UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Investigation of the physiological responses in soybean and common bean to water deficit

By

BERHANU AMSALU FENTA

Thesis submitted in partial fulfilment of the requirements for the degree

PHILOSOPHIAE DOCTOR

Forestry and Agricultural Biotechnology Institute (FABI)

Department of Plant Science

In the Faculty of Natural and Agricultural Sciences

University of Pretoria

Pretoria

SUPERVISORS: PROF KARL J. KUNERT

DR STEPHEN E. BEEBE

December, 2012

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

DECLARATION

I, Berhanu Amsalu Fenta declare that the thesis, which I hereby submit for the degree PhD in Plant Sciences at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or other tertiary institution.

Date………………………………………………

Signed………………………………………………

TABLE OF CONTENTS PAGES

ABSTRACT

Drought causes considerable reduction of legume productivity and significantly threatens the food security, and this situation is expected to be aggravated due to climate change. In soybean and common bean, water resource capturing through plant root architectural plasticity and the role of symbiotic nitrogen fixation have not been investigated in greater detail yet. This study was therefore conducted to identify and apply useful morphological and physiological performance markers (traits) for selection of drought-tolerant common bean and soybean cultivars under both controlled phytotron and field conditions that might be applicable as markers in future legume breeding programs. In soybean, traits related to above ground performance, such as photosynthesis, biomasses, and stomatal conductance, were related to parameters for nitrogen acquisition in nodules. The ability to maintain vigorous shoot growth under drought-induced nitrogen limitation was identified as an important trait that can be used to select for improved drought tolerance. Further, experiments carried out growing different common bean inbred lines under controlled phytotron conditions revealed the importance of growth and gas exchange parameters as well as nitrogen fixing ability as performance markers to select superior performing bean lines for growth under drought. As a further result, the strong association of symbiotic nitrogen fixation with CO_2 assimilation and stomatal conductance was also ascertained. In field experiments the effective use of water through enhanced lateral root development and maintaining the water status of the plant was found to be crucial for enhanced productivity under drought, with root morphology traits (root length, area and volume) as well as root architectural traits (first whorl angle, basal root number and adventitious root branching density) significantly related to seed yield. Measurement of these traits might be added to future

bean varietal improvement programs. Further, a direct relationship between both water use efficiency (WUE) estimated using carbon isotope discrimination (CID) and nitrogen fixation (^{15}N abundance) with root morphological and architectural traits (root length, area and volume, basal root number, 1st as well as 2nd whorl angles) was identified. CID (WUE) and ^{15}N abundance (SNF ability) had a direct relationship with each other and also with productivity traits (seed yield and pod harvest index). Soybean field experiments verified the importance of root system architecture and morphology for providing drought tolerance with root architectural traits, tap and lateral roots (diameter and branching density) and morphological traits (root length, surface area and volume) contributing to better performance under drought. Moreover, the strong association of CID (WUE) with $\delta^{15}N$ (SNF), root traits as well as seed yield in soybean exposed to drought was ascertained. Findings suggested that higher performance in CID under drought stress may be due to higher CO_2 assimilation and better N_2 fixation resulting in better root system architecture and morphology of the drought-tolerant cultivar through maintenance of the water status of the plant for efficient biological activity. Overall the study has generated new knowledge about the use of physiological markers (traits) that can be used widely for legume evaluation under drought suitable for both phytotron and field studies.

THESIS COMPOSITION

Chapter 1 of this thesis provides a summary of the importance of grain legumes and effect of drought in common bean and soybean production. It also provides an overview of previous research on the effect of drought stress on legumes including shoot and root physiological performances as well as symbiotic nitrogen fixation ability. Further, the rationale, aim and objectives of the study are also presented at the end of the chapter. **Chapter 2** reports the results obtained for determining performance of different soybean cultivars under drought conditions in a growth chamber experiment. In particular, this chapter deals with the identification of easily measurable traits, such as gas exchange, plant growth and symbiotic nitrogen fixation, for plants grown under well-watered and drought conditions. **Chapter 3** reports about the physiological performance of different common bean inbred lines with varying degrees of drought tolerance grown in a phytotron under either adequate water supply or drought stress conditions. Performance traits measured under drought for soybean were also measured in order to identify performance traits more widely applicable for legumes. In **Chapter 4** the field performance of common bean inbred lines is reported. Especially this section considers the potential role of root architectural and morphological traits for identifying superior performing bean lines under drought conditions. The relationship of productivity traits with root system traits is also outlined for different nitrogen-fixing lines. **Chapter 5** reports the results obtained for evaluation of common bean lines for water use efficiency and symbiotic nitrogen fixation ability measured using stable carbon isotope discrimination (CID) and ^{15}N natural abundance respectively. Furthermore, the relationships of CID and ^{15}N natural abundance with plant productivity as well as morphological and architectural root traits are outlined in this chapter. **Chapter 6** presents the results obtained for determining the field performance of soybean cultivars. In particular, results of root morphological and architectural traits, plant productivity parameters, WUE (CID) and symbiotic nitrogen fixation (^{15}N natural abundance) performance of these soybean cultivars grown under drought and well-watered are reported. Finally, this chapter also deals with the results obtained on the association of especially CID and ^{15}N natural abundance with root and productivity performance traits. **Chapter 7** summarizes the findings and relevant information generated from this PhD study with the focus of how this study contributed to an advancement of the physiological understanding of the response of the shoot-root system of legumes for drought stress. It further highlights the importance of the use of root system architectural parameters, symbiotic nitrogen fixation traits together with other physiological traits for identification of drought tolerant legumes. Moreover, in this chapter a recommendation for application of performance traits for particular growth condition and legume type is provided. Finally, this chapter also outlines the possible future research activities which might help for using morpho-physiological performance traits for multiple stresses and for wider application in other tropical legumes. This is followed by the **reference** list of the citations used in this thesis and appendix.

ACKNOWLEDGEMENT

First of all I would like to thank God for his incredible gift of love, wisdom and inspiration throughout my entire life.

Sincere gratitude to my supervisor, Prof Karl J Kunert for his guidance, follow up, support and inputs throughout the course of my study. Thank you for the patience and knowledge I have been able to acquire through you. Thank you for all the opportunities you have offered me.

I am very much indebted to my co-supervisor Dr Stephen E Beebe (CIAT) for sponsoring my study, for continued supervision and valuable inputs to my research as well as the thesis. Thank you also for your help for linking my field research to Penn State University, USA root biology research team, which has been very valuable to gain knowledge and experience from the team and to conduct successful field research. Thank you also for facilitating guidance and support from CIAT physiologist (Dr Rao Idupulapati). I am also very much indebted to Dr Rao Idupulapati for all his inputs during the course of my study.

Many thanks to Dr Urte Schlüter who helped me to get a practical training in working with gas chromatograph, to Dr Juan Vorster for his moral and administrative support in our research group. I would like also to extend my sincere gratitude to Dr Nicolette Taylor and Mr Jacques Marneweck (Plant Production and Soil Science Department; UP) sharing with me physiological equipment and also taking their time to give me a practical training on instruments.

My sincere thanks to Prof. Christine Foyer and Simon P. Driscoll at the University of Leeds, UK; who helped me to acquire knowledge and experience in plant physiology measurements and their valuable edition during our publication preparation. Further, I would like also to thank Virginie Mortier, Ghent University, Belgium, who worked with me during her study and for the experience we shared during her stay in South Africa and her recognition of our group work.

A word of appreciation to Prof Jonathan Lynch (Penn State University, USA) who hosted me to conduct my field research at Ukulima Root Biology Center, Limpopo province; South Africa. My special thanks also goes to root physiology research team from Penn State University, USA (Curtis Markle Frederick, Dr Samuel Trachsel, James David Burridge Jr, Katy Barlow, and Patompong Saengwilai) who helped me to grasp basic knowledge and experience on root architecture and morphology analysis. I am grateful to meet and to work with them.

I am grateful to Mr John Lanham and Mr Ian Newton at the University of Cape Town, Archaeology Department for their valuable support for stable isotope analysis.

I am also very grateful to the Departmet of Plant Sciences Head, Prof Marion Mayer, FABI administration (Helen Doman, Martha Mahlangu, Eva Muller), Plant Sciences Department administrative and technical staff (Rene Swart, Elsa vanWyk, Dr Miranda Deutchlander, Natalie Uys, Thomas Nembudani and Garland More) and Nutrilab UP (Elise Ferereira and Truida Smit) who substantially supported me technically and in the administrative matters during my study period. Many thanks to FABI students and staff who helped me during my study; you are a wonderful team to work with.

I am grateful to Ethiopian Institute of Agricultural Research (EIAR) and Melkassa Agricultural Research Center (MARC) administration for giving me a study leave and support during my study. My gratitude also goes to National Bean Research project team at MARC (Dr Setegn Gebeyehu, Kasaye Negash and Kidane Tumssa) for their continuous moral and motivation support during my study period. Especial thanks Mr Kidane Tumassa for his help in Winrhizo software analysis.

To my special childhood friends (Tekle Hable, Getenet Belyneh, Dr Sehalu Demem) for your moral support even in the difficult time of my life. You have a special place in my life.

Profound and endless gratitude and love to my entire family. To my parents (Asmarech Malede, Gezahegn Emeire, Seyum Mesfin, Megbaru Misikir, Tsegaye Misikir, Esynesh Miskir, Asmarech Miskir, Fentansh Miskir, Yigebawl Shumet, Betigest Shumet, Mentamir Bogale, Achameyeleh Getaneh) for your continuous moral support. You are a wonderful family who really shares my vision and make my life to be with full of joy.

Thanks to all my lab mates in South Africa (Abigal Mashamba, Celia Martins, Rosita Endah, Tsholofelo Kibido, Magdeleen Du Plessis, Dr Endale Gebre, Priyen Pillay, Ryhanrd Smit, Stephan Van Wyk, David Talengera, and Eugene Makgoba) who made my study time to be wonderful and you helped from your experience and for the team work as well as your understanding.

I am also grateful to my Ethiopian colleagues (Dr Teferi Yeshitla, Dr Abubekir Hassen, Dr Ahmed Edris, Dr Dambela Gello, Dr Wubetu Bihon, Habtamu Teka, Ms Hyiwot Minkir, Dawit Degefu, Yibeltal Adane, Efrem Kebede, Mihiretu Chernt, Mesay Demel, Esmaeal, Belyneh Yibeltal,) providing me with plenty of support and contributed to make my stay in South Africa simple and interesting. You are really awesome friends!

Tropical legume II has funded this PhD study through International Centre of Tropical Agricultural (CIAT) Cali, Colombia and CIAT-Africa or Pan Africa Bean Research Alliance (PABRA), Uganda. I am very much grateful for the administration of CIAT-Colombia (Dr Stephen E Beebe) and CIAT-Africa (PABRA) (Dr Robin Buruchara, Mathew Abang, and Jolly Basemera).

DEDICATION

I dedicate this thesis to my Mother Mrs. Lakubish Admasu Akeyu who implanted on me the inspiration, wisdom and strength of life and my Sister Mrs. Atalay Amsalu Fenta and Brother-in-Law Mr. Miskir Tashu, who took the vision relay of Mam and who contributed for my performance throughout my life and both passed without seen my success. I would like also to dedicate this thesis to my wife Mrs. Mulu Emerie Anagaw, who shared my academic as well as life vision and supported me with her love, care and inspiration throughout my career and being my source of success. Finally, I would like to dedicate this thesis for my daughter Eyerusalem Berhanu Amsalu, my son Yitbark Berhanu Amsalu for their full support with their love and endurance during my study period.

ABBREVIATIONS AND SYMBOLS

A	Assimilation
ABA	Abscisic acid
Adv.	Adventitious
ANOVA	Analysis of variance
ARA	Acetylene reduction assay
ATP	Adenosine tri-phosphate
ATP	Adenosine triphosphate
Bran.	Branching
C%	Carbon percentage
C:N	Carbon to nitrogen ration
C_2H_2	Acetylene
C_2H_4	Ethylene
CEC	Cation exchange capacity
Ci/Ca	Ratio of intracellular to ambient air CO_2 concentration
CIAT	International Center for Tropical Agriculture
CID	Carbon isotope discrimination
cm	Centimeter
CO_2	Carbon dioxide
CSA	Central Statistics Authority of Ethiopia
d	day
$d^{15}N/^{14}N$ ($\delta^{13}N$)	Delta of 15 nitrogen to 14 nitrogen isotope
df	Degree of freedom
DNA	Deoxyribonucleic acid
DTPA	Diethylene triamine pentaacetic acid
DW	Dry weight
EUW	Effective use of water
FABI	Forestry and Agricultural Biotechnology Institute
FAO	Food and Agriculture Organization of the United Nations

FAOSTAT	Statistics Division of the Food and Agriculture Organization of the United Nations
Fl	Flowering
FW	Fresh weight
G	Stomatal conductance
h	Hour
HI	Harvest index
IAEA	International Atomic Energy Agency
IITA	International Institute of Tropical Agriculture
IWUE	Instantaneous water use efficiency
KCl	Potassium chloride
LSmeans	Least Squares Means
$m^{-2}s^{-1}$	Meter square per second
MC	moisture content
mm	Millimeter
mmol	Millimol
MPa	mega Pascal
MPF	Mid pod filling
N	Nitrogen
na	Not applicable
Ndfa	Nitrogen drived from the aomosphare
NDPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
NH_3	Ammonia
ns	Non significant
NUE	Nitrogen use efficiency
PAR	Photosynthetically active radiation
PCA	Principal component analysis
PEPCase	Phosphoenolpyruvate carboxylase
PHI	Pod harvest index
QTL	Quantitative trait loci
r	Pearson's correlation coefficient

RuBiSCO	Carboxylation of ribulose-1, 5-bisphosphate carboxylase oxygenase
RUBP	Ribulose-1-5- biphosphate
RuBPC-ase	Ribulose-1-5- biphosphate carboxalase
SCMR	SPAD chlorophyll meter readings
SE	Standard error
SEM	Standard error of the mean
SNF	Symbiotic nitrogen fixation
SWC	Soil water content
t	ton
URBC	Ukulima Root Biology Center
USD	United States Dollar
USDA	United States Department of Agriculture
WUE	Water use efficiency
WUE_{intr}	Intrinsic water use efficiency
WW	Well-watered
Y	Yield
$^{13}C/^{12}C$	Ratio of 13 Carbon to 12 Carbon
^{14}N	Nitrogen isotope with molecular mass of 14
^{15}N	Nitrogen isotope with molecular mass of 15
$^{15}N/^{14}N$	Ratio of 15 nitrogen to 14 nitrogen isotope
$\delta^{13}C$	Carbon isotope discrimination
θ_v	Volumetric water content
%Ndfa	Nitrogen derived from the atmosphere
µmol	Micromole
‰	Parts in thousands
^0C	Degree Celsius

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX TEBLES