

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

By

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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.....



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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 CO₂ 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 $\delta^{15}\text{N}$ (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.

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ABBREVIATIONS AND SYMBOLS

A	Assimilation
ABA	Absciscic 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 ₂ H ₂	Acetylene
C ₂ H 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 (δ 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 driven from the aomosphere
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
δ ¹³ C	Carbon isotope discrimination
θ _v	Volumetric water content
%N _{dfa}	Nitrogen derived from the atmosphere
μmol	Micromole
‰	Parts in thousands
⁰ C	Degree Celsius

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