UNIVERSITY OF ADELAIDE, AUSTRALIA

CHARACTERISATION OF GLUTAMINE SYNTHETASE TO MAP NEW REGULATORY LOCI MODULATING NITROGEN USE EFFICIENCY IN HEXAPLOID WHEAT

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Thesis submitted in fulfillment of the requirements for the degree of Doctorate of Philosophy in the Faculty of Sciences at the University of Adelaide, Australia

The Australian Centre for Plant Functional Genomics (ACPFG), Adelaide

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This thesis is dedicated to Catherine and the Kids,
my mum Dora Akleh Djietror and to loving memory of
Samuel Ayiku Djietror

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DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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LIST OF KEY TERMS AND ABBREVIATIONS

AMT: Ammonium transporter

cDNA: complimentary DNA

DNA: Deoxyribonucleic acid

FL: Flag leaf

FL-1: Fully extended leaf next the flag leaf

GDH: Glutamate dehydrogenase

GOGAT: Glutamate 2-oxoglutarate transaminase

GS: Glutamine synthetase

HN: High nitrogen treatment (5.0 mM NO_3^- + NH_4^+)

KASP: Kompetitive allele specific primer

LN: Low nitrogen treatment (0.5 mM NO_3^- + NH_4^+)

N: Nitrogen

NH_4^+: Ammonium

NO_3^-: Nitrate

NUE: Nitrogen use efficiency

NRT: Nitrate transporter

OFB: Older fully extended leaf

PCA: Principal component analysis

PCR: Polymerase chain reaction

PTM: Post translational modification

POTAGE: PopSeq ordered *Triticum aestivum* gene expression

qPCR: Quantitative polymerase chain reaction

SNP: Single nucleotide polymorphism

YEB: Young fully extended leaf

Zadoks stages: distinct phases of cereal growth and development

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GENERAL INTRODUCTION

Each year, cereal crops such as wheat, rice, maize and barley are cultivated and harvested to serve as staple foods that contain calories, dietary fibre, vitamins and minerals in the diets of over 70% of the world population. Wheat is among the most cultivated cereal crops. The major wheat production areas include the Mediterranean production areas of the Middle East (Fertile Crescent Region), Europe, North America, Asia (India and China) and Australia (Fig. G.1). Wheat cultivation involves considerable application of nitrogenous fertilisers to the plants in order to maximise yield. Annually, the global nitrogen (N) fertiliser application in crop production estimates at 85 – 90 mMt; of which 53.3 mMt is applied to cereals. Nitrogen fertilizer is vital for crops as the plants utilise nitrogenous compounds (N_2O, NO, N_2 and NH_3^+) to synthesise amino acids essential for grain development.

Nitrogen is added to soils in the form of inorganic fertiliser and processes such as precipitation, atmospheric nitrogen fixation (lightning and thunderstorms), and the activity of soil micro-organisms in root nodules of leguminous plants. The movements of N out of agricultural soils is by gaseous losses (volatilisation) to atmosphere, leaching from topsoil and uptake in crop plants for growth and physiological development (conversion of N to biomass). Concerns over excess NH_3^+ on the atmosphere and climate, environmental impact of excess nitrogenous fertilisers in croplands and the damaging effects in aquatic

ecosystems have highlighted the need for the introduction of more N-efficient crop varieties into cropping systems.

Nitrogen response in plants can be assessed in terms of grain yield per N supplied per area and in terms of physiological variables including plant height, biomass, chlorophyll content, leaf area (Asplund et al. 2016, Barutçular et al. 2016; Elazab et al. 2016; Singh et al. 2016). These traits are controlled by genetic factors including specific enzymes such as Glutamine synthetase (GS) that affect N uptake in plants and may determine positive correlation between N-uptake genes and plant (grain and stem) N content (Habash et al. 2007). Various isoforms of GS have been shown to catalyse metabolic processes in N uptake and biosynthetic pathways within cereal crops including hexaploid wheat (Sukanya, et al. 1994; Singh & Ghosh, 2013; Urriola & Rathore, 2015; Basuchaudhuri, 2016).

Currently, there are few studies of GS in wheat. Glutamine synthetase studies in related cereal species have, revealed however, crucial links between the GS enzyme and N- related traits. For example, quantitative trait analysis has revealed genetic loci for GLN1 cytosolic GS isoform, whose activity relates to grain production in maize (Hirel et al, 2007; Galais and Hirel 2004) and rice where there is correlation between cytosolic GS protein content and grain number/size (Yamaya et al. 2002, Obara et al. 2004).

This current study attempts to decipher the genetic characteristic of GS in N metabolism principally in hexaploid wheat, but may be applicable to related cereal crop species and non-related species more generally. The objective of this study is to characterise the different isoforms of GS enzymes that are actively involved in nitrogen metabolism in hexaploid wheat.

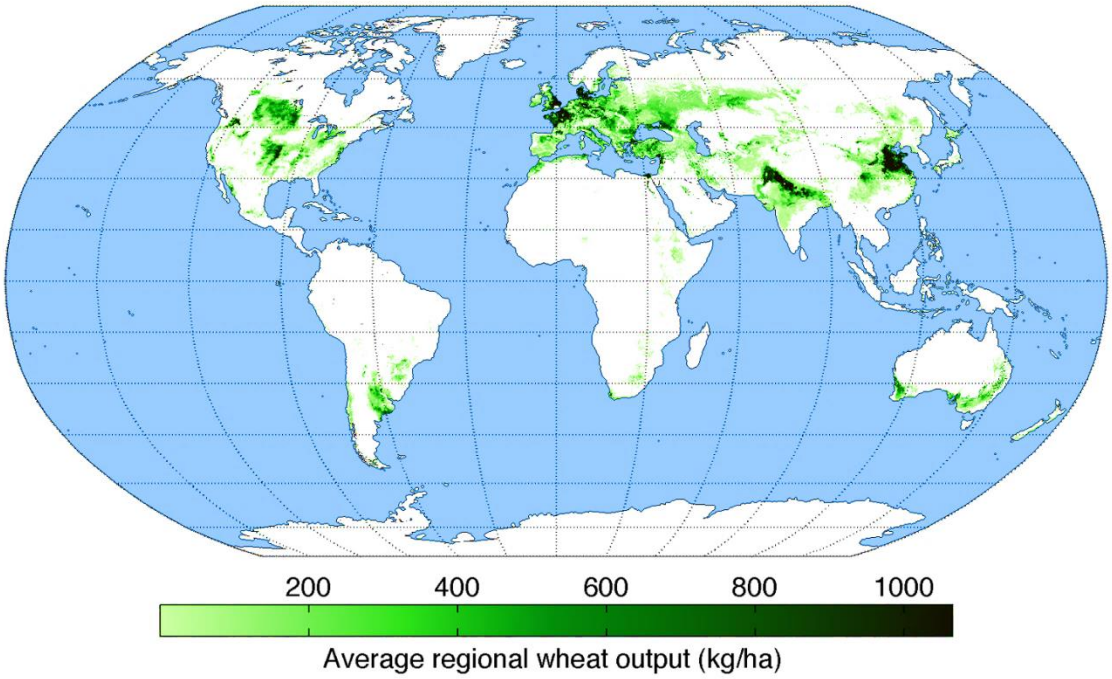

Fig. G.1 Global map of wheat production (mean percentage of cultivation land x mean yield in each grid cell) by the University of Minnesota, Institute of Environment.Source:https://en.wikipedia.org/wiki/International_wheat_production_statistics#/media/File:WheatYield.png.

Specific Aims and Objectives of the Present Study

The main aim of this research project is to identify and characterise genetic variation within a diverse collection of wheat germplasm for key enzymes linked with N metabolism in wheat and related cereal species. Moreover, the study will attempt to address the following research objectives:

- Identity and confirm the genetic loci of GS homologues in hexaploid wheat genome.
- Characterise sequence diversity among different accessions of wheat through phylogenetic analysis.
- Develop molecular markers within the GS conserved domain sequences.
- Assess GS expression by quantifying the transcript abundance under high and low N treatment of wheat plants.
- Evaluate effects of genetic variation on GS activity under high and low N.

The germplasm for this project is sourced from a genetically diverse pool of wheat, adapted for the growth in the major cultivation zones around the world, and represent a unique resource for use in this study.