



**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*

# **Characterisation of Glutamine synthetase to Map New Regulatory Loci Modulating Nitrogen Use Efficiency in Hexaploid Wheat**

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## DECLARATION

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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  $\text{NO}_3^- + \text{NH}_4^+$ )

**KASP:** Kompetitive allele specific primer

**LN:** Low nitrogen treatment (0.5 mM  $\text{NO}_3^- + \text{NH}_4^+$ )

**N:** Nitrogen

**$\text{NH}_4^+$ :** Ammonium

**$\text{NO}_3^-$ :** 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 ( $\text{N}_2\text{O}$ ,  $\text{NO}$ ,  $\text{N}_2$  and  $\text{NH}_3^+$ ) 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  $\text{NH}_3^+$  on the atmosphere and climate, environmental impact of excess nitrogenous fertilisers in croplands and the damaging effects in aquatic

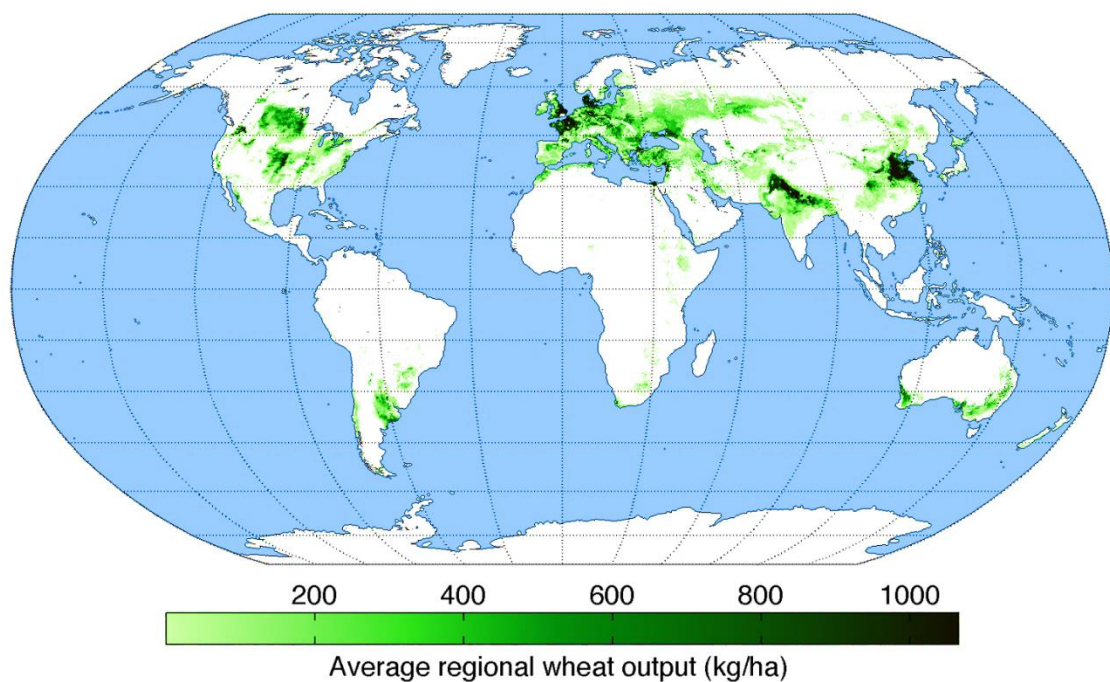
ecosystems have highlighted the need for the introduction of more N-efficient crop varieties into cropping systems.

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](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.