

REPORTING REQUIREMENTS

1.	Principal Investigator's Last Name: Parent	Given Names: Jean-Sebastien	Position: Research Scientist	Date: 05/15/2026
	Institution: Agriculture and Agri-Food Canada		Department: Ottawa Research and Development Center	
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2.	Title of Research Project: Optimizing oat floral architecture to maximize grain yield and quality			
3.	Start Date: 04/01/2025		Completion Date: 03/31/2028	

Part I – Annual Progress Report

- a) **Summary** - include activities during the progress period and status of the project (i.e. is it on schedule), any significant observations related to the progress of the project.

Building on comparative analyses of covered and naked oat, RNA-seq and genomic data have been central to refining our model of the genetic determinants underlying the naked phenotype. These analyses, focused on the master regulator located on chromosome 4D, have enabled the identification of downstream genes involved in oat inflorescence development, particularly those regulating spikelet formation. As the model has evolved, additional candidate genes have been discovered, and a population of contrasting genotypes has been developed to explore variation in floral architecture. Complementing this work, we have established microscopic protocols to examine inflorescence development at the level of the spikelet meristem, providing critical developmental context for gene function.

Guided by these insights, we have prioritized nine candidate genes suspected to play key roles in spikelet development for functional validation. Gene editing of these targets is well underway, with the first batches of edited lines—targeting three genes—already generated and advancing through genotyping and phenotyping pipelines. Overall, substantial progress has been achieved across all project objectives, with gene editing efforts approaching the stage of delivering key functional and applied outcomes.

- b) **Methods** – include approaches, experimental design, methodology, materials, sites, etc. Major changes from original work plan must be indicated and the reason(s) for the change should be specified. Significant changes from the original work plan will require written approval from WGRF.

- **Gene editing:** CRISPR constructs were generated using standard cloning procedures. Seven out of nine constructs have been introduced in the oat genome using a tissue culture method developed at McGill (Singh *et al.* 2025). Regenerated plants were genotyped using SCAP methods and also amplicon sequencing, confirming the presence of edits. No changes to be anticipated for this experiment.
 - **DNA and RNA sequencing:** Results in our study indicated possible genome rearrangements on chromosome 4D where the controlling naked *N1* allele is located in the isogenic lines we were using. Genome sequencing was performed on these lines using the services offered by Genome Quebec which was not originally planned for. Sequencing confirmed massive deletions in some of the lines we were using which had us change the material we were planning to use for transcriptomic experiments. Indeed we decided not to use the isogenic lines because of the genomic rearrangements but instead select a panel of representative covered and naked varieties available at ORDC. Because of this change, the sampling, library preparation and RNA sequencing experiments that were planned will be delayed into the second year of the project.
 - **Microscopy:** We have decided that developing a Scanning Electron Microscopy (SEM) procedure for developing spikelets would be more informative at this early stage of the project. For this, we delayed the development of RNA *in situ* hybridization microscopy that was planned in Objective 2.
- c) **Progress during the reporting period** – (e.g. laboratory, growth chamber, greenhouse, and field experiments; chemical analysis; data analysis; model development). Please briefly indicate what has been done during the reporting period in respect to meeting the stated objectives of the project. Please briefly indicate any variances in the current year in comparison to what was proposed in the research application.

Objective 1 - Creating new alleles to optimize floret number and groat percentage, maximizing milling oat yield and quality: During the first year of the project, we identified a new family of three genes to be added to our proposed list of targets for gene editing (three families of three genes for nine targets total). We do not expect that this will cause significant delay for this objective. The research team at the Ottawa Research and Development Center (ORDC) produced nine CRISPR constructs that were transferred to the team at McGill for oat transformation. A first batch of three constructs was inserted in the oat genome through tissue culture in August 2025. Dozens of plants were regenerated and genotyped confirming the efficacy of the gene editing process. Seeds from the regenerated plants have been transferred to ORDC to grow a second generation which will be used to segregate transgene-less homozygous mutants to associate phenotypes to corresponding gene inactivation. A second batch of four constructs has been transformed in November 2025 and the regenerated plants have been confirmed as edited. The remaining two constructs will be transformed in the near future. This objective is on schedule to be completed as planned.

Objective 2 - Identification of new genetic factors contributing to oat floral development: By performing genome sequencing on the isogenic lines we were planning to use in our study, we discovered that they contained large chromosome deletions precisely at 4D. While this did not affect our model, it encouraged us to consider alternatives to the isogenic lines for transcriptomic experiments. We therefore selected a panel of well characterized covered and naked plants for this part of the objective. The plants are being grown now and will be processed throughout the

summer of 2026 which will delay the production of transcriptomic datasets. On the positive side, the unexpected discovery of large structural rearrangements on chromosome 4D creates the opportunity to study the stability of this particular region as well as the impact of deletions on the overall fitness of the plant.

For this objective, we also realized that studying the developing spikelet meristem at higher resolution would help refine our understanding of the process. We are therefore developing a Scanning Electron Microscopy (SEM) procedure to get exquisite details of the developing meristem. We are developing this procedure instead of the planned RNA *in situ* hybridization as we feel SEM could be more rapidly deployed and provide similarly useful information to the project. Moreover, because ORDC is already equipped with SEM capacity, this change in our methods can be done without additional resources.

Objective 3 - Development and study of a range of multifloral architecture: Based on the other objectives, we identified that crossing the two varieties Gehl (naked) and GS7 (covered) would create a population where new allelic combinations could be combined and studied. We procured those varieties at ORDC, crossed them and are presently growing the F1 individuals. We confirmed the hybridity of the crosses through genotyping and will be able to grow the F2 population in the summer of 2026.

- d) **Project Progress to date** – (e.g., laboratory, growth chamber, greenhouse, and field experiments; chemical analysis; data analysis; model development). Results if available. Please indicate overall project progress since its initiation. Please briefly indicate any anticipated variances which may be encountered in future years in comparison to what was proposed in the research application.

We have discovered that the sum of all differences between covered and naked oat is essentially being controlled by nine *APETALLA2-LIKE* (*AP2L*) genes. Specifically, these genes are downregulated in the spikelet meristem of naked plants. We are well underway to inactivating each gene separately in a covered background (Park) to be able to assign each gene to a specific sub-trait of the naked phenotype. We have developed an SEM protocol to obtain images from the developing spikelet meristem in order to better understand the sequence of events that leads to the different architectures. This technique will also be used to analyse gene edited plants. We also confirmed the crossing of varieties Gehl and GS7 that will be used to create lines with *AP2L* variations in natural populations. We have developed a series of PCR markers to be able to track the different alleles efficiently through generations.

- e) **Extension and communication activities:** (e.g., extension meetings, papers produced, conference presentations made, photos)

Scientific seminars given at Carleton University (2025/09/26), McGill University (2025/10/30) and ORDC (2026/01/09).

- f) **Acknowledgements** – Include actions taken to acknowledge support by WGRF.

Seminars were given by Jean-Sébastien Parent at ORDC, McGill and Carleton University during which WGRF and POGA support was acknowledged.

- g) **Other Administrative Aspects:** personnel involved; equipment bought; project materials developed.

AAFC employees Jean-Sébastien Parent, Wubishet Bekele, Nathan Liang and Asuka Itaya contributed to the project.

Master student from Carleton University Bonnie Rule was directly supported by WGRF funds to contribute to the project.

McGill employees Jaswinder Singh, Rajvinder Kaur and Cali Kaye contributed to the project.

- h) **Appendices** - If necessary, include any materials supporting the previous sections, e.g., detailed data tables, maps, graphs, specifications.

None

- i) **Financial**
- Comprehensive Financial Statement that summarizes the total income and expenditures to date attributable to WGRF.
 - Explanation of variances from budget which are greater than 10%.

Financial statements is accompanying this report.

Part II – Final Research Report

AAFC's receipt of the final financial installment shall be predicated on its submission to **WGRF**, and **WGRF's** acceptance of said submission of a Final Research Report.

Final Research Report – to be provided on or before the date indicated in the Schedule of Payments and Milestones Table in Appendix “C” of the Collaborative Agreement and will be as per the reporting requirements below:

- Abstract/Summary** - Maximum of one page. This must include project objectives, results, and conclusions for use on the WGRF website.
- Introduction** – Brief project background, rationale and objectives.
- Methods** – Include approaches, experimental design, methodology, materials, sites, etc. Major changes from original plan should be indicated and the reason(s) for the change should be specified.
- Results** – Present and discuss project results, including data, graphs, models, maps, design and technology development.

- e) **Conclusions and Recommendations** – Highlight significant conclusions based on the discussion and analysis provided in the previous section with emphasis on the project objectives specified above; also provide recommendations for the application and adoption of the project results and identify any further research, development and communication needs, if applicable.
- f) **Extension and communication activities:** (e.g. extension meetings, papers produced, conference presentations made, photos)
- g) **Acknowledgements** – Include actions taken to acknowledge support by WGRF.
- h) **Literature Cited**
- i) **Other Administrative Aspects:** personnel involved; equipment bought; project materials developed
- j) **Appendices** - If necessary, include any materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications.
- k) **Financial**
 - a. Comprehensive Financial Statement that summarizes the total income and expenditures to date attributable to WGRF
 - b. Explanation of variances from budget which are greater than 10%.

Part III – Final Extension Report and final expenditure statement- to be submitted on or before the date indicated in the Schedule of Payments and Milestones Table in Appendix “C” of the ***Collaborative Agreement***. This final 1-2 page report should summarize extension and KTT activities conducted since the submission of the Final Research Report.