in micro-organelles (Winichayakul *et al.,* 2013; Roberts *et al.,* 2010, 2011; Beechy-Gradwell *et al.,* 2020). These organelles are stable within the leaf and remain during the ensiling process (Winichayakul *et al.,* 2020). The early Arabidopsis research published in 2013 (Winichayakul *et al.,* 2013) speculated that CO_2 recycling led to the enhanced photosynthesis. However, as it has turned out from research in this contract, there is another very novel and important mechanism and this has been one of the most important discoveries from this research.

The allocation of different sources of carbon (sugars and fat) in different tissues is altered, leading to reduced negative feedback of photosynthesis (Beechy-Gradwell *et al.*, 2020; Cooney *et al.*, 2020). This enables the overall plant energy to be increased due to greater fixation of atmospheric CO_2. Increased plant growth rates are also observed, although the rate of increase is affected by competition for light in densely packed sward conditions.

International research on photosynthesis over the last 30 years has focussed on step-by-step incremental improvements of this complex 156 step set of interacting biochemical pathways. Progress was made in various steps but there were two overarching negative feedback mechanisms based on the carbohydrate status of the plant and the plants carbon: nitrogen balance. These negative feedbacks often limited the progress made. The discovery by the AgResearch team from research in this contract identified that one of the negative feedback mechanisms (based on the plant carbohydrate status) was overcome, this has been a major step forward. While there are conditions where HME ryegrass can have significantly greater growth rates, under competition for light and nutrients this diminishes, and the plant allocates the extra photosynthate into greater energy density. The same is seen in soybean with the same technology where occasionally the plants have greater yields, but in general the enhanced photosynthesis leads to increased energy density stored in the seed through increased oil and protein composition.

Nitrogen Utilisation

The plant nitrogen status is linked to photosynthesis. The nitrogen cycle is an important pathway linked to photosynthesis in that it supplies the reductant needed to drive the cycle. It was important to understand the plant response to different forms of nitrogen. It had been observed that the plant responds differently to reduced nitrogen compared to controls. For forages, this links into the farm nitrogen cycle which includes added and recycled nitrogen.

Research in this area is split into grasses and legumes with grasses including ryegrass and rice (as a model species), and legumes including alfalfa and now soybean. The overall goal of this research has been to understand the nitrogen requirement of different species and their responses to different nitrogen forms, nitrate, ammonia and urea. We made significant progress in ryegrass, and this was published in 2018 (Beechy-Gradwell *et al.*, 2018). The key findings were that HME ryegrass utilised all three forms of nitrogen, but the greatest growth responses were to reduced forms of nitrogen (ammonia and urea). Research on soybean has focussed on nitrogen levels within field grown plants and we have demonstrated that plants have some increased leaf nitrogen but not throughout the early reproductive stages and this appears to be then transferred into the seed which may account for the increased seed protein in some lines. It appears the legume symbiosis is sufficient to obtain competitive yields and addition of nitrogen (anhydrous ammonium) only benefits if soil N levels are low.

Nitrogen and Water Use Efficiency

The aim of this research area was to determine if HME trait expression in transgenic plants alters plant nitrogen metabolism. This goal is different from the research in described above on nitrate utilization as it is more encompassing and focuses on overall plant nitrogen metabolism. We are also examining water use efficiency and other stress responses such as light and temperature.

We performed controlled environment experiments on HME ryegrass event ODR4501 and looked at its ability to utilize nitrate, ammonium, and urea. HME ryegrass shoot dry weight increased across the entire nitrogen supply range regardless of nitrogen form, whereas the non-GM control ryegrass shoot dry weight did not significantly increase beyond 7.5 mM nitrogen supply. At 10 mM nitrogen supply, HME ryegrass shoot dry weight was 27-34% greater and root dry weight was 25-45% greater than in the non-GM control ryegrass. Total plant percent nitrogen and the shoot to root ratio was lower for plants supplied with nitrate than with ammonium or urea but did not differ between the non-GM control and HME ryegrass. This suggested that HME ryegrass has a similar nitrogen utilisation efficiency and biomass partitioning.

Of particular interest is the legume species alfalfa and soybean. As these species can form symbioses with the nitrogen fixing bacterium Rhizobium, they are provided with a source of nitrogen in the form of ammonium. Research on soybean has focussed on nitrogen levels within field grown plants and some of

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this is ongoing and being conducted by our partner ZeaKal. This links to the observation mentioned previously that plants have some increased leaf nitrogen throughout the early reproductive stages and this appears to be then transferred into the seed which may account for the increased seed protein in some lines. It suggests that alfalfa and white clover are similarly likely to benefit.

The overall nitrogen utilisation of HME ryegrass is relevant to on farm models currently being used to help establish the value in farming systems. As the program moves into the commercialisation phase from 2022 and beyond, we are repeating our farm and financial models informed from the US based field trial data and recent work on methane mitigation experiments from *in vitro* methane assays.

Breeding in Containment and the Ryegrass Endophyte

The novel breeding approach based in containment facilities the team has developed in collaboration with their seed company partners has enabled seed to be developed for 5 years of overseas field trials and has set the programme up for much larger scale planting for animal nutrition trials. The breeding occurs in crossing cages in PC2 containment and takes 9-10 months per cycle. Once sufficient homozygous familied have been developed and characterised it is possible to scale up to an open flowering and pollination system.

Two methods of plant transformation were used with gene gun plants produced prior to the start of this contract and Agrobacterium plants produced during the contract. We found the Gene Gun plants had multiple loci of integration of the transgene gene cassette and these became problematic during the breeding phase as they segregated, and we ended up with partial HME phenotypes. The Agrobacterium system produced a favourable frequency of single copy, single locus integrations and we were able to recreate many HME ryegrass plants that performed much better in the breeding phase. To ensure we has intact single copy integrations of the T-DNA we performed whole genome sequence analysis (an innovation that became an option in 2018/2019). This enabled us to map the integrations to the genome assemble and identify genic and intragenic insertions. Not all events were easy to map as the genome is not complete and therefore, we adopted a long-read sequencing approach which is still to be fully completed for some events.

The ryegrass endophyte that lives intercellularly within the plant leaves and stem provides protection from various insect predators of ryegrass. As the transgenic HME ryegrass material was developed out of the plant transformation process it was endophyte free (to prevent contamination in tissue culture). The AR1 and AR37 endophytes were introduced in the breeding process and an important question was how this symbiotic fungus would survive in a HME ryegrass plant. This has been answered in containment and field-based experiments and we have found no difference in transmission from seed generation to seed generation, no difference in fungal biomass and a minor reduction of some of the protective alkaloids, although the levels still remain within the seasonal variation range. This will be submitted for publication in 2022.

The proposed animal nutrition trials require one ha of HME ryegrass and one ha of a null control line. This needs 10 to 20 kg of seed for each treatment to be produced in PC2+ containment. Additionally, the three to four cycles of breeding need to be performed in elite industry ryegrass germplasm. We developed a unique system in the new PC2+ containment glasshouses at the Palmerston North Campus. Using a rapid homozygous breeding protocol that minimises in breeding depression while minimising the generations required, we have developed the first batch of seed and by February 2023 we will have produced the 10-15 kg of seed for use in a trial in Australia. This enables both in and off season seed production. Analysis of seed quality indicated it passes the phytosanitary requirements for shipping to Australia. Protocols were co-developed with an industry advisory group and containment measures supervised by the Ministry of Primary Industries. This is the first time this volume of seed has been produced in containment from a genetically modified wind pollenated grass species and highlights the unique facility and capability that has been developed.

Increasing Farmer Awareness and Understanding of HME Forages

In collaboration with NZIER, AgResearch conducted a survey on farmer decision making and the key decision timeframes for a GM HME ryegrass. Key findings were:

- Some farmers are interested in the potential benefits of HME ryegrass. They may adopt it within a

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year or two of its commercial release. However, they want to know more about how it might perform.
- They are likely to want information early, through many channels. The best time to provide information will be after the field trials and before product release. Some farmers want information even earlier. Given the number of information sources that farmers use, and the time frames for decision making, the information should be available in many formats through many channels.
- The use of genetic modification (GM) technology creates additional complexity. Some farmers will not adopt HME ryegrass because it is GM. Other farmers would adopt HME ryegrass but recognise that GM technology is an issue with consumers and in their supply chains.
- HME ryegrass would likely be a minority of total pasture. Among potential adopters, HME ryegrass would likely be one of several cultivars used.

More Recently a Survey of potential Gatekeepers and New Zealand Agrifood Exports (Kaye-Blake *et al.*, 2022) was conducted to understand:

- Whether there is significant gatekeeping behaviour in New Zealand export supply chains
- Where in the supply chains gatekeeping occurs?
- Whether GM technology might be expected to trigger gatekeeping behaviour.

Interviews and an online survey were conducted with respondents involved in export supply chains for meat and milk products from New Zealand. The focus was on perceptions of gatekeeping behaviour and the impact of private standards on the ability to sell GM food to overseas consumers. Gatekeeping and private standards are methods by which companies can exert influence on global value chains. The growth in private standards in particular has been advanced as a limitation on producers and their ability to innovate. Although results did suggest that there is gatekeeping in the export supply chains, they provide little support for the idea that either gatekeeping or private standards significantly impede New Zealand's ability to market GM food to overseas consumers. Instead, government regulation and non-GM demand by some consumers were more important factors. It is planned to publish this research in the journal *New Biotechnology*.

New Zealand Based Nutrition Trial

There is potential to perform a sheep nutrition trial to assess the methane mitigation effects of HME ryegrass from ensiled HME and Control lines grown in PC2 containment. We focussed on methane reductions as published nutritional studies of ruminants suggest a 5% reduction in methane emissions for every 1% increase in dietary fat. We aim to have experimental resolution to measure a 10% reduction in methane emissions.

Previous research on the stability of the fatty acids stored in the leaf micro-organelles (MSc Thesis Beechy-Gradwell, 2015) and more recently in the publication by Winichayakul *et al.,* (2020) looking at *in vitro* methane assays using ensiled ryegrass, suggested ensiling was a viable storage method.

We developed a novel system to grow and ensile ryegrass in batches and store the material for up to 18 months until we have approximately 1000 kg of dry matter needed for a trial. AgResearch is funding the project that will be conducted over the next 18-24 months. This system enables us to generate enough dry matter for both HME and control ryegrass to conduct the trial in sheep at the AgResearch Palmerston North Campus. This means the first animal nutrition study of this technology will be complete by the end of 2024.

USA Field Trials

AgResearch was able to partner with the University of Missouri in a five-year collaborative program to conduct field trial-based assessment of HME ryegrass in regulated field trials. The robust but well-established regulatory system in the USA enabled analysis of genetically modified ryegrass with two restrictions, no seed planted in the field and no plant reproduction. The first two years involved assessing the environmental conditions and developing protocols for transplantation of glasshouse germinated seedlings, comparing space plants vs. sward growth conditions and prevention of reproduction. We initially assessed gene gun derived HME ryegrass which turned out to have problematic multi-loci integrations of the HME gene cassette that segregated during the breeding. Therefore in 2019-2021 we used the superior Agrobacterium derived HME ryegrass. The recent publication from the team (Beechy-

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Gradwell *et al.,* 2022) shows the primary benefits for HME ryegrass (elevated leaf fats and gross energy) reliably translate from the laboratory to the field, as demonstrated across two field seasons and under realistic sward-like growing conditions.

Across the 2019 and 2020 field seasons, HME ryegrass displayed a 25–34% increase in FA delivering up to 0.5 MJ kg^{-1} DW higher gross energy and no penalty to biomass production. If successfully converted to ME, this energy gain is 250% greater than the total ME gain achieved over the last 4 decades by traditional genetic selection. Consequently, these increases are predicted to deliver 30kg MS cow^{-1} $season^{-1}$ (based on FARMAX modelling) and 10% less methane for the New Zealand farmer.

The translation of the secondary benefits for HME technology, elevated photosynthesis, and growth from the laboratory to the field were also examined in this paper. The team showed that HME ryegrass could deliver 18% greater carbon assimilation and up to 13% higher growth, but only in spaced pots, when light competition was low. When grown in dense swards, in either the laboratory or the field, HME ryegrass displayed no increase in photosynthesis or growth rate. Consequently, this paper provides a comprehensive assessment of the reliability of HME technology's primary and secondary benefits, what traits translate to the field (increased fatty acids and gross energy), and which do not (increased growth and photosynthesis).

The final 2021 HME ryegrass field trial in the USA analysed the performance of homozygous families and the assessment of primary and secondary traits. Building on the 2019 and 2020 trials that utilised hemizygous families we were able to demonstrate that gross energy and plant fatty acid composition translated from lab to field. In this line we observed a yield penalty over the season however it is unclear if it was a feature of this line or the propagation conditions. Our initial plan had been to perform animal nutrition studies in the USA, however the environmental risk of the hot summers led to a new strategy.

Row Crop Science Innovation

In addition to the breakthroughs in forages, significant innovation in soybeans has led to major benefits. Translating from a model species and grass into a seed-producing legume required new ways to control gene expression. The timing of gene expression has been critical, as the goal is to provide the plant with sufficient photosynthate for efficient seed development. This has been achieved with increased seed oil and protein composition and an overall increase in both co-products per acre over multiple seasons and sites across the USA. More recently, the ZeaKal team have shown that PhotoSeed™ plants can outperform their controls in non-irrigated settings. About 75% of U.S. soybeans are propagated in non-irrigated conditions. A recent innovation is the use of genes and regulatory elements derived solely from soybean which will assist the deregulation process.

The ZeaKal team has also branched into hemp and developed hemp plants with increased leaf oil. This potentially adds a new application to this crop, one that has lacked the plant breeding developments of other crops due to prohibition. The team has also branched into corn with the potential for greater oil yields per acre.

Intellectual Property

A critical component for raising investment in biotech is a robust and investible patent portfolio. Since 2009, the team has developed a portfolio of thirteen patents around increasing plant oils, protection of plant oils, enhancing photosynthesis, modifying plant architecture and reducing nitrous oxide emissions from crops. These are granted in multiple (92) jurisdictions. Part of the strategy has been extending the life of the patent portfolio by filing more recent methods patents and new applications. To date, this portfolio has led to the raising of nearly $100M of government, industry, and venture capital investment. There is potential that HME ryegrass may provide farmers with a tool for both methane and nitrous oxide reductions and this could be utilised alongside other mitigation measures to make significant reductions to Australasia's greenhouse gas inventory. The new patent filing extends the protection period for this technology well beyond the 2029 expiry date for the cysteine oleosin patent.

Social and Environmental Benefits

HME ryegrass represents a unique opportunity to improve both the productivity and environmental impact

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of pastoral farming. Exemplification of the stable translation in the USA field trials of HME was a major step towards commercialising this technology and delivering these benefits to farmers both domestically and abroad.

Improving the nutritional composition of forages through the accumulation of fatty acids in the grazed portion of the plant provides significant potential productivity and environmental benefits. A forage with increased metabolizable energy provides farmers the opportunity to maintain productivity using less land and potentially fewer animals. There also potential benefits of reduced methane emissions, supported by evidence from several animal nutritional studies and from *in vitro* methane assays. The goal is to achieve a minimum of a 10% reduction in methane emissions.

The improved nutritive quality is expected to reduce nitrogen excretion in ruminants leading to reduced nitrate leaching and nitrous oxide emissions. Recently, evidence from controlled environment experiments suggests another significant reduction in nitrous oxide emissions can be directly derived by the altered plant metabolism and morphology. This can drive up farm profitability and provide farmers with additional tools to manage environmental impacts from pastoral grazing systems. Experiments in controlled environment chambers on HME ryegrass and control mesocosms designed to measure nitrous oxide emissions from plants treated with bovine urine have indicated a novel mitigation potential. Two HME ryegrass events were tested in different industry cultivars. In one case a significant 50% reduction in nitrous oxide emissions was observed over the course of the experiment in cultivar Impact and in the second more modern line there was a clear trend for reduction. We anticipate that in most modern cultivars the reduction would be lower than the 50% seen in CV Impact. This would need to be verified in field conditions and may comprise part of the Australian science plan.

Future Commercialisation Options Beyond the Contract Completion

We are now developing a commercialisation and science plan to progress in Australia in 2023 and are about to seek regulatory approval from the Office of the Gene Technology Regulator. The Australian market is a good opportunity as there is already GM crop production and there is a significant ryegrass market for both the dairy, sheep and beef industries. Australia can become a market leader to inform New Zealand on the benefits of HME ryegrass in pastoral agriculture.

Current activities include developing the costed draft science plan, identifying the ideal business model from four possible options, bringing in new partners and fund raising.

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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 ryegrass 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 exceeds the ability of livestock to utilise it. 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 (2–3.3%) 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 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. In 2021 we demonstrated that 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 for gross energy and total fatty acid content.

Results of the research are published in seven Peer Reviewed Scientific Journal Articles between 2018 and 2022.

The program is now focussing on commercialisation opportunities in Australia.

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Key Achievements

Sequence	Key achievements

1	**Scaled-Up Breeding and Seed Production of GM grasses in PC2+ Containment** Conducting a meaningful nutrition study focussed on methane emissions from large ruminants consuming genetically modified High Metabolizable Energy (HME) ryegrass requires one ha of HME ryegrass and one ha of a null control line. This requires 10 to 20 kg of seed for each treatment to be produced in PC2+ containment. Additionally, the three to four cycles of breeding need to be performed in elite industry ryegrass germplasm. We developed a unique system in the new PC2+ containment glasshouses at the Palmerston North Campus. Using a rapid homozygous breeding protocol that minimises in breeding depression and the generations required, we are close to achieving 10-15 kg of seed needed for a trial in Australia. This enables both in and off season seed production. Analysis of seed quality indicated it passes the phytosanitary requirements for shipping to Australia. Protocols were co-developed with an industry advisory group and containment measures supervised by the Ministry of Primary Industries. This is the first time this volume of seed has been produced in containment from a genetically modified wind pollenated grass species and highlights the unique facility and capability that has been developed.
2	**Completion of USA HME Ryegrass Trials and Commercialisation Strategy for Australia** The final 2021 HME ryegrass field trial in the USA analysed the performance of homozygous families and the assessment of primary and secondary traits. Building on the 2019 and 2020 trials that utilised hemizygous families we were able to demonstrate that gross energy and plant fatty acid composition translated from lab to field. In this line we observed a yield penalty over the season however it is unclear if it was a feature of this line or the propagation conditions. Our initial plan had been to perform animal nutrition studies in the USA, however the environmental risk of the hot summers led to a new strategy. We are now developing a commercialisation and science plan to progress in Australia in 2023 and are about to seek regulatory approval from the Office of the Gene Technology Regulator. The Australian market is a good opportunity as there is already GM crop production and there is a significant ryegrass market for both the dairy and sheep & beef industries. Australia can become a market leader to inform New Zealand on the benefits of HME ryegrass in pastoral agriculture.

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3	**Identification and Patenting of a Novel Approach to Reduce Agricultural Nitrous Oxide Emissions** Experiments in controlled environment chambers on HME ryegrass and control mesocosms designed to measure nitrous oxide emissions from plants treated with bovine urine have indicated a novel mitigation potential. Two HME ryegrass events were tested in different industry cultivars. In one case a significant 50% reduction in nitrous oxide emissions was observed over the course of the experiment and in the second line there was a clear trend for reduction. This would need to be verified in field conditions and may comprise part of the Australian science plan. We filed a provisional patent in 2022 on the use of this technology for reducing nitrous oxide emissions in farming systems. We have successfully obtained funding for a PhD studentship to further elaborate the mechanisms for nitrous oxide reductions. There is potential that HME ryegrass may provide farmers with a tool for both methane and nitrous oxide reductions and this could be utilised alongside other mitigation measures to make significant reductions to Australasia's greenhouse gas inventory. The new patent filing extends the protection period for this technology well beyond the 2029 expiry date for the cysteine oleosin patent.
4	**Development of Forage Ensiling System in PC2 Containment and its Use in Animal Trials** There is potential to perform a sheep nutrition trial to assess the methane mitigation effects of HME ryegrass from ensiled HME and Control lines grown in PC2 containment. We focussed on methane reductions as published nutritional studies of ruminants suggest a 5% reduction in methane emissions for every 1% increase in dietary fat. We aim to have experimental resolution to measure a 10% reduction in methane emissions. We developed a novel system to grow and ensile ryegrass in batches and store the material for up to 18 months until we have the 1000 kg of dry matter needed for a trial. AgResearch funded the project that will be conducted over the next 18-24 months. This system enables us to generate enough dry matter for both HME and control ryegrass to conduct the trial in sheep at the AgResearch Palmerston North Campus. This means the first animal nutrition study of this technology will be complete by the end of 2024.

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5	**New Knowledge on Plant DGAT1 Enzymes Patented, Published and In Commercialisation** The enzyme diacylglycerol O-acyltransferase 1 (DGAT1) is ubiquitous in all eukaryotic organisms and has been well studied in animals where there is a clear model for its function, structure and topology as a membrane bound enzyme. This contract has supported research investigating the function of both monocotyledonous and dicotyledonous plant DGAT1s and the team made a breakthrough understanding the topology of plant DGATs and their function. This has added significantly to the understanding of plant DGAT1s which has previously been thought to have different topology to animal DGAT1s. DGAT1 is a fundamental component of the HME technology in ryegrass and it also has value in increasing oil seed composition. The team has recently filed a new provisional patent on a novel mechanism and uses of plant DGAT1s and published this in ***Frontiers in Plant Science***. The IP developed in a family of four patents is currently in commercialisation in the USA by AgResearch's biotechnology start-up partner ZeaKal Inc. One version may be in the US market in soybean in 2026 once regulatory approval has been obtained.

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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		
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		

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3.2.2	WUE in model grass species	Critical step	30/09/2021	Achieved		
3.2.3	WUE in commercial ready ryegrass	Critical step	30/09/2020	Achieved		
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	Achieved		
4.2.1	T1 Generation	Critical step	30/06/2019	Achieved		
4.2.2	T2 Generation	Critical step	31/03/2021	Achieved		
4.2.3	T3 Generation	Critical step	31/12/2021	Achieved		
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		
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		
5.1.5	Monitoring and evaluation of the Farmer Awareness and Understanding Raising Programme	Critical step	30/09/2021	Achieved		

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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

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

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

RELEASED UNDER THE OFFICIAL INFORMATION ACT

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

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

RELEASED UNDER THE OFFICIAL INFORMATION ACT

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

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

RELEASED UNDER THE OFFICIAL INFORMATION ACT

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

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

RELEASED UNDER THE OFFICIAL INFORMATION ACT

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

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

RELEASED UNDER THE OFFICIAL INFORMATION ACT

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

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

RELEASED UNDER THE OFFICIAL INFORMATION ACT

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

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

RELEASED UNDER THE OFFICIAL INFORMATION ACT

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

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

RELEASED UNDER THE OFFICIAL INFORMATION ACT

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

RELEASED UNDER THE OFFICIAL INFORMATION ACT

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

RELEASED UNDER THE OFFICIAL INFORMATION ACT

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

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

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

RELEASED UNDER THE OFFICIAL INFORMATION ACT

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

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
Achieved

Reason

Action

RELEASED UNDER THE OFFICIAL INFORMATION ACT

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
Achieved

Reason

Action

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

RELEASED UNDER THE OFFICIAL INFORMATION ACT

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

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

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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

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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 Fonterra 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

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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 agrifood 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

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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 9(2)(a) [redacted] to develop the science plan post contract (2022-2025). This allows a farm systems approach for progressing the science.

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Project Deliverable Status (cont)

End user relationship:
Achieved

End user relationship comment:

Over the course of this contract we have had significant support from industry stakeholders. Now the Contract is complete we have reached a major stage where the technology developed is reaching the commercialisation phase. Commercialisation of genetically modified plants have long timelines and face significant regulatory hurdles. We have developed a strategy with industry partners and major industry groups to enter a commercialisation programme in Australia. This market has a track record of GM crop adoption and a significant Dairy and Sheep and Beef industry that would benefit from the key primary and secondary traits offered by HME ryegrass. The opportunity for New Zealand is twofold as investors can gain a return on investment from sales and royalties from the Australian Industry and secondly, help elaborate the value proposition for New Zealand. This will aid informed decision making on the adoption of GM technology.

This contract has helped develop underpinning knowledge of this novel technology and related technologies (e.g. novel DGAT1s). It has supported the industry funded field trials based in the USA and led to new options for the industry. Importantly, the filing of new patents on the novel HME ryegrass mechanism, reduction of nitrous oxide and novel plant DGAT1s extends the investibility of the technology so that new investors supporting the commercialisation can gain a return on investment.

Key personnel:
Achieved

Key personnel comment:

We have been fortunate that there is a diverse set of capability within AgResearch so as new skills are needed they are readily on hand. The core team has grown over time and have developed their skills and knowledge.

Research progress:
Achieved

Research progress comment:

All the research goals that make up the five key areas have been achieved. This has enabled the HME technology to progress towards commercialisation and the research has opened new opportunities for investment in other crops and forage species.

Developing enhanced photosynthesis technologies in forages and crops has lead to world leading capability that has led to commercialisation opportunities in soybean, hemp, corn and forages internationally.

The team was nominated for the Royal Society MacDairmid Medal in 2022 for its outstanding research and the potential benefits to society and the winer will be announced in early November 2022.

Has any Change Event occurred in the Reporting Year?

No

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If YES when was MBIE advised?

Work Programme Conditions

Contract condition # 60762

This project does not give effect to Mātauranga Māori as the work and outcomes have no immediate and distinctive reliance on Māori mātauranga or resources or indigenous taonga species. While there are currently no field trials of genetically modified forages underway in New Zealand there is no ban on them being carried out. Through the Genetic Technologies Enabling Platform AgResearch is actively engaging with a wide range of stakeholders to discuss the risks, benefits and costs to New Zealand's environment, society, culture and economy of utilising - or not utilising - genetic technologies. In the long term (beyond the project), it is expected that new ryegrass cultivars will be generated that contain the HME technology, which will be capable of mitigating climate change and enhancing freshwater quality for NZ and the world. Part of the development and progression of this project will involve the team continuing building relationships with external groups who will help to guide the project as it moves forward, including regulatory and Māori stakeholder groups. Within AgResearch, the team is supported in guiding critical thinking in relation to Vision Mātauranga, te ao Māori and engagement (Te Ara Tika, Māori Research Partnerships group). The research team understands the importance of this research to Māori, in terms of concerns around how genetic technologies can be viewed as interacting with te ao Māori and New Zealand's indigenous and modern landscapes, and the potential risks and benefits to culture, local economies, and century-spanning community sustainability. The HME team has been represented at wānanga (*e.g.* Māori, Genetics, and Genomics, Cambridge, July 2021) where researchers and iwi representatives have discussed the potential impact of new genetic technologies, including HME Ryegrass. The research team is also engaging with the EPA around the type of application and the information that would be required for progressing an application for field trials and/or release in New Zealand. This includes meeting with the EPA's Kaupapa Kura Taiao team to discuss pathways for engaging with Māori.

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Outputs

Knowledge Transfer

ModifiedDate	Knowledge transfer type	Number of Events	Knowledge transfer comments (optional)
11/08/2022	Workshops and hui	9	Meeting with MfE and Environmental Protection Authority on status of research into GM for forages 4th August, 2021. Interview with National Business Review on commercialisation of GM technology 9(2)(a) [redacted] 7th October, 2021. Presentation to Ministry of Primary Production 7th November, 2021. Visit by 9(2)(a) [redacted] NZAGRC and glasshouse tour 26th November, 2021. Presentation to New Zealand - Israel 2022 Agritech Summit 15th February, 2022. Presentation to Dairy NZ and Taranaki farmers and glasshouse tour 8th June, 2022. Presentation to Massey University Agriculture Students 16th June, 2022. Presentation to Ministry of Primary Production staff and glasshouse tour 6th July, 2022. Presentation to Fonterra on value proposition modelling 10th August, 2022.

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Knowledge transfer type
Workshops and hui

Number of Events

9

Knowledge transfer comments (optional)
Meeting with MfE and Environmental Protection Authority on status of research into GM for forages 4th August, 2021.

Interview with National Business Review on commercialisation of GM technology 9(2)(a) [redacted] 7th October, 2021.

Presentation to Ministry of Primary Production 7th November, 2021.

Visit by 9(2)(a) [redacted] NZAGRC and glasshouse tour 26th November, 2021.

Presentation to New Zealand - Israel 2022 Agritech Summit 15th February, 2022.

Presentation to Dairy NZ and Taranaki farmers and glasshouse tour 8th June, 2022.

Presentation to Massey University Agriculture Students 16th June, 2022.

Presentation to Ministry of Primary Production staff and glasshouse tour 6th July, 2022.

Presentation to Fonterra on value proposition modelling 10th August, 2022.

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Non-peer Reviewed Published Articles

Number of non-peer reviewed published articles

0

Non-peer reviewed published articles comments (optional)

New Products, Processes and Services

Number of new products

0

Number of new processes

0

Number of new services

0

New products, processes and services (optional)

Science Quality

Peer-reviewed journal articles in the year they are accepted for publication	2
Number of books or chapters	0
Number of published conference proceedings	0
Awards for science achievement (not open internationally)	0
Awards for science achievement (open internationally)	0
Keynote presentations (not open internationally)	1
Keynote presentations (open internationally)	1
Number of masters or doctoral theses	0

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Science quality comments (optional)

Somrutai Winichayakul, Amy Curran, Roger Moraga, Ruth Cookson, Hong Xue, Tracey Crowther, Marissa Roldan, Greg Bryan, Nick Roberts (2022). An alternative angiosperm DGAT1 topology and potential motifs in the N-terminus. Frontiers in Plant Science, section Plant Proteomics and Protein Structural Biology. Manuscript ID: 951389

Ruth Cookson, Somrutai Winichayakul, Hong Xue, Kim Richardson, Roger Moraga, Aurelie Laugraud, Ambarish Biswas, Greg Bryan, Nick Roberts (2022). Evolution and conserved functionality of organ size and shape regulator PEAPOD. PEAPOD. PLoS ONE 17(2): e0263928. https://doi.org/10.1371/journal.pone.0263928

Beechey-Gradwell Z, Kadam S, Bryan G, Cooney L, Nelson K, Richardson K, Cookson R, Winichayakul S, Reid M, Anderson P, Crowther T, Zou X, Maher D, Xue H, Scott R, Allan A, Stewart A, Roberts N (2022). *Lolium perenne* engineered for elevated leaf lipids exhibits greater energy density in field canopies under defoliation. Field Crops Research 275 (108340)

Cooney, L.J., Beechey-Gradwell, Z., Winichayakul, S., Richardson, K.A., Crowther, T., Anderson, P., Scott, R.W., Bryan, G., Roberts, N.J. (2021). Changes in leaf-level nitrogen partitioning and mesophyll conductance deliver increased photosynthesis for *Lolium perenne* leaves engineered to accumulate lipid carbon sinks. *Frontiers in Plant Science* 12, article 641822.

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 CO2. J. Experimental Botany 71:2351-2361

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.

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.

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Provisional Patent and PVR Applications

Number of Patent or Plant Variety Right (PVR) applications

2

Provisional patent and PVR applications comments (optional)

Two new patent applications in last 12 months on Nitrous oxide reduction and Novel DGAT1s.

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.
92 patents granted since 2009.

Photosynthesis/HME

Cysteine oleosins: 26

Reducing WSC: 1

Increasing carbon dioxide and root oil: 6

DGAT1s

DGAT1 N/C Chimera: 19

DGAT1 Modified n-term: 9

DGAT ZM-long: 6

Diarginine EDGAT: 1

PeaPod

PeaPod in monocots: 12

PeaPod in dicots: 12

Jurisdictions:

Indonesia; Iraq; India; Canada; Paraguay; Philippines; Japan; Chile; Mexico; New Zealand; South Africa; Germany; Spain; France; China; Australia; Brazil; Europe; USA; Argentina; India; Malaysia; Thailand; Uruguay; Venezuela.

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Revenue and Contracting

Co-funding and Subcontracting

Reporting financial year: 2021 (This report covers the period 01/07/21 - 30/06/22)

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	Grasslanz Technology Limited	Yes	Direct	Cash	$12,500.01	$50,000.00	There was no change in the total commitment from GTL of $50,000 p.a. for 5 years ($250,000), the additional $37,500 is based on payment dates in the Collaborative Agreement contract between AgResearch and its co-investors, and they were invoiced $25,000 in November 2021 and $25,000 in April 2022.
Co-Funding	Dairy NZ	Yes	Direct	Cash	$187,500.00	$0.00	The final invoice of $187,500 was sent to Dairy NZ in May, 2021. The overall commitment by Dairy NZ has not changed. The discrepancy is due to the timing of invoicing as stipulated in the Collaborative Agreement contract between AgResearch and the co-investors.
Co-Funding	PGG Wrightsons	Yes	Direct	Cash	$24,999.99	$0.00	The final invoice was sent to PGG Wrightsons in May 2021. There has been no change in their commitment of $100K p.a. over five years ($500K). The discrepancy of $25K is due to timing of invoicing and payments.

Reporting financial year: 2021 (This report covers the period 01/07/21 - 30/06/22)

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Organisation
Grasslanz Technology Limited

Select type
Co-Funding

Listed in the contract
Yes

Listed amount (NZD excl GST)
$12,500.01 (Excl. GST)

Type
Direct

Cash or In-kind
Cash

Actual amount (NZD excl. GST)
50,000.00

(Excl. GST)

Percentage of listed funding achieved:
400%

Comment

There was no change in the total commitment from GTL of $50,000 p.a. for 5 years ($250,000), the additional $37,500 is based on payment dates in the Collaborative Agreement contract between AgResearch and its co-investors, and they were invoiced $25,000 in November 2021 and $25,000 in April 2022.

Reporting financial year: 2021 (This report covers the period 01/07/21 - 30/06/22)

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Organisation
Dairy NZ

Select type
Co-Funding

Listed in the contract
Yes

Listed amount (NZD excl GST)
$187,500.00 (Excl. GST)

Type
Direct

Cash or In-kind
Cash

Actual amount (NZD excl. GST)
0.00
(Excl. GST)

Percentage of listed funding achieved:
0%

Comment

The final invoice of $187,500 was sent to Dairy NZ in May, 2021. The overall commitment by Dairy NZ has not changed. The discrepancy is due to the timing of invoicing as stipulated in the Collaborative Agreement contract between AgResearch and the co-investors.

Reporting financial year: 2021 (This report covers the period 01/07/21 - 30/06/22)

Organisation
PGG Wrightsons

Select type
Co-Funding

Listed in the contract
Yes

Listed amount (NZD excl GST)
$24,999.99 (Excl. GST)

Type
Direct

Cash or In-kind
Cash

Actual amount (NZD excl. GST)
0.00

(Excl. GST)

Percentage of listed funding achieved:
0%

Comment

The final invoice was sent to PGG Wrightsons in May 2021. There has been no change in their commitment of $100K p.a. over five years ($500K). The discrepancy of $25K is due to timing of invoicing and payments.

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Formal Collaborations

Collaborations by Country

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

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Capability Building

Students

Number of students obtaining Masterate qualifications

0

Number of students obtaining Doctoral qualifications

1

Number of students obtaining Post-Doctoral qualifications

0

Secondments to or from end users

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
Dairy NZ	Dairy NZ represent the interests of the NZ diary sector. Dairy NZ provide governance of the overall programme as a member of the Programme Steering Group.	9(2)(a) [redacted]	[redacted]	[redacted]
PGG Wrightson Seeds	PGG Wrightson Seeds provide governance of the overall programme as a member of the Programme Steering Group. They have provided elite ryegrass germplasm, collaborate on breeding and are part of the pathway to market.	9(2)(a) [redacted]	[redacted]	[redacted]
Grasslanz Technology Limited	Grasslanz Technology provide governance of the overall programme as a member of the Programme Steering Group. They collaborate on breeding and are part of the pathway to market.	9(2)(a) [redacted]	[redacted]	[redacted]
Barenbrug Agriseeds	Barenbrug Agriseeds have provided elite ryegrass germplasm. They collaborate on breeding. They are part of the pathway to market.	9(2)(a) [redacted]	[redacted]	[redacted]

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Spinouts and Startups

Spinouts and Startups (super-users only)

Spinouts and startups

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COVID-19 Information

COVID-19 Information

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

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Declaration

Declaration

The Contractor declares that:

I Agree

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