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** (2020/04/01 to 2021/03/31):
Project Start Date (2019/04/01):	**Project End Date** (2022/03/31):

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)	2	Two MSc students: 1. Thomas Donoso, MSc Plant Science, McGill University 2. Annis Fatmawati, MSc Plant Science, McGill University
2.	Training/knowledge transfer events		
	2.1 Number of training/knowledge transfer events organized by the recipient		
	2.2 Number of presentations made in training/knowledge transfer events	1	***Transposon- assisted biotechnology in small grain cereals***, IV International Scientific Conference, "Latest Achievements of Biotechnology", National Aviation University**,** Kyiv Ukraine, September 22-23, 2020 (**Plenary lecture**, delivered remotely due to Pandemic).
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		

Performance Measure		Results Achieved	Provide a brief description of each final result achieved during the reporting period.
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: ____ / 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. **Uncovering the *TLP8* homologs in *Avena sativa*** The Pepsico OT3098 sequenced genome was used to find homologs of the *HvTLP8* in oat using BLASTN (Figure 1). The assembled genome has assorted the sequences into one of the three genomes, making it possible to determine which homeolog is found on which genome. Interestingly, the homeolog on the D-genome has a 60bp deletion. The deletion includes the sugar-binding motif previously described (Singh et al., 2017). Considering OT3098 is a variety high in grain beta-glucan, this 60bp deletion may indicate the antagonistic effect of *TLP8* homeologs on soluble fibre content. Using the Pepsico data, specific primers were designed for each genome copy, allowing for the sequencing of the *TLP8* homeologs in the oat variety Park. In this variety, which does not have a high amount of soluble fiber, there is no 60bp deletion in the D-genome homeolog.

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Consensus       CATCAACGTGCCGGCCGGCACGACVAGCGGGCGCGTGTGGGCGCGCACGGGCTGCAACTT  227
OT3098-AsTLP8-D CATCAACGTGCCTGCCGGCACGACGGGCGGGCGCGTGTGGGCGCGCACGGGCTGCAACTT  227
OT3098-AsTLP8-C CATCAACGTGCCGGCCGGCACGACAAGCGGGCGCGTGTGGGCGCGCACGGGCTGCAACTT  227
OT3098-AsTLP8-A GGTCGAGGTGCCGGCCGGCACGACCAGCGGGCGCGTGTGGGCGCGCACGGGCTGCAACTT  227
AsTLP8-D-Park   CATCAACGTGCCTGCCGGCACGACGGGCGGGCGCGTGTGGGCGCGCACGGGCTGCAACTT  227
AsTLP8-C-Park   CATCAACGTGCCGGCCGGCACGACAAGCGGGCGCGTGGGGGCGCGCACGGGCTGCAACTT  227
AsTLP8-A-Park   GGTCGAGGTGGCGGCCGGCACGACCAGCGGGCGCGTGTGGGCGCGCACGGGCTGCAACTT  227

Consensus       CGACGGCAGCGGCAACGGGCGGTGCCAGACGGGCGACTGCGGCGGCAAGCTGCAGTGCAC  287
OT3098-AsTLP8-D CGACGGCAGCGGC------------------------------------------------  240
OT3098-AsTLP8-C CGACGGCAGCGGCAACGGGCGGTGCCAGACGGGCGACTGCGGCGGCAAGCTGCAGTGCAC  287
OT3098-AsTLP8-A CGACGGCAGCGGCAACGGGAAGTGCGAGACGGGCGACTGCGGCGGCAAGCTGCAGTGCAC  287
AsTLP8-D-Park   CGACGGCAGCGGCAACGGGCGGTGCCAGACGGGCGACTGCGGCGGGAAGCTGCAGTGCAC  287
AsTLP8-C-Park   CGACGGCAGCGGCAACGGGCGGTGCCGGACGGGCGACTGCGGCGGCAAGCTGCAGTGCAC  287
AsTLP8-A-Park   CGACGGCAGCGGCAACGGGAAGTGCGAGACGGGCGACTGCGGCGGCAAGCTGCAGTGCAC  287

Consensus       GCAGTACGGGCAGGCGCCCAACACGCTGGCCGAGTTCGGGCTCAACAAGTTCAAC-AA--  344
OT3098-AsTLP8-D ------------GCGCCGAACACCCTGGCCGAGTTCGGGCTCAACAAGTTCAAC-AA--  284
OT3098-AsTLP8-C GCAGTACGGGCAGGCGCCCAACACGCTGGCCGAGTTCGGGCTCAACAAGTTCAAC-AA--  344
OT3098-AsTLP8-A GCAGTACGGGCAGGCGCCCAACACGCTGGCCGAGTTCGGGCTCAACCAGT---ACGAAGG  344
AsTLP8-D-Park   GCAGTACGGGCAGGCGCCGAACACCCTGGCCGAGTTCGGGCTCAACAAGTTCAAC-AA--  344
AsTLP8-C-Park   GCAGTACGGGCAGGCGCCCAACACGCTGGCCGAGTTCGGGCTCAACAAGTTCAAC-AA--  344
AsTLP8-A-Park   GTAGTACGGGCAGGCGCCCAACACGCTGGCCGAGTTCGGGCTGAACCAGT---ACGAAGG  344

Consensus       CCTCGACTTCTTCGACATCTCCCTCATCGACGGCTTCAACGTGCCCATGAACTTCCTGCC  404
OT3098-AsTLP8-D CCTCGACTTCTTCGACATCTCCCTTATCGACGGCTTCAATGTGCCCATGAACTTCCTGCC  344
OT3098-AsTLP8-C CCTCGACTTCTTCGACATCTCCCTCATCGACGGCTTCAACGTGCCCATGAACTTCCTGCC  404
OT3098-AsTLP8-A CCAGGACTTCATCGACATCTCCGTCATCGACGGCTTCAACGTGCCCATGGACTTCCTGCC  404
AsTLP8-D-Park   CCTCGACTTCTTCGACATCTCCCTTATCGACGGCTTCAATGTGCCCATGAACTTCCTGCC  404
AsTLP8-C-Park   CCTCGACTTCTTCGACATCTCCCTCATCGACGGCTTCAACGTGCCCATGAACTTCCTGCC  404
AsTLP8-A-Park   CCAGGACTTCATCGACATCTCCGTCATCGACGGCTTCAACGTGCCCATGGACTTCCTGCC  404
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Figure 1: Multiple nucleotide sequence alignment of *AsTLP8* homeologs. Nucleotides 227 to 404 are shown to demonstrate the deletion in v. OT3098 *AsTLP8-D*. The 60bp deletion is highlighted in red. Data was generated using the MUSCLE alignment tool on the Geneious software.

Analysis of *TLP8* homeologs in *Avena sativa* v. Park

The amino acid sequences between homeologs and species have distinct differences. As previously discussed, the D-genome *TLP8* in v. OT3098 is missing the sugar binding motif of CQTGDCGG altogether (Singh et al., 2017). The A-genome homeolog in both varieties demonstrate a mutation in the second residue of the sugar binding motif. This change is glutamine to glutamic acid (Q → E, Figure 2). Considering glutamic acid has a negatively charged side chain, this may affect the sugar binding potential of this motif. Diploid A-genome species usually have a high amount of grain beta-glucan when compared to other species, and hints that *AsTLP8* homeologs may play a part in the regulation of beta-glucan content (Welch et al., 2000). The second difference is a change in the same residue of glutamine to arginine (Q → R) within v. Park's C-genome. Arginine has a positively charged side chain, and may also affect the action of the sugar-binding domain. Interestingly, this mutation is not found within the diploid C species *Avena eriantha* (AeTLP8-C-AA, Figure 2). In every homeolog, there are residues conserved in enzymes containing beta-glucanase activity, further hinting towards the antagonistic relationship of *AsTLP8* homeologs on the beta-glucan content (Grenier et al., 2000).

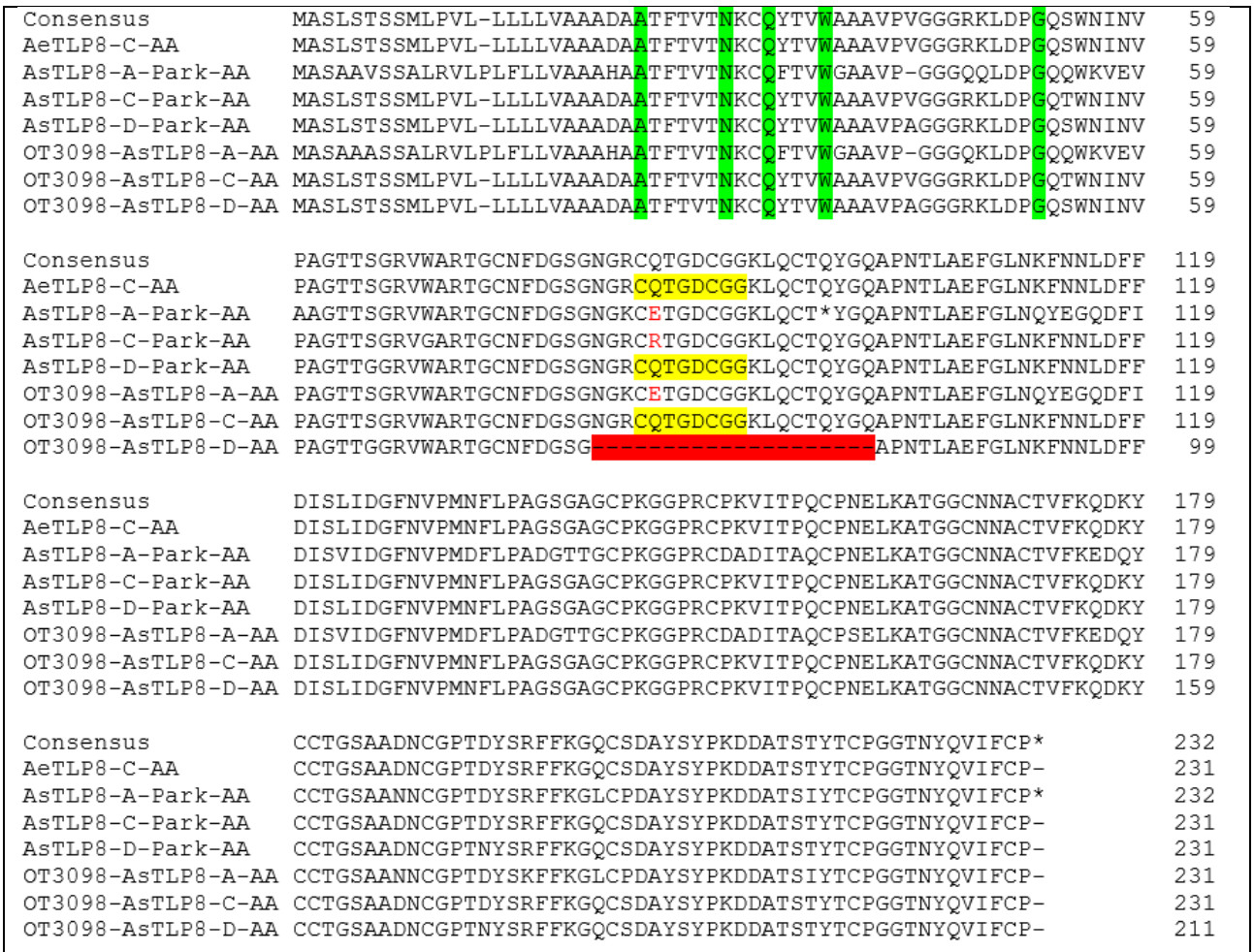

Figure 2: Multiple amino acid sequence alignment of homeologs in *AsTLP8* and homolog of *Avena eriantha*. The sugar-binding motif described by Singh et al. (2017) is highlighted in yellow. Any residues that are mutated within the motif is written in red, and deletions highlighted in red. The residues highlighted in green represent residues conserved in enzymes demonstrating beta-glucanase activity (Grenier et al., 2000).

sgRNA design

The three homeologs had guide RNA designed separately to specifically target each. Potential sgRNA were produced using the Geneious software with the Pepsico OT3098 sequenced genome used as an off-target reference. Guide RNA were ranked based on the parameters of specificity (Hsu et al., 2013) and activity (Doench et al., 2016). A total of three sgRNA were designed per homeolog (Table 1) with a 100bp+ gap in between their positioning. The reason for this gap is to remove the whole sugar binding motif, and to visualize a successful knockout on a gel.

Table 1: Guide RNA designed to target *AsTLP8* homeologs in oat variety Park

Genome	sgRNA	Sequence	Position (bp)
A	TLP8A-sg1	GCAACAGTGGAAGGTCGAGG	156
A	TLP8A-sg2	CTTGTTGGTGACGGTGAACG	74
A	TLP8A-sg3	TGAAGTCCTGGCCTTCGTAC	333
C	TLP8C-sg1	CGTGCCCATGAACTTCCTGC	384

C	TLP8C-sg2	TGGAACATCAACGTGCCGGC	163
C	TLP8C-sg3	AGCTTCCTGCCCCCGCCGAC	124
D	TLP8D-sg1	TGGAACATCAACGTGCCTGC	163
D	TLP8D-sg2	GTTGAGCCCGAACTCGGCCA	311
D	TLP8D-sg3	GTGCCTGCCGGCACGACGGGCGG	175

Construct design

The product of the gene editing constructs was made possible through a Golden Gate cloning (Kumar et al., 2018). Each construct targets a different genome, and therefore have all the sgRNA of their respective genome in Table 1. The backbone contains the *Cas9* protein and Hygromycin resistance (*hph*), promoted by pZmUbi and CaMV 35s promoters, respectively. The guide RNA are regulated by the *pHvU3* (Figure 3, 4, and 5).

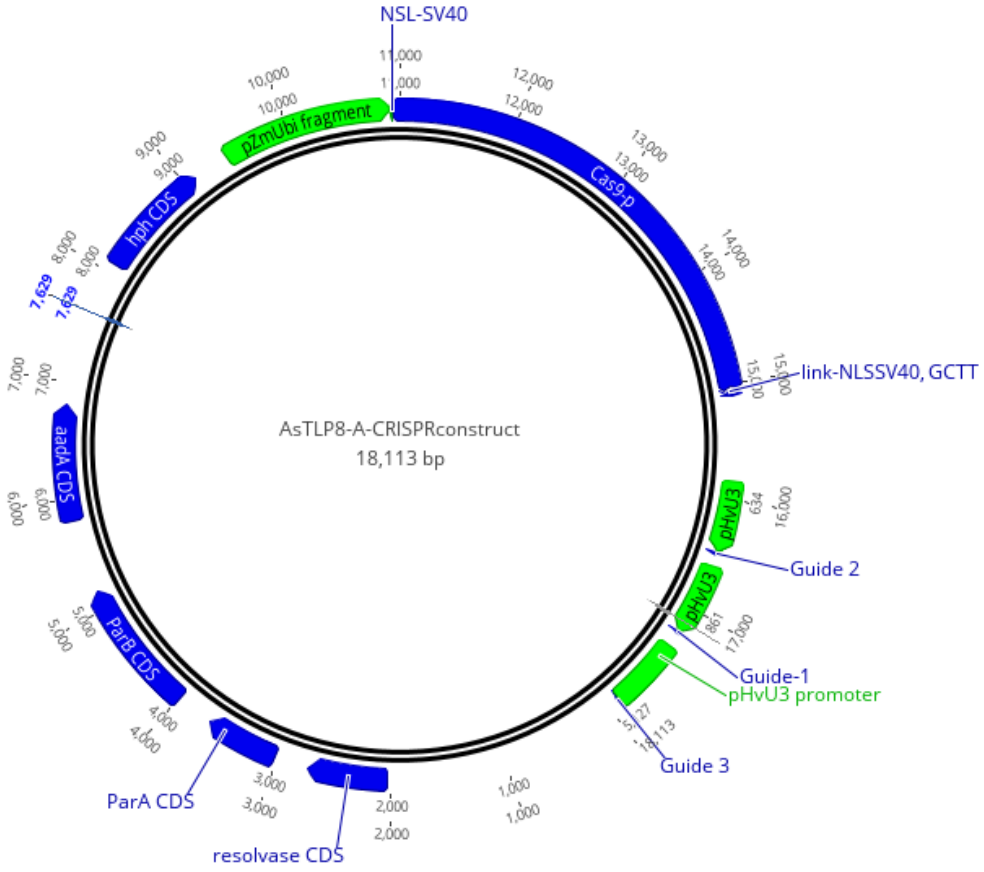

Figure 3: CRISPR construct designed to target the *AsTLP8* homeolog on the A-genome. *Cas9p* represents the *Cas9* protein, under the regulation of the *Zea mays* ubiquitin promoter. *hph* represents the Hygromycin-B-Phosphotransferas gene, instilling hygromycin resistance and under the regulation of the CaMV 35S promoter. Guide 1 represents TLP8A-sg1 in Table 1, and so on. Each guide RNA is expressed through the *HvU3* promoter.

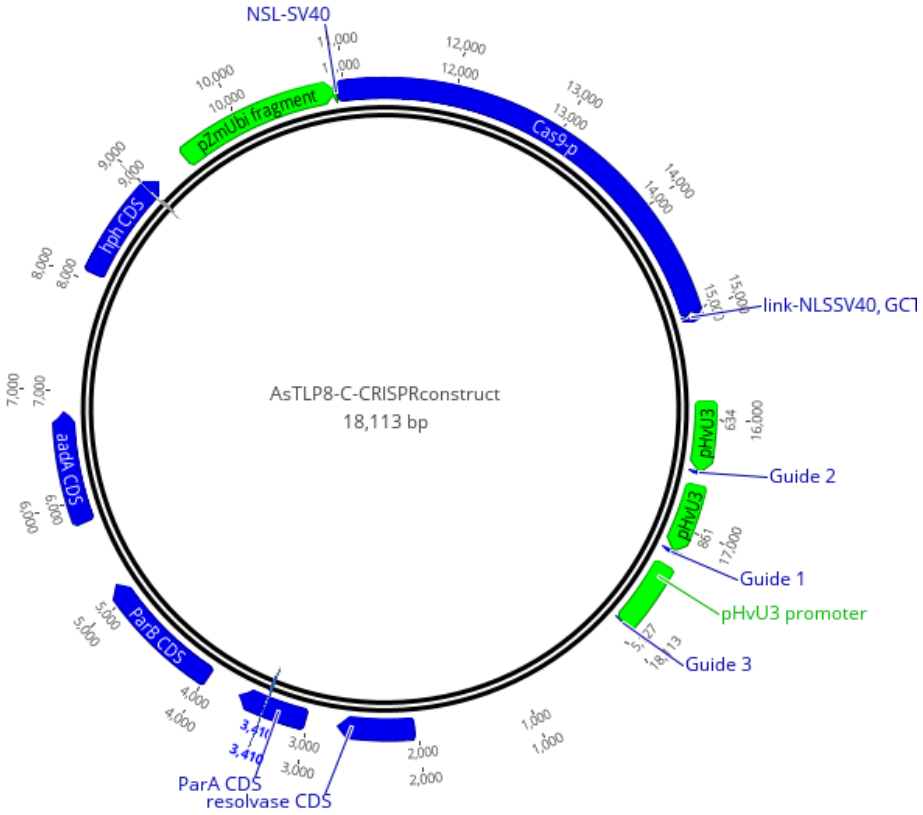

Figure 4: CRISPR construct designed to target the *AsTLP8* homeolog on the C-genome. *Cas9p* represents the *Cas9* protein, under the regulation of the *Zea mays* ubiquitin promoter. *hph* represents the Hygromycin-B-Phosphotransferas gene, instilling hygromycin resistance and under the regulation of the CaMV 35S promoter. Guide 1 represents TLP8C-sg1 in Table 1, and so on. Each guide RNA is expressed through the *HvU3* promoter.

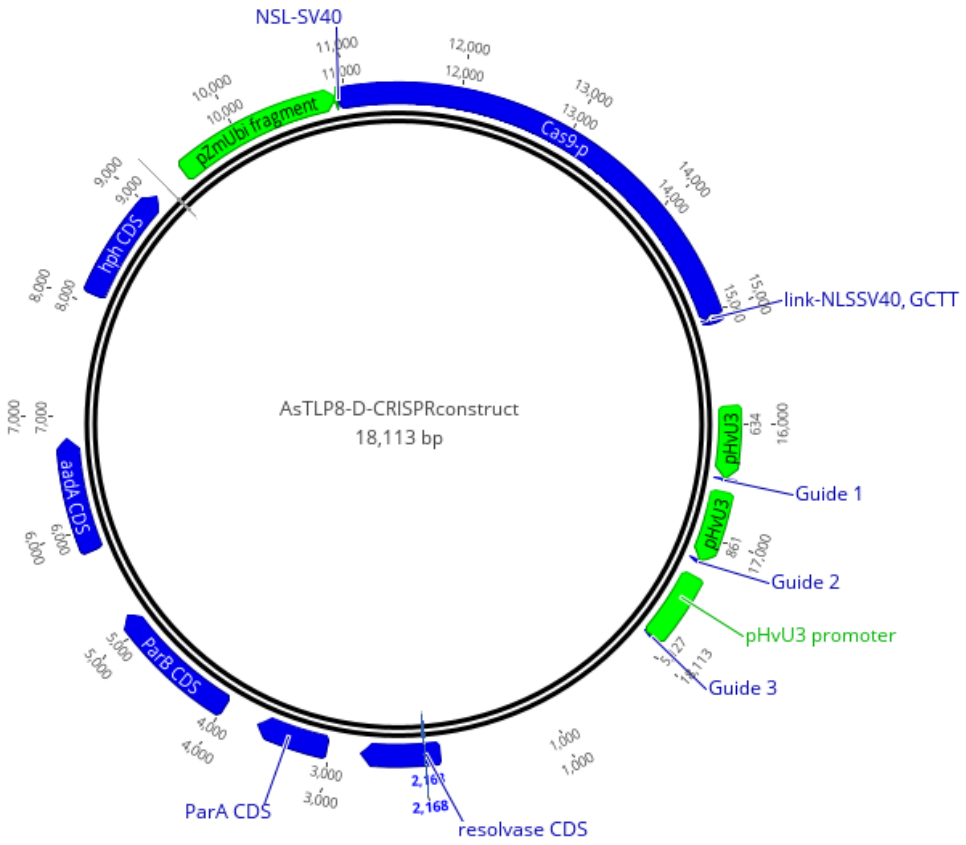

Figure 5: CRISPR construct designed to target the *AsTLP8* homeolog on the D-genome. *Cas9p* represents the *Cas9* protein, under the regulation of the *Zea mays* ubiquitin promoter. *hph* represents the Hygromycin-B-Phosphotransferas gene, instilling hygromycin resistance and under the regulation of the CaMV 35S promoter. Guide 1 represents TLP8D-sg1 in Table 1, and so on. Each guide RNA is expressed through the *HvU3* promoter.

Transformation progress

The oat variety Park was transformed with the CRISPR constructs through microprojectile bombardment. Over 500 calli were bombarded. This bombarded calli undergo two rounds of negative selection using 10mg/L of Hygromycin. Each round takes approximately two weeks. Around 100 calli have undergone two rounds of selection.

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:

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?

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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.