COGGO	Final Report COGGO Research Fund for 2022 projects
Council of Grain Grower Organisations Limited ACN 091 122 039	**A project completion report covering the project. The acceptance of a satisfactory report against the objectives of the project, and agreement on the sharing of any commercial returns and/or IP will trigger payment within 4 weeks, by COGGO for any outstanding payments.**

This Final Report should be completed with reference to the Research and Intellectual Property Agreement (the Research Agreement) signed between the proponent and COGGO Pty Ltd.

1. Project information

Project title	**Using high throughput sequencing technology to transform disease surveillance**
Commencement Date	13 April 2022
Completion Date	13 April 2024

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Project Number	

Date Received	

2. Project results	This section provides a final report against the Project Aim and the Planned Outputs for the Project.

Achievement of the Project Aim	Brief statement of achievement in relation to the aim of the project

Overall objective

Project aim - Utilize high throughput sequencing technology to transform disease surveillance for the Western Australia's broadacre grains industry. Our model pathogen class for this project will be plant viruses.

Outcome - A new protocol was developed utilizing high-throughput genome sequencing technology for comprehensive and unbiased plant virus detection in a single bulk sample per crop. Therefore, the project aim was achieved.

Project Outputs		Please provide a report on the achievement, or otherwise, of the project outputs as per the planned outputs provided in the Project Proposal.
1	-	Output 1 (from Project proposal) A new protocol utilizing high-throughput genome sequencing (HTS) technology for comprehensive and unbiased plant virus detection in a single bulk sample per crop.
		Comment: COGGO funding provided crucial support to DPIRDs diagnostic laboratories, including the provisions of flow cells for genome sequencing, RNA extraction kits, PCR reagents and ELISA antibodies required to validate the HTS protocol. In the first 6 months of the project, we developed a high-throughput sequencing (HTS) workflow protocol to handle samples collected. This was adapted from Fowkes et al 2021 "Integrating High throughput Sequencing into Survey Design Reveals Turnip Yellows Virus and Soybean Dwarf Virus in Pea (*Pisum Sativum*) in the United Kingdom" published in MDPI Viruses to suit our particular objective and laboratory set-up. The HTS workflow underwent a validation experiment to test whether it could detect a single virus infected leaf in a bulk extraction of up to 99 uninfected leaves to mimic field situations and sensitivity detected the virus. The 2022 sequencing protocol was done using a MiSeq (Illumina, USA), and the 2023 protocol was done using DPIRDs new NextSeq (Illumina USA) which offered even faster and more comprehensive extraction of data from each sample. The bioinformatics pipeline was developed by DPIRDs Dr Asad Prodhan. 100 leaves per crop were collected from 3 cereal, 3 oilseed and 4 grain legume crops during the 2022 growing season and 4 canola, 4 cereal and 2 grain legume crops in the 2023 growing season (20 crops total). These samples were then split up into smaller subsamples and underwent a series of crop-specific diagnostic tests using the traditional strategy combining RNA extraction, PCR and serology to test for known endemic viruses. The cereal crops were tested for barley yellow dwarf virus (BYDV-PAV strain), wheat streak mosaic virus (WSMV) and cereal yellow dwarf virus (CYDV). The canola crops were tested for turnip yellows virus (TuYV) and turnip mosaic virus (TuMV). For pulses, lupins were tested for bean yellow mosaic virus (BYMV) and cucumber mosaic virus (CMV), lentils, field peas, faba beans and vetches were tested for potyviruses (BYMV and pea seedborne mosaic virus (PSbMV), CMV, alfalfa mosaic virus (AMV) and 'luteoviruses; (TuYV, soybean dwarf virus (SbDV), phasey bean mild yellows virus (PBMYV)).

Then samples were tested using the HTS method: total RNA extraction (QIAGEN, RNeasy) was conducted on all 100 leaves from each crop at once in a single bulk RNA extraction. Library preparation, sequencing and bioinformatic analysis was then conducted on each bulk extraction.

In 2022, endemic viruses were detected in 3/10 crops by the traditional method and 5/10 crops by the HTS method (Table 1). Not only did the new method detect 100% of the viruses detected by the traditional method (TuYV in samples 4, 5 and 6) but also detected 'brassica yellows virus' BrYV in sample 6 (a rare strain of TuYV), TuYV associated RNA (aRNA) in sample 4 (satellite virus with an unknown function), and cucumber mosaic virus (CMV) in samples 2, 5 and 10. The CMV detections in particular illustrate two key benefits of the new approach; (i) although CMV in canola has been reported, it was thought to be very rare, (ii) the PCR test used for CMV is likely to be too specific as it did not detect CMV in samples 2 or 10 (this would may been reported as negative to the grower). A follow up test confirmed these CMV detections using a loop-mediated isothermal amplification assay on the bulk extractions.

In 2023, endemic viruses were detected in 5/10 crops by the traditional method and 4/10 crops by the HTS method (Table 1). Initially, TuYV and SbDV was detected in samples 5 and 7, respectively, using the traditional method but were virus negative using the HTS method. When the bulk extracts used for HTS were follow up tested for TuYV and sample using specific PCR assays, no virus was detected suggesting the HTS method did not produce a false negative. This suggests one of the following – (i) the infected leaves did not make it into the bulk extraction, or (ii) the initial detection was a false positive. Again, TuYV was the most commonly detected virus with crop incidences of up to 95% and TuYV aRNA was detected in 3 of 4 TuYV infected canola crops. TuYV was detected by HTS in sample 7 even though it was not detected by HTS, demonstrating the increased sensitivity of the HTS method.

Table 1. Summary results table for samples tested from the 2022 and 2023 growing season.

		NEW HTS METHOD		TRADITIONAL METHOD	
Season	**Crop sample**	**Common virus detected**	**Rare viruses detected**	**Viruses tested**	**Virus positive**
2022	1. Oat	None	No	BYDV-PAV/CYDV/WSMV	None
	2. Faba bean	CMV	No	Poty/CMV/Luteo	None
	3. Oat	None	No	BYDV-PAV/CYDV/WSMV	None
	4. Canola	TuYV + aRNA	No	TuYV/TuMV	TuYV (70%)
	5. Canola	CMV; TuYV	No	TuYV/TuMV	TuYV (50%)
	6. Canola	TuYV; BrYV	Yes	TuYV/TuMV	TuYV (10-20%)
	7. Barley	None	No	BYDV-PAV/CYDV/WSMV	None
	8. Lupin	None	No	Poty/CMV	None
	9. Lentil	None	Yes	Poty/CMV/Luteo/AMV	None
	10. Lentil	CMV	No	Poty/CMV/Luteo/AMV	None
2023	1. Canola	TuYV + aRNA	No	TuYV/TuMV	TuYV (15%)
	2. Canola	TuYV + aRNA	No	TuYV/TuMV	TuYV (20%)
	3. Wheat	none	No	BYDV-PAV/CYDV/WSMV	None
	4. Canola	TuYV + aRNA	No	TuYV/TuMV	TuYV (95%)
	5. Pea	none	No	Poty/CMV/Luteo/AMV	TuYV (11%)
	6. Wheat	none	No	BYDV-PAV/CYDV/WSMV	None
	7. Canola	TuYV	No	TuYV/TuMV	None
	8. Faba bean	none	No	Poty/CMV/Luteo/AMV	SbDV (4%)
	9. Wheat	none	No	BYDV-PAV/CYDV/WSMV	None
	10. Wheat	none	No	BYDV-PAV/CYDV/WSMV	None

Lastly, the other benefit of the HTS method listed in the project proposal was the identification of rare, or unknown viruses. Rare or unknown plant viruses were detected in 2 of the samples, and a number of viral sequences from various fungi infecting viruses were detected in the majority of samples.

Project results	*Please provide brief statements on the results of the Project* The protocol was developed and validated on a field sample simulation of 1 infected leaf in 100 and proved highly sensitive. When applied to field samples, it was just as sensitive as traditional molecular diagnostic techniques such as PCR. This means that, when a sample is negative for a given virus, the HTS test can provide valid confirmation and save time and money on the resources associated with traditional testing to confirm negative infection status. One of the primary benefits of the protocol, was its ability to detect unexpected viruses or strains of known viruses. For example, it detected CMV in canola, which is thought to rarely infect canola and therefore is not usually tested for. However, as it was found in several samples, and follow up testing confirmed its prevalence. Further research is now justified on the impact of this virus in canola and whether infection in canola plays a role in CMV epidemiology in narrow-leafed lupins or lentils. The protocol also detected many phytoviruses and mycoviruses with unknown biology, demonstrating how complex and diverse the grain crop virome is in Western Australia and how much we still do not know. Some of these viruses are likely to be either pathogenic, neutral or synergistic with the plant and reporting their presence will provide a valuable resource if they become a target of biological research in the coming years. The approach to crop surveillance developed in this project serves as a template for other systems and pathogen types such as fungal or bacterial disease in grains or horticulture. Our economic analysis showed that, even at this stage, this new HTS approach to virus surveillance is far cheaper than the traditional approach and will continue to become cheaper over time. Total cost to sequence and analyze 20 samples was $10,000. As a comparison, for DPIRDS Diagnostic Laboratory to test the same 20 samples for 10 endemic viruses in sample groupings low enough to enable detection it would cost ~$24,000. Future work should examine other HTS technologies as they come on the market, and whether similar results can be achieved with less intensive sequencing, allowing greater sample number, and reduced time in sequencing and bioinformatics. Furthermore, once they are identified, funding should be allocated to conduct the biological research necessary to determine the function and transmission modalities of the many obscure viruses found in WA grain crops. It is possible that these could be 'immune boosting' agents that provide crops with improves stress tolerance to biotic and abiotic stress. They could also be drivers of novel disease emergence in the future, and so should be monitored. Genomic sequencing technology has already greatly benefited the WA Grains Industry, and this is just another application that will provide a stepwise increase in data extracted from growers crops. Coupled with biological research, this will yield powerful information that will have expected benefits addressed above, but also likely unforeseen benefits. This approach will be further expanded in a subsequent GRDC-funded project and then published in a peer-reviewed journal article.

This section should cover aspects identified in *Section 7.3* of the Research Agreement

- the results of the Project, including discoveries made and other achievements (including any Project IP and Project Confidential Information);
- the potential application of the outputs of the Project to the Western Australian grains industry and broader community;

- the actual or potential economic benefits flowing to the Western Australian grains industry and broader community from the Project;
- the difficulties encountered;
- the conclusions reached;
- the Researcher's recommendations for any further research;
- a list of scientific papers or publications resulting from the Project; and
- attach copies of any photos, diagrams or other artworks (including, if requested by COGGO, negatives, bromides or the like) which the Researcher has and which may be of assistance to COGGO in the dissemination of information concerning the Project to COGGO's stakeholders.

3. Project resources	This section describes use of the funding listed in the initial plan and any refunds due to COGGO

Expenditure of funds requested from COGGO	$ Total funds budgeted	$ Total funds expended (actual)	$ Total funds requested from COGGO*	$ Total COGGO funds expended	$ Refund due to COGGO of any unexpended COGGO funds
Salary/Contractors	0	0	0	0	
Operating costs	$73,270.96	$67,519.93	$73,270.96	$67,519.93	$5,751.07
Capital	0	0	0	0	
TOTAL	$73,270.96	$67,519.93	$73,270.96	$67,519.93	$5,751.07

*Funding provided by COGGO.

IMPORTANT: Return of unused funds to COGGO is required as per *Clause 3.3* of the Research Agreement.

4. Commercialisation	Insert details of the proposed commercialisation process,as applicable, with reference back to the planned commercialisation plan in the project proposal) for any outputs from the project. This should include recommendations for the commercialisation of the results of the project and the registration or other protection of Project IP and Project Confidential Information as per the Research Agreement.

No IP was generated in this study

It is understood that this may require further discussion and agreement with COGGO via its' agent GIWA, as per the undertakings given and terms agreed, in the project proposal. This can be the subject of an appended letter and attachments. In all cases such discussion and subsequent agreements need to be governed by *Section 8 Project IP, Improvements and Project Confidential information* of the Research Agreement.

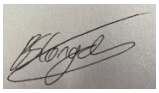
5. Communication/	Insert details of how the communication and extension of the project outcomes has been achieved to date and

Extension	recommendations for future activities to disseminate and promote adoption of the results of the Project.
	In 2021, this project was announced via platforms such as the Farm Weekly and Countryman. Similarly, the project results will be included in a media release in 2024. The protocol will utilised in a subsequent project in which it will used to test grower samples. This approach, or something like it, will likely become the standard in DPIRDs diagnostic laboratory service.

Note: As per *Clause 7.3 (b) (ii)* of the Research Agreement COGGO may require the Researcher to produce an edition of the Final Report in a form suitable for general distribution. If so required by COGGO, the Researcher must produce a non-confidential version of the Final Report within 28 days of receiving a request to that effect from COGGO.

6. Certification

The Project Supervisor and the Research Organisation certify that all information contained in, and forming part of, this final project report is complete and accurate. The project supervisor and research organisation further warrant that the project complied with all the relevant guidelines affecting the conduct of research, for example in relation to ethics, bio-safety, environmental legislation, GMAC or National Health and Medical Research Council Codes.

Project Supervisor's signature 

Name (in Capitals)

BENJAMIN CONGDON Date: 8 April 2024

Research Organisation signature 

Name and title of authorised signatory (in Capitals)

HELEN SPAFFORD, MANAGER CROP PROTECTION Date: 12 APRIL 2024

Completed Final Project reports

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COGGO representative

For the purpose of this Project agreement contract, COGGO will be represented by Grains Industry Association of Western Australia (GIWA), or such other representative that is nominated by COGGO as authorised to operate on behalf of COGGO.

PROJECT SYNOPSIS SUITABLE FOR GENERAL PUBLICITY AND COGGO WEBSITE

Genome sequencing has revolutionized science by giving researchers access to the code of life. Sequencing technology has been evolving at a breathtaking pace over the past decade. For example, the first human genome cost approximately $1 billion, but now it costs less than $1000 and continues to get cheaper. The human genome is millions of times larger than the average virus so sequencing virus genomes is even faster and cheaper. More information about the virus can be generated in a much shorter time. This leads to powerful applications being rapidly realised. For example, this technology has been vital during the COVID-19 pandemic to track virus evolution and the emergence of new strains that have important biological differences such as their transmissibility and reaction to vaccines. Now that genome sequencing is no longer cost-prohibitive to agricultural research, its extraordinary power can be leveraged to open up novel avenues for its use. The crop diagnostic and surveillance approach developed in this project marked the first of its kind in Western Australia.

Plant surveillance involves taking a representative sample from an affected crop (commonly 100 leaves), and testing the sample in groups of 2 to 10 (i.e. 10 to 50 sub-samples per crop) for a handful of known endemic viruses to enable estimation of infection rate. Depending on the crop and the viruses that are known to infect it, sometimes multiple different diagnostic platforms such as PCR (molecular) and ELISA (serological) are needed, each with their own unique leaf extraction, costs and technical skill requirements. In many cases, just one virus is detected in the crop and so significant resources are expended testing for the others. Sometimes, due to their specific nature, these tests can miss genetic variants of a species. Furthermore, this process completely misses unexpected or novel viruses that can be pathogenic, neutral, or even synergistic with the crop.

In this project, we collected 100 leaves from 20 grains crops (7 cereal, 7 canola, 6 pulses) during the 2022 and 2023 growing season to develop and validate a completely new way of conducting crop diagnosis and surveillance using cutting edge genome sequencing technology – the Illumina MiSeq (2022 samples) and the Illumina NextSeq (2023 samples). Using this high-throughput sequencing (HTS) method, all 100 samples from each crop were tested in a single test. To validate the new approach, 13 different virus species across the three crop types were tested for using the traditional approach. Turnip yellows virus (TuYV) infection in canola was by far the most common virus detected in the study and is currently a species of current research investigation. The HTS approach detected viruses detected by the traditional approach, and many others that would never have been detected by the traditional approach. Rare strains of TuYV and TuYV satellite RNA with unknown but likely important function were detected. Although reported previously but considered to be rare, cucumber mosaic virus (CMV) was detected in canola raising questions on its impact on canola and the epidemiological importance of canola to CMV infection in lupin and lentil. Lastly, rare or unknown plant viruses with unknown roles were detected in 2 of the samples, and a number of viral sequences from various fungi-infecting viruses were detected in the majority of samples. The HTS approach also proved to be far more efficient and cost-effective than the traditional approach as sub-sampling to confirm infection rate need not be done for viruses that are not present and can be focused on those that are.

Moving forward, this method will exponentially increase the amount of data obtained from field activities in plant virology and provide assumption-free detection of viruses and other microorganisms in grains crops. Genetic variants, new strains, and new viruses can be detected early allowing researchers and industry to make the appropriate adaptations more rapidly. This project also provides a proof-of-concept template for similar surveillance of beneficial and pathogenic species of bacteria, nematodes, phytoplasma and fungi, and beneficial and pest species of arthropods and the symbiotic or pathogenic microorganisms inside them. This greatly facilitates identification of biocontrol agents for some of our most notorious arthropod pests that are increasingly difficult to control with pesticides e.g. nuclear polyhedrosis virus used to control insecticide resistant fall army worm and is now marketed by AgBiTech as Fawligen.