

Unravelling the Physiology and Genetics of Salinity Tolerance in Chickpea (*Cicer arietinum* L.)

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Structure of the thesis

This thesis is presented in six chapters preceded with an Abstract which sets out the context and gives an overview of the thesis. Chapter 1 is made up of a Literature review which gives a broad background of the work presented in the subsequent chapters. Chapters 2, 3, 4 and 5 are experimental papers which have either been published, under review or unpublished work written in manuscript format shortly to be submitted for peer review. Each of the experimental papers contains an Abstract, Introduction, Materials and Methods, Results, Discussion and References. Additionally, they are prefaced by a statement of authorship that describes the contribution of each author and a link page that ties in the chapter with this thesis. General discussion and future research directions are presented in Chapter 6. This thesis is in agreement with the specification of “thesis by publication” format of the Adelaide Graduate Centre Higher Degree by Research, University of Adelaide, South Australia.

Abstract

Chickpea (*Cicer arietinum* L.) is a nutritious legume predominantly grown in semi-arid environments under rain fed conditions, but is highly sensitive to soil salinity. Until recently, there has been slow progress in the application of molecular genetics in chickpea breeding. This is primarily because the available genetic variation in international chickpea germplasm collections has not been extensively characterised due to a lack of available genomics tools and high-throughput phenotyping resources.

Molecular genetic approaches are needed to identify key loci with the potential to improve salinity tolerance in chickpea. In this project, genetic analysis was conducted on two populations: A recombinant inbred line (RIL) population of 200 individuals developed from a cross between Genesis836 and Rupali which are known to contrast in their tolerance to salinity and a diversity panel consisting of 245 chickpea accessions of diverse genetic background from ICRISAT. For phenotyping, an image-based high-throughput phenotyping platform was used. Data on growth rate, water use, plant senescence and necrosis, and agronomic traits were collected under both control and saline conditions (40 mM for diversity panel and 70 mM NaCl for RIL). In depth studies including differential metabolite accumulation and senescence detection were carried out to increase our understanding of the response of chickpea to salinity.

Genesis836 and Rupali differentially accumulated metabolites associated with the TCA cycle, carbon and amino acid metabolism. Higher senescence scores were recorded in Rupali compared to Genesis836. On average, salinity reduced plant growth rate by 20%, plant height by 15% and shoot biomass by 28%. Additionally, salinity induced pod abortion and inhibited pod filling, which consequently reduced seed number and seed yield by 16% and 32%, respectively. Path analysis was utilised to understand the intricate

relationship existing between the traits measured and aided in the identification of those most related to salinity tolerance. This analysis showed that seed number under salt was highly related to salinity tolerance in chickpea.

To identify Quantitative Trait Loci (QTL) underlying salinity tolerance in chickpea, two complimentary genetic analysis approaches were used: genome-wide association studies (GWAS) and linkage mapping. Phenotypic data was combined with genotypic data from both the diversity panel (generated through whole-genome resequencing) and RIL population (from DArTseq). Linkage mapping and GWAS identified a total of 57 QTL and 54 marker-trait associations (MTAs), respectively. The loci identified were linked to growth rate, yield, yield components and ion accumulation. A novel major QTL for relative growth rate on chromosome 4 that explained 42.6% of genetic variation, was identified by both genetic analyses. This QTL co-located with several other QTL identified, including those associated with projected shoot area, water use, 100-seed weight, the number of filled pods, harvest index, seed number and seed yield under salt. Near-isogenic lines will be developed to allow for targeted fine mapping that will help identify candidate genes for molecular analysis. Molecular markers tightly linked to this QTL will be validated as a selection tool in breeding to improve salinity tolerance in chickpea.

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.

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Judith Akinyi Atieno

February, 2017

Acknowledgements

Look at a stone cutter hammering away at his rock, perhaps a hundred times without as much as a crack showing in it. Yet at the hundred-and-first blow it will split in two, and I know it was not the last blow that did it, but all that had gone before. ~Jacob A. Riis

This PhD journey has had its fair share of ups and downs just like most things in life. I managed to reach this far due to the people I interacted with in the course of this research. I would like to express my gratitude to everyone who supported me during my PhD.

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List of publications

1. Dias D.A, Hill C.B, Jayasinghe N.S, Atieno J, Sutton T, Roessner U (2015) Quantitative profiling of polar primary metabolites of two chickpea cultivars with contrasting responses to salinity. *Journal of Chromatography B* 1000:1-13. doi:10.1016/j.jchromb.2015.07.002.
2. Cai J, Okamoto M, Atieno J, Sutton T, Li Y, Miklavcic SJ (2016) Quantifying the Onset and Progression of Plant Senescence by Color Image Analysis for High Throughput Applications. *PLoS ONE* 11(6): e0157102. doi:10.1371/journal.pone.0157102.

List of awards

1. Best poster Award (2013)

Best poster presentation at Pulse Breeding Australia inaugural pulse conference held at the Sebel Playford, Adelaide.

2. Best presentation at The School of Agriculture, Food and Wine Postgraduate Symposium (2014)

Best presentation in agronomy and broad-acre agriculture awarded by the Crop Science Society South Australia.

3. The Max Tate Prize (2014)

Best presentation at the School of Agriculture, Food and Wine postgraduate symposium awarded by Dr Max Tate.

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