

**Project Title:** Genetic Improvement of Photosynthetic Efficiency and Capacity to Improve Grain Yields in Wheat Cultivars

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**Project Context:** Wheat is a very important and widely grown staple food crop globally and in Canada. This crop is facing several challenges including yield gaps and low economic returns to wheat farmers. It is increasingly important that the grain yields have to be improved significantly to overcome these limitations. These challenges also present opportunities for development of new approaches and innovative technologies to improve grain yields in wheat. In this context, improvements in photosynthesis hold enormous potential for increasing yield potential in wheat crop and also by contributing positively to global wheat supply.

**The Challenge:** The photosynthesis is a fundamental process in plants and the carbon fixed during this process is the major contributor to the plant growth and development and to the overall yield and performance in a crop context. The photosynthetic efficiency in most crops is less than 2% and even a small increase in this has potential to improve biomass and yield. It has been recognized that the currently grown wheat cultivars globally including Canadian are not performing optimally from the photosynthetic potential point of view. Addressing this challenge requires discovery of superior alleles functioning in photosynthetic efficiency and capacity. Among different factors and processes that contribute to photosynthetic efficiency and capacity, the components of photosynthetic machinery, regulators of carbon partitioning, mesophyll conductance, radiation use efficiency, canopy temperature, shoot biomass, spike photosynthesis, spike fertility and the sink capacity in the grain – are potential targets for substantial improvements (Long et al., Cell 2015; Lin et al., Nature 2014; Parry et al., Jour Expt. Botany 62:653, 2011; Reynolds et al., Jour Expt. Botany 62:439, 2011; Zhu et al., Annual Rev. Plant Biology 61:235,2010).

**The Opportunity:** It has been well documented that agro-climatic conditions influence physical and physiological characteristics governing the overall crop performance and its productivity. From a Canadian perspective, though wheat is primarily grown as a spring crop with a short growing season, extended daylight offer better conditions for photosynthesis and carbon fixation during the important grain filling phase. Further, the cellular machineries that provide the templates for many of the biochemical reactions including photosynthesis hold some key controls to determine the efficiency (Long et al., 2015). As the light dependent reactions are closely connected and regulated by the associated networks and interactions - their outputs likely also influence the photosynthetic efficiency. These complexities though present a major challenge; also present an opportunity for making new advances with potential applied implications. Interrogation of these key contributing factors and processes from a perspective specific to wheat will create new critical insights into photosynthesis associated gene targets for improving performance and yield.

**The Rationale:** Towards improving photosynthetic efficiency in wheat crop, in this project, we propose to evaluate the wheat cultivars, genotypes and other relevant germplasm for photosynthetic efficiency and capacity. The research performed at CIMMYT and several other labs demonstrated that architecture; flag leaf and spike traits are important contributors of improved photosynthetic efficiency during grain filling stage. Our preliminary results from analysis of cell and developmental aspects of selected lines developed by CIMMYT identified several unique features revealing the underpinning developmental programs connected to the observed superior photosynthesis phenotypes. This project is developed to exploit these observations and the unique resources developed by CIMMYT – to create new opportunities in the form of discovery of new gene targets for

improving photosynthetic efficiency in wheat. The research advances and outcomes of this project will present opportunity to apply the findings in Canadian wheat cultivars and also has potential to make significant contributions to other related global/international (e.g. new IWYP initiatives) wheat improvement programs including the related efforts at CIMMYT and UK.

### **The Project Goals and Objectives:**

1. Genetic and physiological assessment of photosynthetic efficiency and capacity in Canadian wheat cultivars;
2. Selection of genotypes and new germplasm (e.g. developed at CIMMYT) that display photosynthetic efficiency characteristics relevant and useful for addressing the bottlenecks identified in wheat cultivars;
3. Phenotypic and physiological assessment of photosynthetic efficiency and capacity in these lines and identify best performers;
4. Define the molecular identity of the underlying genetic regulatory factors and their respective functions in photosynthetic efficiency and capacity in wheat;
5. Deployment of these gene targets and their corresponding appropriate alleles into elite cultivars to critically assess respective genetic potential under field conditions.

**The Research Plan and Timelines:** The major goal of this project is to improve wheat crop yields by targeting photosynthesis. To achieve this goal, under “Activity-1”, we will systematically assess relevant Canadian wheat cultivars to identify any bottlenecks or limitations associated with photosynthesis. In “Activity-2” we will explore discovery of genes associated with programs in architecture, flag leaf and spike photosynthesis using lines identified and developed by CIMMYT researchers and related leads from research findings in CWA projects. In the “Activity-3”, the gene targets and desirable alleles identified in this project will be deployed in wheat cultivars for assessing their functions to improve grain yields. The detailed research plans to achieve these goals are described under these three activities:

### **Activity 1: Phenotypic and physiological of assessment photosynthesis in selected Canadian wheat cultivars:**

**Year 1-2:** As the Canadian climate and growth conditions are unique, the proposed experiments and critical evaluations will be performed using field grown plants. We will use selected Canadian wheat cultivars for this field study. The objective of this study is to critically assess in these Canadian cultivars photosynthetic efficiency at different stages of growth including at the important grain filling phase. For example, (a) the lines that accumulate larger biomass during vegetative phase have potential to fix more carbon and make more photosynthate; (b) the lines with better (e.g. up-straight) architecture are well positioned to capture more light energy to use in the photosynthesis; (c) the lines that display better photosynthetic efficiency in “flag leaf” and “spikes” during grain filling stage have potential to produce higher grain yields. These and other key contributors to photosynthetic efficiency and capacity will be systematically assessed in all these lines to identify which of the desirable traits are present among these Canadian genotypes and cultivars. Towards this, we will employ optimized protocols and standard systems (e.g. Li-COR 6400XT photosynthesis system) to measure photosynthesis at different stages of development in the key contributing tissues. These include: (a) vegetative phase; (b) grain filling phase; (c) target tissues representing the leaf, flag leaf and spike - because these tissues are the major contributors to photosynthesis and also to the overall crop productivity and grain yield.

The large datasets coming from this field study will be analysed to identify cultivars that perform the best in field conditions in terms of photosynthetic efficiency. We will also analyse datasets of individual tissues (e.g. flag leaf, spike) to identify best performers for a given target tissue. Together, these analyses will identify the lines that potentially carry the best allele(s) for a given tissue or whole plant. It is possible that the best alleles for different tissues identified in the cultivars are

present in different lines, but not all in any one cultivar. In such cases, we will perform appropriate crosses to bring them together in right combinations, and for further performance evaluation. The expected outcome of these studies is development of comprehensive datasets capturing photosynthetic efficiencies in different wheat cultivars. Furthermore, these studies will also identify relatively better performing cultivars in photosynthesis.

## **Activity 2: Discovery of gene targets and desirable alleles associated with architecture, flag leaf and spike photosynthesis:**

**Year 1:** To complement the above studies, and to identify new gene targets and alleles relevant to improved photosynthetic efficiency, we will investigate the lines developed by “CIMMYT and University of Lancaster” that display desirable architecture, flag leaf and spike photosynthesis traits. As it is not known how these lines perform under unique Canadian conditions, we will conduct critical field evaluation of these to confirm and also establish comparisons with the best lines identified from the “Activity-1” studies with Canadian cultivars. These experiments will produce critical and important comparative field datasets to identify potential bottlenecks and limitations associated with Canadian cultivars. This critical knowledge will be used to further to assess potential gaps in the photosynthetic performance especially during grain filling stage in Canadian cultivars.

Photosynthesis is a complex biological process involving regulation at different levels that include biochemical pathways, metabolic and inter-connected genetic networks and developmental programs. To identify and reveal the underpinning genes and metabolites implicated in the CIMMYT and UK lines that display desirable architecture, flag leaf and spike photosynthesis; we will apply systematic cell biology and development targeted analyses to determine the underpinning programs connected to the photosynthesis phenotypes. To capture the genetic and molecular components associated with relevant developmental stages, total RNA samples will be collected and subjected to RNA seq based genome-wide profiling studies. From these genomic studies, in the context of genes specifically regulated differentially between the lines displaying photosynthetic efficiency compared to the controls (that do not show these phenotypes), target genes will be identified that display both up-regulation or down regulation. These include protein coding genes with potential functions in core photosynthetic machinery as well as regulatory components that facilitate and modulate its efficiency and capacity. We will perform more detailed analyses (gene specific qPCR, segregation and linkage) for these putative target genes to identify which of these genes are associated and/or linked to specific photosynthetic efficiency phenotypes in each of the architecture, flag leaf and spike photosynthesis lines. Though the focus of the genomic studies outlined above is on protein coding genes, as several recent studies highlight the importance of non-coding RNAs (including microRNAs) as regulatory factors, we will also use the RNA seq based genomic datasets to mine for these in differentially regulated transcripts. Findings from this analysis will be tested further for any potential connections between the non-coding RNAs and photosynthetic efficiency. The proposed global metabolomics studies will complement these genomic studies to capture and reveal differentially accumulated metabolites including photosynthesis related and that are connected to the observed phenotypes in the lines investigated.

**Year 2-3:** To define the molecular identity of the genes controlling architecture, flag leaf and spike photosynthesis phenotypes – we will use the available genetic map linkage datasets developed by CIMMYT researchers. The corresponding co-ordinates will be connected with transcriptome datasets to identify putative target gene transcripts. Integrated analyses of these datasets will assist to identify a short list of potential candidates for these independent photosynthesis phenotypes. For these genes, we will perform combination of bioinformatics and function based evaluations, to identify the respective targets. We will use heterologous and transgene based functional studies to validate and confirm that the identified candidate genes are indeed the targets for the observed photosynthesis phenotypes. We will also develop closely linked molecular markers for each of these traits to assist in molecular cloning of the corresponding genes; will also explore similar strategies to

identify more lines with desirable photosynthesis phenotypes by screening additional germplasm. Together the objective of this activity is to identify new gene targets for the photosynthesis and develop target gene based and/or closely linked genetic markers.

The outcomes of this activity will reveal the molecular identities of respective gene targets that confer (a) desirable architecture, (b) improved flag leaf photosynthesis, (c) improved spike photosynthesis. Additionally, the proposed studies will contribute to the biochemical, molecular and functional understanding of these genes in the context of improved photosynthetic efficiency – these findings will have broader applied implications.

### **Activity 3 – Deployment of new genes and desirable alleles for improved photosynthetic efficiency in wheat cultivars:**

**Year 2-3:** The proposed approach as outlined in this project will apply genomics, metabolomics in combination with phenotyping and field physiology to generate new gene based tools for improving photosynthetic efficiency in wheat cultivars. The outcomes coming from “Activities 1 and 2” will represent discovery of genes for improving architecture, flag leaf and spike photosynthesis programs. Along with these, the results of these studies also will likely illuminate functions of specific genes, pathways and the biology behind the improved efficiency in photosynthesis.

The genes and products in the form of metabolites identified in this project will generate several important tools (e.g. genetic markers, diagnostic metabolites). To exploit these valuable datasets further, we will perform pathway analysis to reveal the biochemical reactions and pathways and the regulatory programs underlying differential photosynthetic efficiency in wheat genotypes investigated in this project. The objective of this study is to identify which biochemical pathways are involved in the observed photosynthetic efficiency phenotypes. The outcomes of this will directly contribute to establishing not only link between the phenotype and genotype but also revealing the underlying biochemical pathways and their regulation. These studies will identify the genes and alleles (in the lines investigated in this project) along with metabolites in relevant pathways that confer better photosynthetic efficiency.

To capture applied potential for these findings, promising genes and alleles from this study will be used as gene targets for photosynthetic efficiency. Accordingly, these alleles will be introduced into elite wheat lines to further evaluate their roles (synergistic and additive) to improve photosynthetic efficiency. Additionally, we will specifically address the limitations as identified in wheat cultivars (Activity-1), by deployment of the new gene targets and their relevant alleles identified in this project. Together, this project will take systematic and comprehensive approach to bring together appropriate alleles to improve the genetic potential of photosynthetic efficiency in wheat cultivars. Promising lines coming from this project will be evaluated critically for performance and grain yield under field conditions. After validation and confirmatory studies, these lines will be explored further in breeding and to develop new elite wheat lines. These lines will be further evaluated and critically tested at different sites for extending the key findings of this project to wheat improvement breeding programs in Canada, CIMMYT and Lancaster University.

### **The Projected Outputs:**

1. Evaluated Canadian wheat cultivars for photosynthetic efficiency and identified bottlenecks and limitations
2. Defined molecular identity of new genes functioning in improved photosynthetic efficiency
3. Developed gene targets for improving photosynthetic performance in wheat cultivars

Advancing critical knowledge base for improving photosynthetic efficiency in wheat crop has tremendous potential. The discovery of genes and alleles could lead to development of new

molecular tool set for wheat breeding in Canada, at CIMMYT and Lancaster University (UK). We will also explore possibilities for participation and close interaction with IWYP mission and goals.

**The Research Team:**

(a) Curtis Pozniak and Richard Cuthbert will contribute to this project with their extensive expertise in wheat breeding, genomics, field testing and new varietal development;

(b) Jas Singh has extensive expertise in physiology and photosynthesis will contribute evaluation of wheat lines developed in this project;

(c) Raju Datla has extensive expertise in genomics, molecular biology, signaling and development, and metabolomics; will contribute to the developmental analysis, gene discovery, and functional evaluation studies;

(d) Matthew Reynolds and Gemma Molero are the leading international researchers with extensive expertise in wheat field physiology and photosynthesis. CIMMYT will contribute advanced lines and new germplasm resources and their field evaluation and generation of photosynthesis related datasets;

(e) Martin Parry - Internationally recognized expert in photosynthesis with extensive related broad expertise in biochemistry, biophysics, physiology, genetics, genomics and crop biotechnology. Martin team will contribute advanced lines and evaluation with reference to performance of core photosynthetic machinery and components.