AgriScience Program - Projects Component

Annual Performance Report

Name of Recipient: Jaswinder Singh	
Project Title: Tuning the Oat Genome with CRISPR-based systems	
Project Number: ASP-061	**Period Covered by the Report** (2019/04/01 to 2020/03/31):
Project Start Date (2019/04/01):	**Project End Date** (2021/03/31):

1. Performance Measures – Project Level

In the performance measures table below, please provide the results and achievements that were finalized during the reporting period, that combines all the CA and CRDA activities. Do not include results that are not final or that will continue to be developed. It is quite possible that in the first year or two, there may not be any results to report. Please see Annex A for a description of each performance measure.

	Performance Measure	**Results Achieved**	Provide a brief description of each final result achieved during the reporting period.
1.	Number of highly qualified personnel (HQP) working on funded activities (HQP refers exclusively to current Master and PhD students)	3	Two MSc students and one part time Research Associate
2.	Training/knowledge transfer events		
	2.1 Number of training/knowledge transfer events organized by the recipient	NA	
	2.2 Number of presentations made in training/knowledge transfer events	2	**1.** Fatmawati, A., Mahmoud, M., Donoso, T., Chen, W-Y., Kaur, R., Tinker, N., **Singh, J**. (2019) Genetic transformation of oat to elucidate a gene associated with beta-glucan. Plant Canada 2019, July 7-10, Guelph, Ontario, Canada **(Poster)** **2.** Fatmawati, A., M. Mahmoud, T. Donoso, W.Y. Chen, R. Kaur, N.A. Tinker, **J. Singh**. (2020) Towards an understanding of beta-glucan regulation in oat. PAG XXVIII, Jan. 11-15, San Diego, California, USA (**Oral**).

Performance Measure		Results Achieved	Provide a brief description of each final result achieved during the reporting period.
3.	Number of participants at training/knowledge transfer events		
4.	Number of new knowledge transfer products developed		
5.	Number of papers published in peer reviewed journals		
6.	Number of new technologies (new products, practices, processes and systems) that are developed		
7.	Number of new technologies (new products, practices, processes and systems) that are assessed under research conditions		
8.	Number of new technologies (new products, practices, processes and systems) that are demonstrated on-farm or in-plant		
9.	Number of new technologies (new products, practices, processes and systems) that attain Intellectual Property (IP) protection.		
10.	Number of new technologies (new products, practices, processes and systems) that are utilized		

2. Activity-level Information

In this section, please complete one table for each activity. For activities with both a CA and CRDA component, please integrate the results into one table.

CA Activity Number: __1__ / CRDA Activity Number: ___
Name(s) of Activity: Development of oat genome specific gene constructs for genome editing
Principal Investigator: Jaswinder Singh
Summary of Activity Please provide a high-level summary of this activity that includes an introduction, objectives, methodology, deliverables, results and discussion. Technical language can be used in this section.
In this activity, expression of oat TLP8 was observed from different oat varieties. The oat TLP sequences were analysed to identify appropriate genic targets for genome editing. CRISPR-basd method in oat is being standardized using specific gene constructs involving TLP8 gene associated with β-glucan regulation. The genome editing will simply alter/ exchange existing genes with other versions of the gene from within existing oat germplasm and expected to create improved versions of the genes in the oat genome with great precision.

Methodology:

RNA Synthesis. The samples were used in this experiment were *Avena sativa* var.Hifi, *Avena sativa* var. CDC, *Avena sativa* var. Goskin, *Avena sativa* var. AcMorgan, *Avena sativa* var. CDC, *Avena sativa* var. Park, and *Avena strigosa*. Mature seeds were imbibed for 16 hours. The seeds were submerged in liquid nitrogen as soon as possible to prevent the RNA degradation. Afterward, the samples were ground in mortar to yield fine powder by applying liquid nitrogen. To lyse tissue samples, 700 µl of lysis solution/2-ME mixture were added to tissue powder followed by vortexing it immediately and vigorously as 30 seconds. The sample was then incubated at room temperature for 3-5 minutes. The tubes were then centrifuged at maximum speed for 3 minutes to pellet cellular debris. The supernatant was taken into a filtration column seated in a 2-ml collection tube by positioning the pipette tip at the bottom of the tube but away from the pellet. The column was then centrifuge to filtrate solution at 12000 rpm for 1 minute.

The binding solution as 700 µl was added to the filtrate lysate and mixed immediately by pipetting at least 5 times. The mixture was then placed into binding column followed by centrifugation for 1 minute to bind RNA. The flow through liquid was decanted and the collection tube was tapped in clean absorbant paper. The column was then returned into collection tubes. This process was followed by two times washing steps using washing solution 2. The final step was elution by adding 50 µl elution solution directly into the center of the binding matrix inside the coloumn.

cDNA synthesis. RNA which has been synthesized was then treated using DNAse and was ready for cDNA synthesis using iScript cDNA synthesis kit (Bio-Rad). 6 µl of DNAse-treated RNA was mixed with 10 µl master mix, 3 µl oligo dT primer, and 1 µl enzyme to form 20 µl final reaction. The amplification condition was performed as 42^0 C for 5 minutes, 55^0C for 15 minutes, and 95^0C for 5 minutes. The cDNA was generated and could be either stored in -20°C for next PCR or directly used for PCR analysis.

RT-PCR. The PCR was performed in 20 µl solution containing 1 µl cDNA template, 10 µl green master mix, 7 µl molecular water, 1 µl forward primer, and 1 µl reverse primer. The primer used was AsTLP8rsF (CGGACCGACTAGTCCGGCGGGGGCC) and AsTLP8rsR (CCTAGGGAGCTCCGGGCAGGAAGTCCATGG) to amplify the Thaumatin Like Protein (*TLP8)* gene which was expected to show band at 304bp. The amplification conditions applied was 95 °C for 2 min, followed by 30 cycles at 94 °C for 30 s, 63 °C for 30 s, and 72 °C for 30 s. The PCR product was then analyzed on 1% agarose gel. The actin was chosen as housekeeping gene. The primers used for Actin amplification were AsActinF (GAGACCTTCAATGTTCCAGCCATG) and AsActin R (ATACTTCCTCTCGGGCGGTG) which band size expected was 600bp.

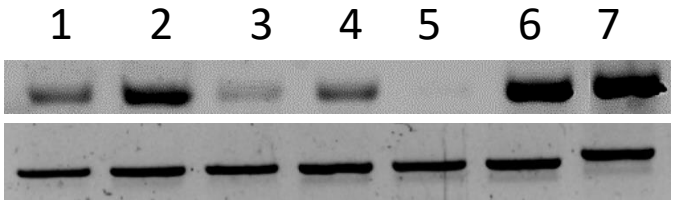
TLP sequences and their analysis:

Using the sequence of hexaploid species *Avena byzantina*, cluster analysis was done on the homologs of barley *TLP8* in oat. First, the thaumatin family profile was searched for in the sequences.

Results:

The Semi-Quantitative PCR experiment was conducted after 16 hours of seed imbibition of 6 samples (*Avena sativa* var.Hifi, *Avena sativa* var. CDC, *Avena sativa* var. Goskin, *Avena sativa* var. AcMorgan, *Avena sativa* var. CDC, *Avena sativa* var. Park, and *Avena strigosa)* with *Actin* was chosen as the housekeeping gene. The primer used was AsTLP8rsF (CGGACCGACTAGTCCGGCGGGGGCC) and AsTLP8rsR (CCTAGGGAGCTCCGGGCAGGAAGTCCATGG)

to amplify the Thaumatin Like Protein (*TLP8)* gene with the amplification at 304bp. This approach revealed the differential expression of *AsTLP8* of six samples in which, *Avena strigosa,* the only diploid oat in the samples, showed the highest expression. Whereas, the least *AsTLP8* expression was discovered in hexaploid oat *Avena sativa* var. Park. Hifi has considerably the same expression profile as AcMorgan, which presented slightly higher than Goskin.



1. Hifi
2. CDC
3. Goskin
4. AcMorgan
5. Park
6. Avena strigosa
7. Control (genomic DNA)

Sequence Analysis:

```
CLUSTAL multiple sequence alignment by MUSCLE (3.8)


Astrigosa       WGAAVP-GGGQKLDPGQQWKVDVPAGTTSGRVWARTGCNFDGSGNGKCETGDCGGKLQCT
AbTLP8-3        WGAAVP-GGGQKLDPGQQWKVEVPAGTTSGRVWARTGCNFDGSGNGKCETGDCGGKLQCT
HvTLP8          WAAAVPAGGGQKLDAGQTWSINVPAGTTSGRVWARTGCSFDGAGNGRCQTGDCGGKLRCT
AbTLP8-1        WAAAVPVGGGRKLDPGQTWNINVPAGTTSGRVWARTGCNFDGSGNGRCQTGDCGGKLQCT
AbTLP8-2        WAAAVPAGGGRKLDPGQSWNINVPAGTTGGRVWARTGCNFDGSGNGRCQTADCGGKLQCT
                *.**** ***.***.** *.::*******.*********.***:***.*:*.******.**
                :**************
```

Figure 2: Thaumatin family profile in each respective species. The thaumatin family profile is G-X-[GF]-X-C-X-T-[GA]-D-C-X-(1,2)-G-X-(2,3)-C where X refers to any amino acid and is highlighted.

Required amino acids are shown in red. *AbTLP-1,2,3* refer to the three homologs of barley *TLP8* in *Avena byzantina*. *Astrigosa* is the homolog in the diploid oat species *Avena strigosa. HvTLP8* refers to the TLP8 in barley (*Hordeum vulgare*).

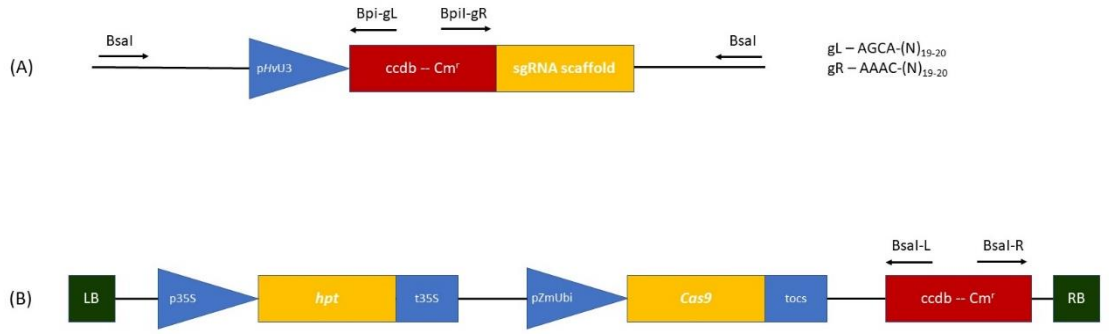
Every sequence searched had the thaumatin family profile, suggesting that they are thaumatin-like proteins (Figure 2). However, this does not proves function of the sequences. So, the same sequences were searched for the sugar-binding domain (CQTGDCGG).

```
Astrigosa       WGAAVP-GGGQKLDPGQQWKVDVPAGTTSGRVWARTGCNFDGSGNGKCETGDCGGKLQCT
AbTLP8-3        WGAAVP-GGGQKLDPGQQAWKVEVPAGTTSGRVWARTGCNFDGSGNGKCETGDCGGKLQCT
HvTLP8          WAAAVPAGGGQKLDAGQTWSINVPAGTTSGRVWARTGCSFDGAGNGRCQTGDCGGKLRCT
AbTLP8-1        WAAAVPVGGGRKLDPGQTWNINVPAGTTSGRVWARTGCNFDGSGNGRCQTGDCGGKLQCT
AbTLP8-2        WAAAVPAGGGRKLDPGQSWNINVPAGTTGGRVWARTGCNFDGSGNGRCQTADCGGKLQCT
                *.**** ***.***.** *.::*******.*********.***:***.*:*.******.**
```

Figure 3: Sugar binding domain in each respective species. The sugar binding domain is CQTGDCGG and is highlighted in yellow (Singh et al. 2017). Mutated amino acids in other species are in red. *AbTLP-1,2,3* refer to the three homologs of barley *TLP8* in *Avena byzantina*. *Astrigosa* is the homolog in the diploid oat species *Avena strigosa. HvTLP8* refers to the TLP8 in barley (*Hordeum vulgare*).

Only the first homolog in *Avena byzantina* and *HvTLP8* contain an intact sugar-binding domain. As shown in Figure 3, only *AbTLP8-1* and *HvTLP8* contain an intact sugar-binding domain. Interestingly, the sugar-binding domain is mutated in the A-genome diploid species *Avena strigosa,* which is typically associated with a higher beta-glucan content (Welch et al. 2000). The

presence of a sugar-binding domain hints at the interaction between this protein and beta-glucan. Modification this genic region through gene editing will be valuable to further understand this interaction. For this purpose, constructs are being developed using Golden Gate system. Construct plan is described below



Issues

- Describe any challenges or concerns in achieving the results and deliverables of this activity during the reporting period. How were they overcome or how do you plan to overcome?
- Describe any potential changes to the work plan and the budget during the reporting period. How were or how will they be managed?

COVID-19 outbreak resulted in lab closed on March 14, 2020 so plasmid creation was interrupted and options are being explored.

Key Achievements

A key achievement represents a significant achievement or tangible result that could potentially be applied either by farmers or industry or the science community. In one to three paragraphs, please provide key achievements that meet one of the following criteria:

1) The item has commercial potential (all testing and piloting has been completed);
2) The item has been commercialized; or
3) The item has been adopted by the sector.

Examples of tangible results could include increased sustainability (beneficial management practice), reduced costs, improved productivity or increased profitability. Please note that the information provided will be used for communication purposes only.

If no key achievements have been realized at this stage, please leave this section blank.

CA Activity Number: ____ / CRDA Activity Number: ___
Name(s) of Activity:
Principal Investigator:
Summary of Activity Please provide a high-level summary of this activity that includes an introduction, objectives, methodology, deliverables, results and discussion. Technical language can be used in this section.
Issues • Describe any challenges or concerns in achieving the results and deliverables of this activity during the reporting period. How were they overcome or how do you plan to overcome? • Describe any potential changes to the work plan and the budget during the reporting period. How were or how will they be managed?
Key Achievements A key achievement represents a significant achievement or tangible result that could potentially be applied either by farmers or industry or the science community. In one to three paragraphs, please provide key achievements that meet one of the following criteria: 4) The item has commercial potential (all testing and piloting has been completed); 5) The item has been commercialized; or 6) The item has been adopted by the sector. Examples of tangible results could include increased sustainability (beneficial management practice), reduced costs, improved productivity or increased profitability. Please note that the information provided will be used for communication purposes only. If no key achievements have been realized at this stage, please leave this section blank.

CA Activity Number: ____ / CRDA Activity Number: ___

Name(s) of Activity:
Principal Investigator:
Summary of Activity Please provide a high-level summary of this activity that includes an introduction, objectives, methodology, deliverables, results and discussion. Technical language can be used in this section.
Issues • Describe any challenges or concerns in achieving the results and deliverables of this activity during the reporting period. How were they overcome or how do you plan to overcome? • Describe any potential changes to the work plan and the budget during the reporting period. How were or how will they be managed?
Key Achievements A key achievement represents a significant achievement or tangible result that could potentially be applied either by farmers or industry or the science community. In one to three paragraphs, please provide key achievements that meet one of the following criteria: 7) The item has commercial potential (all testing and piloting has been completed); 8) The item has been commercialized; or 9) The item has been adopted by the sector. Examples of tangible results could include increased sustainability (beneficial management practice), reduced costs, improved productivity or increased profitability. Please note that the information provided will be used for communication purposes only. If no key achievements have been realized at this stage, please leave this section blank.

Please add additional tables here as required

Annex A

Performance Measures Table	
Performance Measures	**Description**
1. Number of highly qualified personnel (HQP) working on funded activities	This includes only individuals who are registered in Master or PhD programs and are working on activities that receive funding through the Canadian Agricultural Partnership. They are only counted in their first year working on projects. For each reported HQP, please provide the following: the name of the student, level of degree, field of study and name of the academic institution.
2. Training/knowledge transfer events	
2.1. Number of training/knowledge transfer events organized by the recipient	This includes events completed in the reporting year that were organized under the project to share results of the activities with audiences who may use that knowledge in the future. Examples could include training events, scientific meetings, symposia, conferences, workshops, industry meetings, field days or webinars. Annual General Meetings do not normally qualify for this category as they are considered to be part of normal day-to-day business. For each reported item, please provide the following: name of the event, name of the organizer and organization, location, and year/month/day.
2.2. Number of presentations made in training/knowledge transfer events	This includes oral presentations and poster presentations at events that are not organized by the recipient, for example, conferences, symposiums or training events. For each reported item, please provide the following: name of presenter, title of presentation, name of the event, location, and year/month/day.
3. Number of participants at training/knowledge transfer events	This includes individuals who attend the events listed and who may use that knowledge in the future.
4. Number of new knowledge transfer products developed	New knowledge could include, but is not limited to: 1) newly acquired knowledge that differs significantly from previously acquired knowledge; 2) existing knowledge that is enhanced to meet different requirements; 3) existing knowledge that is applied in different situations. These are knowledge transfer materials created under the project that have been disseminated to transfer information to audiences who may use that knowledge in the future. Examples could include brochures, factsheets, flyers, guides, articles in trade magazines, technical bulletins and social media items. Only the number of products developed should be reported, not the number of copies that were printed and disseminated. For each reported item, please provide the following: author(s), title of the item, type of the reported item (e.g. brochure), name of the trade

	magazine/publisher and page number(s) if applicable, and year/month/day.
5. Number of papers published in peer reviewed journals	This includes scientific papers that are published in peer reviewed journals. Papers that are not yet published (ex. manuscripts in preparation, under review or accepted) should not be reported. For each reported item, please provide the following: author(s), year of publication, article title, title of journal, volume (issue), and page number(s). If the item is a book or a book chapter, add name of publisher. If the item is an article for conference proceedings, add title of published proceedings, location, and year/month/day.
6. Number of new technologies (new products, practices, processes and systems) that are developed	A new technology could include, but is not limited to: 1) a newly created technology that differs significantly from existing technologies; 2) an existing technology that is modified to meet different requirements; 3) an existing technology that is tested in different situations. New products are goods and services that differ significantly in their characteristics or intended uses from products previously produced and used. Examples could include equipment, software, novel foods or consumer goods. New practices are new agronomic techniques or methods that can be applied directly by producers. New processes are the set of operations performed by equipment in which variables are monitored or controlled to produce an output in labs or processing facilities. New systems are the set of detailed methods, procedures and routines created to carry out a specific activity, perform a duty, or solve a problem. Development consists of the creation of a new product, the generation of a new practice, or the demonstration of utility of a new process or system. This category does not include new varieties. New varieties are only reported under 'Number of new technologies that attain Intellectual Property protection' and/or 'Number of new technologies that are utilized'. Gene sequences, breeding lines and populations are not eligible under this category. To avoid duplication, for any new technologies, only set a target that represents the last stage in the innovation process. For example, a new technology is either developed, or assessed, or demonstrated or utilized.
7. Number of new technologies (new products, practices, processes and systems) that are assessed under research conditions	See the definition of new technologies under #6. Are assessed: when new technologies are evaluated or tested under research conditions.

	This category does not include new varieties. New varieties are only reported under 'Number of new technologies that attain Intellectual Property protection' and/or 'Number of new technologies that are utilized'. Gene sequences, breeding lines and populations are not eligible under this category. To avoid duplication, for any new technologies, only set a target that represents the last stage in the innovation process. For example, a new technology is either developed, or assessed, or demonstrated or utilized
8. Number of new technologies (new products, practices, processes and systems) that are demonstrated on-farm or in-plant	See the definition of new technologies under #6. Are demonstrated: when new technologies are presented to the sector by experiments, prototypes, examples or pilot on-farm or in-plant. This category does not include new varieties. New varieties are only reported under 'Number of new technologies that attain Intellectual Property protection' and/or 'Number of new technologies that are utilized'. Gene sequences, breeding lines and populations are not eligible under this category. To avoid duplication, for any new technologies, only set a target that represents the last stage in the innovation process. For example, a new technology is either developed, or assessed, or demonstrated or utilized.
9. Number of new technologies (new products, practices, processes and systems) that attain Intellectual Property (IP) protection	See the definition of new technologies under #6. Examples for IP protection could include, but are not limited to: plant breeder rights, patents filed, registered trademarks and copyrights, and registered germplasms and released varieties (excluding breeding lines and gene sequences). For each new variety, please provide the registration number, the variety name, and year/month/date.
10. Number of new technologies (new products, practices, processes and systems) that are utilized	See the definition of new technologies under #6. Are utilized: when new technologies are adopted or implemented for use within the sector. Examples may include, but are not limited to: a signed license agreement, a signed letter of intent, a new product that is available on the market, and a new practice which is adopted by farmers. Gene sequences, breeding lines and populations are not eligible under this category. To avoid duplication, for any new technologies, only set a target that represents the last stage in the innovation process. For example, a new technology is either developed, or assessed, or demonstrated or utilized.