Summary

Summary

Client report summary:

Key:	CONT-47267-CRFRP-AGR C10X1603-CR-1
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:	01/07/2016 to 30/06/2017
Contract total value:	$11,500,000.00
Team:	

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Progress reporting

Annual update

2016-17 Annual Update
Background

The High Metabolisable Energy (HME) technology confers enhanced photosynthesis in plant species with C3 photosynthesis (95% of all plans species). This transgenic technology has been validated in perennial ryegrass, rice, soybean, alfalfa (lucerne), white clover and the model species *Arabidopsis thaliana*. The enhanced photosynthesis leads to increased plant yields, increasing seed yield in soybean and increasing plant biomass in perennial ryegrass. In addition, for forages the technology also increases the metabolisable energy available for grazing ruminants. Biophysical modelling and *in vitro* experimentation suggests this improved nutritional quality is likely to lead to reduced urine nitrogen excretion, reduced methane emissions, increased liveweight gains and milk solids prodiction.

This programme aims to better understand the basis for the increased/enhanced photosynthesis (up to 24%) and the corresponding plant metabolic processes that enable this. The programme will also support industry-funded, overseas field trials and animal nutrition trials. As perennial ryegrass is an obligate out-crossing species we will use the self-fertile grass species (rice) as a rapid and efficient model to improve understanding this technology and apply these findings to New Zealands most important pasture species. To conduct plant field trials, we need to develop homozygous populations of seed that express the HME trait. We refer to this as fixing the trait and it is conducted in PC2 glasshouses under artificial environmental conditions, taking 36 months and four generations to transition from a single transgenic HME ryegrass plant (referred to as an 'event') to a population of homozygous seed. We also need to field trial a range of independently generated HME ryegrass events as we have shown that there is an ideal range of trait expression. As we increase the plant lipid levels to over 7% of the dry weight we begin to reduce the enhanced growth rates. We also know that as we move from heterozygous plants to a homozygous population the trait expression level increases. Therefore we are taking one high, two medium and one low level expressing events through the trait fixing process. Performing field trials with genetically modified plants requires regulatory approval and carefully designed field experiments to assess plant performance.

Therefore for this year we decided to move forward our plan to conduct industry-funded overseas field trials so that we can test all steps of the process and establish all the protocols. This has accelerated the programme and means we are in a better position to progress our understanding in the 2018 trials and beyond.

The HME forage project has progressed rapidly over the last 12 months as AgResearch and industry stakeholders developed a collaborative agreement to commercialise HME ryegrass. We have secured five years of industry co-funding in addition to this five ear contract from the MBIE Endavour fund. This has enabled AgResearch to begin field trials one year earlier than planned.

We have three HME lines progressing toward overseas-based field trials in 2018 with activities including trait fixing and seed production. Some of these lines have up 50% faster growth rates than control lines due to 20% increased photosynthesis. We have also assessed the growth of the ryegrass endophyte in HME ryegrass and found no difference in its growth and seed transmission. Controlled environment testing has shown that HME ryegrass has 9% increased actual water use efficiency compared to control ryegrass.

Implementation Pathway

Fixing the HME trait in a homozygous population of perennial ryegrass

We need to develop the seed suitable for field trials and this means developing seed where the transgene is in a homozygous state (a copy of the transgene in each of the pair of chromosomes for thich the transgene is inserted). Ryegrass is an out-crossing species so every seed is genetically distinct and we need to effectively overlay the effects of the transgene expression over the genetic diversity of the seed population (this is different from many crops e.g. rice that are inbred species and every single seed of a

cultivar is genetically uniform). Each HME ryegrass primary transgenic plant produced is referred to an event and the goal is to field test one high, two medium and one low level expressing event.

As the primary transgenic events come out of the pipeline the plants with single copy insertions of the HME expression cassette transgene are hemizygous (a single copy in one of the chromosome pairs). It is necessary to breed homozygous progeny to fix the trait (so all seeds contain the transgene in a homozygous state). This complex process in an obligate out-crossing species needs to be performed in containment glasshouses using a process designed to avoid in-breeding depression. It takes four crosses and 36 months to develop a batch of homozygous seed. We will have homozygous seed for a medium level expressing event ODR4501 in February. Three additional events are mid-way through the process and homozygous seed will be available for the 2019 field trial.

US Based field trials to establish value proposition

US based field and animal nutrition trials funded by industry co-funders will inform NZ industry and NZ regulators on the costs and benefits of NZ field trials. The initial plan was to perform the first field trial in 2018. However due to rapid technical progress and by agreement from the stakeholders we have already completed the first field trial a full year early. The decision was made to enable us to test all aspects of the seed production, regulatory approval, plant propagation and transplanting into the field, test the suitability of the environment and give the research providers the opportunity to develop and test all protocols prior to a scale up in 2018. This has validated our approach and provided confidence that the field trial location is suitable. It has helped build a relationship with the US regulators USDA-APHIS and provide them assurance that we are able to manage reproduction. Preliminary analysis of data available to date has shown us that the plants are likely to perform in the field as expected.

Development of a Stakeholder Co-Funding Collaborative Agreement

The industry co-funders Dairy NZ, PGG-Wrightsons Seeds and Grasslanz Technology have been negotiating with AgResearch to develop a collaborative agreement to manage to overall programme that encompasses the MBIE funding, AgResearch SSIF funding and the industry co-funding. The programme will have a Programme Steering Group and a Technical Committee that will work with the science team. The combined group will develop a commercialisation plan to enable commercialisation of the technology in perennial ryegrass once the MBIE programme is completed. The collaborative agreement is not yet finalised but will be close to signing once the seed companies develop a plan to provide elite ryegrass germplasm needed for the NZ phase in 2022.

Engagement with Industry and communication with farmers

The adoption practices of end users are critical to the success of any new technology such as HME ryegrass. In conjunction with Dairy NZ we have initiated a pastoral industry engagement strategy which in 2017 involved presentations to farmers at the Dairy NZ Farmer Forums at five venues across NZ which reached over 900 farmers. Presentations have also been made to various other agricultural based forums including the Mystery Creek Field Days.

Research, Science and Technology Benefits to New Zealand

A new collaborative partnership with the University of Missouri, USA

AgResearch has developed a new collaborative partnership with the University of Missouri. The reason this has eventuated is that in 2010 AgResearch and Kapyon Ventures established a biotech subsidiary ZeaKal. The HME technology and a second technology encompassing enhanced DGAT enzymes for improving triacylglyceride production in seeds was licensed to Zeakal for a number of the row crop species (e.g. soybean) while the forages remained with AgResearch for New Zealand benefit. Over the last few years, Zeakal developed partnerships with a number of US universities and two AgResearch Scientists 9(2)(a) and 9(2)(a) work for both AgResearch and ZeaKal. ZeaKal developed a soybean biotechnology programme with the University of Missouri and has conducted soybean field trials in 2016 and 2017.

AgResearch has been able to leverage off the ZeaKal network to establish a 5 year collaborative agreement with the University of Missouri to conduct HME ryegrass field trials and animal nutrition trials. The same teams that provide plant physiology, biochemistry and field trial capability in soybean now also provide a new ryegrass capability. This effectively expands our research and development capability and adds the essential field trial capability. We have also been able to utilise the ZeaKal regulatory affairs officer 9(2)(a) to help develop and apply for import and release permits with the USDA-APHIS regulator in the US. The real value of these new partnerships is that we were able to start field trials a whole year earlier than planned.

Enhancement of research collaboration with Professor Arnold Bloom, UC Davis, USA

Arnold Bloom is internationally recognised for his research on plant responses to increasing CO_2 environments. 9(2)(a) from AgResearch and 9(2)(a) from Lincoln University have had an ongoing collaboration with 9(2)(a). This year, AgResearch hosted 9(2)(a) and 9(2)(a) and 9(2)(a) on have developed an expanded research collaboration in a model HME grass (rice) project to better understand nitrogen metabolism. An AgResearch PhD student will spend a few months in UC Davis as part of the project. This collaboration will enhance our capability and provide access to isotope analysis expertise. The reason this collaboration is so important is that there are a number of similarities with HME plants with enhanced photosynthesis and plants grown in high CO_2 environments in Free Air Carbon Dioxide Experiments (FACE). The HME technology provides a valuable model to study plant responses to increasing atmospheric CO_2 levels and this collaboration will contribute to international efforts to adapt crops to rapidly increasing atmospheric CO_2.

First HME Ryegrass Field Trials

The first field trials of the HME technology in ryegrass were conducted this year. Very positive results from a ZeaKal soybean trial with the same technology in 2016 had provided confidence that results obtained in glasshouses would successfully transfer to the field. However, this was the first opportunity in ryegrass and while the trial has been terminated, all plant analysis is still underway we were pleased to be able to measure an increase in photosynthesis HME ryegrass plants compared to control plants despite the very young age of the plants (8 weeks post germination and 4 weeks in the field). We are also able to report that plant growth was completely normal with both HME ryegrass and control plants.

Normal Endophyte Symbiosis

With 25-50% increased growth rates of HME ryegrass, an obvious question is whether or not the ryegrass endophyte is able to form a stable symbiosis in a plant. Initial analysis in the T_2 progeny of one HME ryegrass event from glasshouse grown plants with a single commercial endophyte strain showed a high rate of seed transmission and a normal association in the seedlings. This will need to be verified in HME ryegrass events with even greater growth rates and also in field trials.

Publicly available information

Publicly available information

DO NOT INCLUDE CONFIDENTIAL INFORMATION HERE

The HME forage project has progressed rapidly over the last 12 months as AgResearch and industry stakeholders developed a collaborative agreement to commercialise HME ryegrass. We have secured five years of industry co-funding in addition to this five year contract from the MBIE Endeavour fund. This has enabled AgResearch to begin field trials one year earlier than planned.

The first HME ryegrass field trial was completed on the 10th July 2017. This was conducted in the US and the first trial was designed to test all steps of the process including regulatory approval, seed production, pre-germination in containment, transfer to the field and the various experimental processes needed to assess the efficacy of the HME ryegrass. The trial used a single HME ryegrass event that has a mid-level expression of the HME trait. In-field photosynthesis measurements were consistent with glass house measurements. The trial demonstrated that establishment of HME ryegrass was equivalent to control plants and that the environment was suitable for plant growth.

We have three HME lines progressing toward overseas based field trials in 2018 with activities including trait fixing and seed production. Some of these lines have up to 50% faster growth rates than control lines due to 20% increased photosynthesis. We have also assessed the growth of the ryegrass endophyte in HME ryegrass and found no difference in its growth and seed transmission. Controlled environment testing has shown that HME ryegrass has 9% increased actual water use efficiency compared to control ryegrass.

Five key achievements

Sequence	Key achievements
1	**Demonstration that the HME trait is stably inherited in successive generations in multiple HME ryegrass events.** The transition from primary transgenic HME ryegrass plants (shown to have increased photosynthesis, growth and metabolisable energy) to a homozygous population where the trait is genetically fixed into the population takes 36 months and 4 generations. This complex process in conducted in crossing cages in PC2 glasshouses. Homozygous seed is needed for the industry funded field trial and animal nutritional trials in the US. We have taken four separate HME ryegrass transgenic lines (called events) through two cycles of out-crossing with elite ryegrass cultivars. In each and every case the HME transgene has been stably inherited and expressed in the progeny with the appropriate genetics. We are on track to generate the first homozygous population of seed in February 2018. This is an important achievement as it demonstrates the viability and appropriate inheritance of this trait during the breeding process as well as developing seed for field trials.

2	**Demonstration that HME ryegrass has 9% actual increased water use efficiency.** During analysis of plant photosynthesis and transpiration it was demonstrated that HME ryegrass has a theoretical increase in water use efficiency (called intrinsic water use efficiency or iWUE). It was unclear how iWUE translated to actual WUE and the significance of this to field conditions. We obtained the first actual WUE measurements in controlled environment chambers and found that HME ryegrass was 9% more efficient than control non GM ryegrass. Later in the programme we plan to determine what this means in the field and how this will benefit farmers, but it is expected that this may lead to an improved response to water stress.
3	**Completion of first HME ryegrass trial in Mid-West USA.** This is probably the most significant achievement of the programme. It was intended to conduct the first field trial in 2018, however we decided to run a 2017 trial to test all steps of the process from US regulatory approval, testing plant responses to the environment that trials would be conducted in and the performance of the HME ryegrass plants compared to control plants. Importantly, this would enable the research providers to establish and test all protocols. The trial was conducted from May to July with a T_2 (second generation) population of HME ryegrass Event ODR4501. ODR4501 was an intermediate expressing event with over 10% more photosynthesis and 20% increased growth rates (in the containment glasshouse). Data is still being collected but preliminary outcomes are that the regulatory process was successful, we measured increased photosynthesis in the field and the plants performed well in the environment.
4	**Demonstration that despite greatly increased plant growth rates (25-50%), the ryegrass endophyte AR1 is capable of a stable symbiosis and transmission via seed.** The ryegrass endophyte is an essential component of ryegrass pastures providing insect protection. Because HME ryegrass has greatly accelerated growth rates an obvious question is can the endophyte form a stable symbiosis with HME ryegrass and be successfully seed transmitted? This year we demonstrated that the AR1 endophyte symbiosis and seed transmission is normal in the intermediate expressing HME ryegrass event ODR4501 in plants grown in the containment glasshouse. We will test additional HME ryegrass events and endophyte strains during the programme. This will include field grown material.

5	**Communication and engagement with end users** The Plant Biotechnology team scientists teamed up with Dairy NZ to present to over 900 dairy farmers at five venues (Southland, Canterbury, Palmerston North, Taranaki and Waikato) between 4th and 25th May at the Dairy NZ Farmers Forums. This was for many farmers, the first real exposure to the science and potential future opportunity of HME ryegrass with increased growth rates and metabolisable energy. They were particularly interested in learning about the potential environmental benefits including reductions in total nitrogen load on pasture and reduction in greenhouse gas emissions.

Performance indicators

End User relationship:
On track with issues

End User relationship comment:

We have yet to sign the collaborative agreement between AgResearch, Dairy NZ, PGG Wrightsons and Grasslanz Technology. The collaborative agreement is essentially complete and close to sign off. PGG Wrightsons Seeds and Agriseeds need to reach agreement around elite ryegrass germplasm to be used in the programme. The delay in signing the agreement means we received $500,000 (from Dairy NZ) of co-funding rather than the $675,000 in total planned from the three organisations. This has not had a negative impact on the programme as the costs this year for the overall programme were in line with the level of co-funding received. Our co-funders have committed to providing the full level of co-funding over the length of the contract, and as we get to the industry funded animal nutrition trials in years 4 and 5 the costs increase so their commitment ensures we remain on track.

Key personnel:
On track

Key personnel comment:

We hired a Plant Physiology Post Doctoral Scientist early 2017, 9(2)(a) who will provide valuable expertise for both field and controlled environment analysis of HME forages.

Research progress:
On track

Research progress comment:

The research has progressed well and we are ahead of plan with the first field trials conducted in the US a year early.

Success story worth communicating has been generated:
Yes

Comment:

The first field trial of HME ryegrass has been completed in the mid-West USA and we have demonstrated that HME ryegrass plants had elevated photosynthesis in the field, confirming measurements taken in the glasshouse. The trial was conducted a year earlier than planned and the goal was to test all steps of the process including gaining regulatory approval, transferring seed, testing the growing environment and developing the experimental procedures. We tested a medium level expressing HME ryegrass event that was previously demonstrated in glasshouse studies in established plants to have 15% elevated photosynthesis and 20% increased growth rates. The HME ryegrass seedlings grew as expected and during the growing period in the field and transitioned through what we describe as an acclimation phase where the new seedlings transition to a high carbon dioxide growing state before accelerating their growth and out growing control plants. During this time the plants had elevated photosynthesis (approximately 10%) compared to control ryegrass. This growth phenotype was exactly matched by a mirror set of plants grown in the glasshouse in AgResearch. The trial was terminated in July as planned and we are now in a position to have a greatly expanded trial with four events and over a longer period in 2018.

Has any change event occurred in the Reporting Year?

No

If YES when was MBIE advised?

Project deliverable status

Deliverable Status:

Click on the deliverable to enter a status

Sequence	Short title	Type	Status	Reason	Action
1	Carbon Dioxide Recycling in HME Ryegrass	Impact statement	On track	This research area is progressing as planned with initial focus on critical step 1.1.1, the analysis of HME reygrass. AgResearch hired a Post Doctoral Scientist Plant Physiologist in early 2017 and analysis of both controlled environment and field grown ryegrass is underway. Analysis of alfalfa and rice will be initiated once suitable material expressing the HME trait are available.	
1.1	Infra-Red Gas Analysis	Research aim	On track		

1.1.1	IRGA analysis of Ryegrass	Critical step	On track	Both controlled environment and field IRGA analysis have been conducted. Controlled environment analysis has been used to identify HME ryegrass Events with a range of enhanced photosynthesis (from 10% to over 20%). These will be used in field trials in 2018. A single mid level expressing event was tested in the field this year and shown to have elevated photosynthesis.	More detailed analysis in the field with 4 HME ryegrass events is planned in 2018.
1.1.2	IRGA analysis of alfalfa	Critical step	On track with issues	Initial alfalfa plants showed poor HME expression so new improved gene constructs have been generated and we are developing new alfalfa events. Material will be available mid 2018.	Over the length of the 5 year contract we will be able to generate HME alfalfa events with appropriate expression levels and fix the trait in homozygous populations. We decided not to progress with the first set as we know it is possible to obtain greater expression levels of HME and therefore greater photosynthesis (nearing 24%). We are fortunate to be able to leverage off the Zeakal soybean programme which does have proven legume gene constructs and so this saves significant time.
1.1.3	IRGA analysis of rice	Critical step	On track	Several HME rice events with good expression levels have been developed and we are developing homozygous populations of seed. IRGA analysis can be initiated in mid 2018.	

1.2	Isotope partitioning of metabolic pathways	Research aim	On track	While this research is due to be initiated in late 2018, we have developed an enhanced research collaboration with 9(2)(a) from UC Davis, USA. We plan to use HME rice in the collaboration and our PhD student will spend time in UC Davis on the project. Initial analysis of HME ryegrass has shown a change in nitrogen isotope discrimination, validating our experimental approach.	
1.2.1	Isotope partitioning in model species	Critical step	On track	HME rice will be available for project.	
1.2.2	Isotope partitioning in forage species	Critical step	On track	Initial analysis of HME ryegrass shows a change in nitrogen isotope discrimination but not carbon isotope discrimination. This validates our experimental approach and shows that isotope feeding studies can be used to understand changes in nitrogen metabolism resulting from the enhanced photosynthesis.	
2	Nitrate Utilization in HME Ryegrass and other species	Impact statement	On track		
2.1	Nitrate utilization in C3 plant species	Research aim	On track	We have confirmed that HME ryegrass with enhanced photosynthesis has increased growth compared to controls when grown with ammonium or urea as the sole nitrogen source. Growth with nitrate or glutamine as the sole nitrogen source is the same as controls. This will enable us to identify the best nitrogen management regime for HME ryegrass and the findings from controlled environment studies will be validated in field trials in 2019 and 2020.	

2.1.1	Nitrogen utilization in model species	Critical step	On track	We are progressing toward homozygous HME rice. This requires 3 generations and further analysis to select HME rice events with appropriate expression levels and phenotype (Enhanced photosynthesis). Once appropriate material is identified, detailed nitrogen utilisation experiments can be initiated.	
2.1.2	Nitrate utilization in forage species	Critical step	On track	As part of the PhD programme the first ryegrass nitrogen preference study has been completed. HME ryegrass has superior growth when either ammonuim or urea is the sole nitrogen source. Growth is idential to non HME ryegrass when nitrate or glutamine are the sole nitrogen source.	Continue as planned.
2.1.3	Appropriate Fertilizer Composition	Critical step	On track	Reliant on 2.1.1 and 2.1.2 which are both on track.	
2.1.4	Effects on rhizobium symbiosis	Critical step	On track	This is not due to start until late 2018. We have had to generate new HME alfalfa events as the initial HME alfalfa showed poor trait expression. The new events will be available in time. We also can use soybean expressing the HME trait as they will be gifted by ZeaKal and have already been tested in field trials and shown to have normal Rhizobium symbiosis.	

3	Nitrogen and water use efficiency in HME plant species	Impact statement	On track	We have initially focussed on water use efficiency as research is conducted in controlled environment chambers. IRGA analysis predicted a 30% increase in intrinsic Water Use Efficiency (iWUE). This is a theoretical calculation. We have since shown for one HME ryegrass event that we have a 9% actual increase in WUE. The significance of this will be tested in field conditions in 2019 and 2020.	
3.1	Nitrogen use efficiency	Research aim	On track	In progress.	
3.1.1	Assess stomatal conductance in grass species	Critical step	On track	This has been initiated with detailed assessment of HME ryegrass in controlled environment. The analysis supports the results from other species (Arabidopsis thaliana and soy bean) that the HME technology is a carbon concentrating mechanism and leads to enhanced photosynthesis.	
3.1.2	Measurement of NUE	Critical step	On track	This research has only just been initiated with the testing of four different forms of nitrogen as sole nitrogen sources. We will be starting different combinations of nitrogen as part of our collaboration with 9(2)(a) at Lincoln University.	
3.2	Water use efficiency	Research aim	On track		
3.2.1	WUE in Ryegrass	Critical step	On track	We have measured a 9% increase in actual water use efficiency in one HME ryegrass event.	Compare to other species when data available. Test in field conditions in later years.

3.2.2	WUE in Legumes	Critical step	On track	Not due to start until early 2018. Will start late (late 2018) due to need to develop new HME alfalfa. No issues completing the research by proposed end date of 30/9/19.	
3.2.3	WUE in model grass species	Critical step	On track	Not due to start until July 2018. HME rice development is on track and we have several events that will be suitable once homozygous seed is available.	
3.2.4	WUE in commercial ready ryegrass	Critical step	On track	Will not start until mid 2019. The commercial ready HME ryegrass transformations have started and we are on track to have the trait in elite germplasm. This is supported by the AgResearch SSIF funding which has recently been confirmed for 5 years to match the MBIE funding.	
4	Creating genetic material and knowledge for overseas field trial assessment of HME forages	Impact statement	On track	Fixing the HME trait in homozygous ryegrass requires 4 generations to obtain a pool of homozygous seed. The full process takes 36 months. This is due to the out-crossing nature of perennial ryegrass and we have a validated system to complete this and avoid in breeding depression. We plan to progress 4 independent HME ryegrass events (one low, two medium and one high level expressing line). We are progressing these four events as we have shown that trait expression increases as we obtain homozygous plants. This is to ensure we field trial plants with appropriate levels of expression. As we complete this process we include the ryegrass endophyte in the crossing partners. We are then able to examine the symbiosis and confirm that the ryegrass endophyte can form stable associations with HME ryegrass.	

4.1	Ryegrass HME Trait Fixing	Research aim	On track		
4.1.1	T1 Generation	Critical step	Achieved	This was achieved for all 4 HME ryegrass Events.	
4.1.2	T2 Generation	Critical step	On track	Avhieved for 2/4 HME ryegrass events with second set of 2 to be completed as scheduled.	
4.1.3	T3 Generation	Critical step	On track	We have one T3 generation (ODR4501) that will be completed in February 2018. This will be tested in field trials in 2018. The T3 seed for the remaining events is due to be available as planned in time for the 2019 field trials.	
4.2	HME Alfalfa trait fixation	Research aim	On track with issues	We have started new transformations with HME alfalfa as the current events showed low levels of expression. We have a new gene construct called CORD that looks very promising in soy bean (Zeakal Inc data). This means we will be delayed starting the trait fixing process however we should remain on track to field trial some events in 2019 and the remainder in 2020.	We can still meet our goal of developing homozygous alfalfa with increased metabolisable energy and enhanced photosynthesis by the end of the contract. We will also be able to complete all the required analysis in this time. We have a clear understanding of how to improve the gene construct for legumes as this has already been validated in soybean by Zeakal. The first constructs did actually work in soybean, although it appears the new CORD constructs are even better.

4.2.1	T1 Generation	Critical step	On track with issues	See 4.2	
4.2.2	T2 Generation	Critical step	On track with issues	See 4.2	
4.2.3	T3 Generation	Critical step	On track with issues	See 4.2	
4.3	Commercial Ready HME Ryegrass trait fixation	Research aim	On track	The development of commercial ready HME ryegrass is supported by the SSIF funding recently confirmed for 5 years. Transformations are underway and we expect to have material for the trait fixing programme as scheduled. The seed industry stake holders will supply elite breeding material for the trait fixing process.	
4.3.1	T1 Generation	Critical step	On track		
4.3.2	T2 Generation	Critical step	On track		
4.3.3	T3 Generation	Critical step	On track		
4.4	In vitro digestion and GHG assays	Research aim	On track	This research is on track and the first results come from in vitro methane assays and show that HME ryegrass may decrease methane emissions by 15-23%. This is consistent with scientific literature looking at fat in ruminant diets.	
4.4.1	Analysis of first generation Ryegrass	Critical step	On track		
4.4.2	Assays on alfalfa	Critical step	On track with issues	Due to the delay in developing alfalfa with appropriate levels ofHME trait experssion we may need to compress the time lines for the research, however we expect to complete close to the planned completion date of September 2019.	

Deliverable Status:

Click on the deliverable to enter a status

Short title
Carbon Dioxide Recycling in HME Ryegrass

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

This research area is progressing as planned with initial focus on critical step 1.1.1, the analysis of HME reygrass. AgResearch hired a Post Doctoral Scientist Plant Physiologist in early 2017 and analysis of both controlled environment and field grown ryegrass is underway. Analysis of alfalfa and rice will be initiated once suitable material expressing the HME trait are available.

Action

Deliverable Status:

Click on the deliverable to enter a status

Short title
Infra-Red Gas Analysis

Due Date
30/09/2019

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Deliverable Status:

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
On track

Reason

Both controlled environment and field IRGA analysis have been conducted. Controlled environment analysis has been used to identify HME ryegrass Events with a range of enhanced photosynthesis (from 10% to over 20%). These will be used in field trials in 2018. A single mid level expressing event was tested in the field this year and shown to have elevated photosynthesis.

Action

More detailed analysis in the field with 4 HME ryegrass events is planned in 2018.

Deliverable Status:

Click on the deliverable to enter a status

Short title
IRGA analysis of alfalfa

Due Date
31/10/2018

Achievement measure
No achievement measure available

Status
On track with issues

Reason

Initial alfalfa plants showed poor HME expression so new improved gene constructs have been generated and we are developing new alfalfa events. Material will be available mid 2018.

Action

Over the length of the 5 year contract we will be able to generate HME alfalfa events with appropriate expression levels and fix the trait in homozygous populations. We decided not to progress with the first set as we know it is possible to obtain greater expression levels of HME and therefore greater photosynthesis (nearing 24%). We are fortunate to be able to leverage off the Zeakal soybean programme which does have proven legume gene constructs and so this saves significant time.

Deliverable Status:

Click on the deliverable to enter a status

Short title
IRGA analysis of rice

Due Date
30/09/2019

Achievement measure
No achievement measure available

Status
On track

Reason

Several HME rice events with good expression levels have been developed and we are developing homozygous populations of seed. IRGA analysis can be initiated in mid 2018.

Action

Deliverable Status:

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
On track

Reason

While this research is due to be initiated in late 2018, we have developed an enhanced research collaboration with 9(2)(a) from UC Davis, USA. We plan to use HME rice in the collaboration and our PhD student will spend time in UC Davis on the project. Initial analysis of HME ryegrass has shown a change in nitrogen isotope discrimination, validating our experimental approach.

Action

Deliverable Status:

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
On track

Reason

HME rice will be available for project.

Action

Deliverable Status:

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
On track

Reason

Initial analysis of HME ryegrass shows a change in nitrogen isotope discrimination but not carbon isotope discrimination. This validates our experimental approach and shows that isotope feeding studies can be used to understand changes in nitrogen metabolism resulting from the enhanced photosynthesis.

Action

Deliverable Status:

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
On track

Reason

Action

Deliverable Status:

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
On track

Reason

We have confirmed that HME ryegrass with enhanced photosynthesis has increased growth compared to controls when grown with ammonium or urea as the sole nitrogen source. Growth with nitrate or glutamine as the sole nitrogen source is the same as controls. This will enable us to identify the best nitrogen management regime for HME ryegrass and the findings from controlled environment studies will be validated in field trials in 2019 and 2020.

Action

Deliverable Status:

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
On track

Reason

We are progressing toward homozygous HME rice. This requires 3 generations and further analysis to select HME rice events with appropriate expression levels and phenotype (Enhanced photosynthesis). Once appropriate material is identified, detailed nitrogen utilisation experiments can be initiated.

Action

Deliverable Status:

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
On track

Reason

As part of the PhD programme the first ryegrass nitrogen preference study has been completed. HME ryegrass has superior growth when either ammonuim or urea is the sole nitrogen source. Growth is idential to non HME ryegrass when nitrate or glutamine are the sole nitrogen source.

Action

Continue as planned.

Deliverable Status:

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
On track

Reason

Reliant on 2.1.1 and 2.1.2 which are both on track.

Action

Deliverable Status:

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
On track

Reason

This is not due to start until late 2018. We have had to generate new HME alfalfa events as the initial HME alfalfa showed poor trait expression. The new events will be available in time. We also can use soybean expressing the HME trait as they will be gifted by ZeaKal and have already been tested in field trials and shown to have normal Rhizobium symbiosis.

Action

Deliverable Status:

Click on the deliverable to enter a status

Short title
Nitrogen and water use efficiency in HME plant species

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

We have initially focussed on water use efficiency as research is conducted in controlled environment chambers. IRGA analysis predicted a 30% increase in intrinsic Water Use Efficiency (iWUE). This is a theoretical calculation. We have since shown for one HME ryegrass event that we have a 9% actual increase in WUE. The significance of this will be tested in field conditions in 2019 and 2020.

Action

Deliverable Status:

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
On track

Reason

In progress.

Action

Deliverable Status:

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
On track

Reason

This has been initiated with detailed assessment of HME ryegrass in controlled environment. The analysis supports the results from other species (Arabidopsis thaliana and soy bean) that the HME technology is a carbon concentrating mechanism and leads to enhanced photosynthesis.

Action

Deliverable Status:

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
On track

Reason

This research has only just been initiated with the testing of four different forms of nitrogen as sole nitrogen sources. We will be starting different combinations of nitrogen as part of our collaboration with 9(2)(a) at Lincoln University.

Action

Deliverable Status:

Click on the deliverable to enter a status

Short title
Water use efficiency

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Deliverable Status:

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
On track

Reason

We have measured a 9% increase in actual water use efficiency in one HME ryegrass event.

Action

Compare to other species when data available. Test in field conditions in later years.

Deliverable Status:

Click on the deliverable to enter a status

Short title
WUE in Legumes

Due Date
30/09/2019

Achievement measure
No achievement measure available

Status
On track

Reason

Not due to start until early 2018. Will start late (late 2018) due to need to develop new HME alfalfa. No issues completing the research by proposed end date of 30/9/19.

Action

Deliverable Status:

Click on the deliverable to enter a status

Short title
WUE in model grass species

Due Date
30/06/2019

Achievement measure
No achievement measure available

Status
On track

Reason

Not due to start until July 2018. HME rice development is on track and we have several events that will be suitable once homozygous seed is available.

Action

Deliverable Status:

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
On track

Reason

Will not start until mid 2019. The commercial ready HME ryegrass transformations have started and we are on track to have the trait in elite germplasm. This is supported by the AgResearch SSIF funding which has recently been confirmed for 5 years to match the MBIE funding.

Action

Deliverable Status:

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
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

Fixing the HME trait in homozygous ryegrass requires 4 generations to obtain a pool of homozygous seed. The full process takes 36 months. This is due to the out-crossing nature of perennial ryegrass and we have a validated system to complete this and avoid in breeding depression. We plan to progress 4 independent HME ryegrass events (one low, two medium and one high level expressing line). We are progressing these four events as we have shown that trait expression increases as we obtain homozygous plants. This is to ensure we field trial plants with appropriate levels of expression. As we complete this process we include the ryegrass endophyte in the crossing partners. We are then able to examine the symbiosis and confirm that the ryegrass endophyte can form stable associations with HME ryegrass.

Action

Deliverable Status:

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
On track

Reason

Action

Deliverable Status:

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

This was achieved for all 4 HME ryegrass Events.

Action

Deliverable Status:

Click on the deliverable to enter a status

Short title
T2 Generation

Due Date
22/12/2017

Achievement measure
No achievement measure available

Status
On track

Reason

Avhieved for 2/4 HME ryegrass events with second set of 2 to be completed as scheduled.

Action

Deliverable Status:

Click on the deliverable to enter a status

Short title
T3 Generation

Due Date
31/05/2018

Achievement measure
No achievement measure available

Status
On track

Reason

We have one T3 generation (ODR4501) that will be completed in February 2018. This will be tested in field trials in 2018. The T3 seed for the remaining events is due to be available as planned in time for the 2019 field trials.

Action

Deliverable Status:

Click on the deliverable to enter a status

Short title
HME Alfalfa trait fixation

Due Date
01/05/2019

Achievement measure
No achievement measure available

Status
On track with issues

Reason

We have started new transformations with HME alfalfa as the current events showed low levels of expression. We have a new gene construct called CORD that looks very promising in soy bean (Zeakal Inc data). This means we will be delayed starting the trait fixing process however we should remain on track to field trial some events in 2019 and the remainder in 2020.

Action

We can still meet our goal of developing homozygous alfalfa with increased metabolisable energy and enhanced photosynthesis by the end of the contract. We will also be able to complete all the required analysis in this time. We have a clear understanding of how to improve the gene construct for legumes as this has already been validated in soybean by Zeakal. The first constructs did actually work in soybean, although it appears the new CORD constructs are even better.

Deliverable Status:

Click on the deliverable to enter a status

Short title
T1 Generation

Due Date
30/11/2017

Achievement measure
No achievement measure available

Status
On track with issues

Reason

See 4.2

Action

Deliverable Status:

Click on the deliverable to enter a status

Short title
T2 Generation

Due Date
31/08/2018

Achievement measure
No achievement measure available

Status
On track with issues

Reason

See 4.2

Action

Deliverable Status:

Click on the deliverable to enter a status

Short title
T3 Generation

Due Date
30/04/2019

Achievement measure
No achievement measure available

Status
On track with issues

Reason

See 4.2

Action

Deliverable Status:

Click on the deliverable to enter a status

Short title
Commercial Ready HME Ryegrass trait fixation

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

The development of commercial ready HME ryegrass is supported by the SSIF funding recently confirmed for 5 years. Transformations are underway and we expect to have material for the trait fixing programme as scheduled. The seed industry stake holders will supply elite breeding material for the trait fixing process.

Action

Deliverable Status:

Click on the deliverable to enter a status

Short title
T1 Generation

Due Date
30/06/2019

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Deliverable Status:

Click on the deliverable to enter a status

Short title
T2 Generation

Due Date
31/03/2020

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Deliverable Status:

Click on the deliverable to enter a status

Short title
T3 Generation

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Deliverable Status:

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
On track

Reason

This research is on track and the first results come from in vitro methane assays and show that HME ryegrass may decrease methane emissions by 15-23%. This is consistent with scientific literature looking at fat in ruminant diets.

Action

Deliverable Status:

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
On track

Reason

Action

Deliverable Status:

Click on the deliverable to enter a status

Short title
Assays on alfalfa

Due Date
30/09/2019

Achievement measure
No achievement measure available

Status
On track with issues

Reason

Due to the delay in developing alfalfa with appropriate levels ofHME trait experssion we may need to compress the time lines for the research, however we expect to complete close to the planned completion date of September 2019.

Action

Work programme conditions

Work Programme Conditions
Condition 1. Progress toward vision Matauranga policy

AgResearch is utilising its relationship with Maori Agribusiness to develop an engagement plan. This will involve workshops on a regular basis to discuss issues relevant to Maori agribusiness. It is hoped that workshop attendees and contributors will then communicate progress and outcomes to the community. This is being coordinated by 9(2)(a) who has experience in previous engagement exercises with Maori entities around land use options. We have allocated budget resources for this exercise and hope to hold the first workshops in late 2017. They will run for the full contract, probably on a biannual basis.

Condition 2. Post Contract outcomes.

This has been completed and the post contract outcomes provided to MBIE.

Outputs

Knowledge transfer

Knowledge transfer

ModifiedDate	Knowledge transfer type	Number of Events
01/08/2017	Substantive information sharing or advice	2
02/08/2017	Workshops and Hui	12

Knowledge transfer

Knowledge transfer type
Substantive information sharing or advice

Number of Events

2

Knowledge transfer

Knowledge transfer type
Workshops and Hui

Number of Events

12

Non peer reviewed published articles

Number of non peer reviewed published articles.

5

New products, processes and services

Number of new products

0

Number of new processes

0

Number of new services

0

Science quality

Peer reviewed journal articles accepted for publication

2

Number of books or chapters

0

Number of published conference proceedings

0

Awards for science achievement (not open internationally)

1

Awards for science achievement (open internationally)

0

Keynote presentations (not open internationally)

0

Keynote presentations (open internationally)

0

Number of masters or doctorate theses

1

Provisional patent and PVR applications

Number of Patent or Plant Variety Right applications

0

Number of Patent Cooperation Treaty (PCT) applications

0

Number of Patent/s or Plant Variety Rights applied for in all countries

0

Patent and PVR grants

Number of Patent or Plant Variety Rights that have been granted

0

Number of Patent/s or Plant Variety Rights granted in NZ

0

Number of Patent/s or Plant Variety Rights granted in EU

0

Number of Patent/s or Plant Variety Rights granted in Japan

0

Number of Patent/s or Plant Variety Rights granted in US

0

Number of other countries where a Patent or Plant Variety Right has been granted

0

Revenue and subcontracting

Co-funding and subcontracting

Reporting financial year: 2016 (This report covers the period 01/07/16 - 30/06/17)

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	$37,499.99	$0.00	We are yet to sign the collaborative agreement between AgResearch, Dairy NZ, PGG Wrightsons and Grasslanz Technology. The collaborative agreement is essentially complete and close to sign off. GTL is one of the parties in the draft agreement. GTL have committed the full level of co-funding over the length of the contract.

Co-Funding	Dairy NZ	Yes	Direct	Cash	$562,500.00	$500,000.00	We are yet to sign the collaborative agreement between AgResearch, Dairy NZ, PGG Wrightsons and Grasslanz Technology. The collaborative agreement is essentially complete and close to sign off (September 2017). The $62,500 reduction from Dairy NZ was mutually agreed and has not had a negative impact on the programme as the costs this year for the overall programme were in line with the level of co-funding received. This co-funding supports the overseas field trials and they were at a small scale this year as we were testing all steps in the process. DNZ have committed to the full level of co-funding over the length of the contract.

Co-Funding	PGG Wrightsons	Yes	Direct	Cash	$75,000.01	$0.00	We are yet to sign the collaborative agreement between AgResearch, Dairy NZ, PGG Wrightsons and Grasslanz Technology. PGG Wrightsons will invest once the agreement is signed and this is essentially complete and close to sign off. PGGWS have accrued funding into the next financial year and the overall level of funding will be as projected. A step to complete is for PGG Wrightsons Seeds and Agriseeds need to reach agreement around elite ryegrass germplasm to be used in the programme. This does not impact the overall programme as costs were lower this year and we do not need the elite germplasm until late 2017.

Reporting financial year: 2016 (This report covers the period 01/07/16 - 30/06/17)

Organisation
Grasslanz Technology Limited

Select type
Co-Funding

Listed in the contract
Yes

Listed amount (NZD excl GST)
$37,499.99 (Excl. GST)

Type
Direct

Cash or In kind
Cash

Actual amount (NZD excl. GST)

0.00

(Excl. GST)

% of listed funding achieved:
0%

Comment

We are yet to sign the collaborative agreement between AgResearch, Dairy NZ, PGG Wrightsons and Grasslanz Technology. The collaborative agreement is essentially complete and close to sign off. GTL is one of the parties in the draft agreement. GTL have committed the full level of co-funding over the length of the contract.

Reporting financial year: 2016 (This report covers the period 01/07/16 - 30/06/17)

Organisation
Dairy NZ

Select type
Co-Funding

Listed in the contract
Yes

Listed amount (NZD excl GST)
$562,500.00 (Excl. GST)

Type
Direct

Cash or In kind
Cash

Actual amount (NZD excl. GST)

500,000.00

(Excl. GST)

% of listed funding achieved:
89%

Comment

We are yet to sign the collaborative agreement between AgResearch, Dairy NZ, PGG Wrightsons and Grasslanz Technology. The collaborative agreement is essentially complete and close to sign off (September 2017). The $62,500 reduction from Dairy NZ was mutually agreed and has not had a negative impact on the programme as the costs this year for the overall programme were in line with the level of co-funding received. This co-funding supports the overseas field trials and they were at a small scale this year as we were testing all steps in the process. DNZ have committed to the full level of co-funding over the length of the contract.

Reporting financial year: 2016 (This report covers the period 01/07/16 - 30/06/17)

Organisation
PGG Wrightsons

Select type
Co-Funding

Listed in the contract
Yes

Listed amount (NZD excl GST)
$75,000.01 (Excl. GST)

Type
Direct

Cash or In kind
Cash

Actual amount (NZD excl. GST)

0.00

(Excl. GST)

% of listed funding achieved:
0%

Comment

We are yet to sign the collaborative agreement between AgResearch, Dairy NZ, PGG Wrightsons and Grasslanz Technology. PGG Wrightsons will invest once the agreement is signed and this is essentially complete and close to sign off. PGGWS have accrued funding into the next financial year and the overall level of funding will be as projected. A step to complete is for PGG Wrightsons Seeds and Agriseeds need to reach agreement around elite ryegrass germplasm to be used in the programme. This does not impact the overall programme as costs were lower this year and we do not need the elite germplasm until late 2017.

Formal collaborations

Collaborations by country

Country	Level	Number of collaborations	Comment
United States of America (the)	Strong	3	The major collaboration that significantly benefits the programme is the collaborative agreement with the University of Missouri (UMC). This is a new partnership for AgResearch. Two AgResearch employees, 9(2)(a) and 9(2)(a) who are involved in this MBIE programme also work for a spin out biotechnology company ZeaKal inc (A US based company formed in 2010). The relationship with UMC was part of the ZeaKal network and provided expertise in soybean biotechnology and field trial capability. AgResearch was able to leverage of these relationships to develop a Master Research Services Agreement with UMC. UMC provides on a fee for service basis Plant Biochemistry, Plant Physiology and Field Trial capability. All IP remains under AgResearch ownership. This enabled AgResearch to conduct the first Industry funded HME ryegrass field trial in Missouri this year.
Thailand	Medium	1	

Capability building

Students

Number of students obtaining Doctorate qualifications

0

Number of students obtaining Masterate qualifications

1

Number of students obtaining Post-Doctorate qualifications

0

Secondments to or from end users

Number of secondments from an end user organisation

0

Number of secondments to an end user organisation

0

End user relationships

End user contact details

Organisation	Briefly describe how you are working with this organisation	Contact person	Contact phone	Contact email
Dairy NZ	Dairy NZ are co-funders and part of the collaborative agreement. They have a representative on the Programme Steering Group that has governance over the programme. DNZ work directly with dairy farmers, manage the Forage Value Index and work closely with processing companies. DNZ will be a key decision maker on how to proceed in New Zealand once the overseas field and animal nutrition trials are complete.	9(2)(a)		
PGG Wrightson Seeds	PGG Wrightson Seeds are co-funders and part of the inplementation pipeline as they provide a route to market. They will have a representative on the programme steering group.	9(2)(a)		

		9(2)(a)		
Grasslanz Technology Limited	Grasslanz Technology Ltd are co-funders and part of the inplementation pipeline as they provide expertise in endophyte commercialisation and the management of nucleus seed for the seed industry. They will have a representative on the programme steering group.			

Spinouts and startups

Spinouts and startups (super-users only)

Organisation	Current annual sales	Current annual export	FTE	Industry sector
Grasslanz Technology Limited	$11,441,493.00	$2,187,891.00	12	82 Plant Production And Plant Primary Products
Grasslands Innovation Ltd	9(2)(b)(ii)		0	82 Plant Production And Plant Primary Products
Farmax Ltd	9(2)(b)(ii)		6	83 Animal Production And Animal Primary Products
Phytagro Corp	$0.00	$0.00	0	82 Plant Production And Plant Primary Products
Phytagro NZ Ltd	$0.00	$0.00	0	82 Plant Production And Plant Primary Products
Phytagro Inc	$0.00	$0.00	0	82 Plant Production And Plant Primary Products
EnCoate Holdings Ltd	$0.00	$0.00	0	82 Plant Production And Plant Primary Products
AgResearch(USA) Ltd	$326,017.00	$326,017.00	2	82 Plant Production And Plant Primary Products
Covita Limited	$0.00	$0.00	0	83 Animal Production And Animal Primary Products
Phytagro LLC	$0.00	$0.00	0	82 Plant Production And Plant Primary Products
AgResearch (PPGR Consortia) Ltd	$0.00	$0.00	0	82 Plant Production And Plant Primary Products
AgResearch (Pastoral Genomics Consortia) Ltd	$0.00	$0.00	0	82 Plant Production And Plant Primary Products
AgResearch (Johnes Disease Research Consortium) Ltd	$0.00	$0.00	0	83 Animal Production And Animal Primary Products
Celentis Ltd	$0.00	$0.00	0	83 Animal Production And Animal Primary Products

Declaration

Declaration

Click the check box to acknowledge that the information you have given is true, correct and complete