Grapevine reproductive performance: the role of amines, and the effects of salt and silicon.

by

Tintu Baby

Thesis submitted to

The University of Adelaide

in fulfilment of the requirements for the degree of

Doctor of Philosophy

November, 2014

Grapevine reproductive performance: the role of amines, and the effects of salt and silicon.

By:

Tintu Baby

Supervised by:

Dr Cassandra Collins,
Senior Lecturer, School of Agriculture, Food and Wine.
The University of Adelaide

Dr Matthew Gilliham,
Associate Professor, School of Agriculture, Food and Wine.
The University of Adelaide

Prof Stephen Donald Tyerman,
Professor, School of Agriculture, Food and Wine.
The University of Adelaide

Thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy

School of Agriculture, Food and Wine
Faculty of Science,
The University of Adelaide
Waite Research Institute, Glen Osmond, SA 5064
Email: tintu.baby@adelaide.edu.au

Table of Contents

Abstract

Optimising reproductive performance of the grapevine is one of the major difficulties faced by Australian viticulturists due to a physically and economically challenging environment. It is well-documented that salt stress is one of the challenges that causes significant damage to grapevine vegetative and reproductive performance. One of this project's aims was to investigate the yield reduction caused by salt stress during and post flowering. While the ability of silicon (Si) to enhance salt tolerance and yield performance is well-known in many crops especially cereals, the use of Si as a tool for improving grapevine reproduction under saline conditions is inconclusive. This study demonstrated that salt stress reduces fruit set by increasing flower abscission and interrupting normal berry development, which results in more live green ovaries and seedless berries in a bunch. The poor berry development due to impaired fertilization correlated with poor pollen tube growth in the style, while pollen viability and stigma receptivity were not affected by salinity. A significantly higher amount of Na^+ and Cl^- was found both in leaves and flowers after salt treatment and was not affected by additional Si application. The inability of Si to restrict Na^+ and Cl^- ions in the reproductive organs of grapevines correlated with its inability to ameliorate the deleterious effects of salinity on the reproductive performance of grapevines. However, this study identified the possible role of Si in improving water use efficiency of non-stressed vines.

Bioactive amines are a group of growth regulators which are reported to have major roles in many aspects of grapevine reproductive development as well as stress tolerance. The reproductive performance of three red winegrape cultivars commonly used in Australian viticulture; Shiraz, Cabernet Sauvignon and Merlot were investigated in relation to the occurrence of different amines in the reproductive organs. Amine profiles of the flowers and developing berries significantly differed among these three cultivars. Significantly higher

amounts of diaminopropane (DAP) were found in Merlot and Cabernet Sauvignon and correlated with a higher proportion of underdeveloped berries. An aromatic amine phenylethylamine (PEA) not previously reported for grapevine was found to be the major free amine in the flowers of Merlot, which is a cultivar susceptible to poor fruit set. To the best of our knowledge, this is the first study to indicate that PEA may have a role in the reproductive performance of grapevines. Exogenous application of amines was also investigated as a way to manipulate the endogenous levels of each targeted amine and to manipulate fruit set. Results from this investigation were inconclusive and as such further studies are required to determine the concentration and timing of application that have an effect on different cultivars.

To undertake controlled environmental experiments small fruiting grapevines were used; we further developed a method described by Mullins and Rajasekaran (1981) into a technique designed to obtain optimal growth in controlled conditions to produce experimental grapevine plants with optimal nutrition and adequate and consistent reproductive performance.

This research led to significant advances in our understanding of grapevine reproductive biology, the impact that salt stress has upon flowering, fruit set and ultimately yield, and the involvement of amines in the reproductive performance of grapevines. Based on these results new research avenues are proposed to further our understanding.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any University or any other tertiary institution and, to the best of my knowledge and belief it contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis when deposited in the University Library, being available for loan and photocopying, subject to the provisions of the Copyright Act 1986.

The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder of those works. 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 catalogue, The Australian Digital Thesis Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

…….. …….

Tintu Baby Date

Publications as part of this Research

Baby, T., Hocking, B., Tyerman, S.D., Gilliham, M. and Collins, C. (2014) Modified method for producing grapevine plants in controlled environments. American Journal of Enology and Viticulture. 62:2, 261-267. (Presented in chapter 3).

Baby, T., Collins, C., Tyerman, S.D. and Gilliham, M. (2014) Salinity negatively affects grapevine fruit set, and cannot be ameliorated by silicon. (Prepared manuscript for submission to the American Journal of Enology and Viticulture. Presented in chapter 4).

Baby, T., Tyerman, S.D., Gilliham, M. and Collins, C. (2014) Differential fruit set between grapevine cultivars is related to differences in pollen viability and amine concentrations in flowers. (Prepared manuscript for submission to the Australian Journal of Grape and Wine Research. Presented in chapter 5).

Each of these manuscripts is displayed in this thesis in either published or submitted form according to the instructions to author of the specific journal

This Thesis has been prepared according to the University of Adelaide's specifications for "Combination of conventional and publication format".

Acknowledgments

All praise, glory and honour of my Lord Jesus Christ for the wisdom and strength he bestowed on me to proceed successfully through this research journey to the accomplishment of this thesis.

The first person I would sincerely like to thank is my principal supervisor, Dr Cassandra Collins, for her excellent support, guidance, feedback and encouragement throughout my PhD. I particularly thank her for giving me the freedom to try experiments of interest to me and to work in a flexible way. That really improved my enthusiasm and confidence in my ability to conduct research. I am extremely grateful to my co-supervisors, Dr Matthew Gilliham and Professor Stephen Tyerman for ongoing support of my project with their wealth of knowledge. I sincerely acknowledge each of their contributions without which this achievement would not have been possible.

I would also like to warmly acknowledge my external advisor Dr Rob Walker for giving me advice and support along the way.

I gratefully acknowledge funding of the project by Australia's grape-growers and winemakers through their investment body, the Grape and Wine Research and Development Corporation (GWRDC, now known as the Australian Grape and Wine Authority, AGWA).

I would like to take this opportunity to thank my colleagues at the University of Adelaide for their friendship and advice during my PhD.

Special thanks to Benjamin Pike and Paul Ingram for their help in setting up my experiments. I sincerely acknowledge Associate Professor Amanda Able and Dr Chris Ford for letting me use their laboratories and the help of the lab members. I am grateful to Dr Gwen Mayo, Annette James, Teresa Fowles, Meagan Mercurio and Natoiya Lloyd for their help with various parts of my experiments.

I would like to sincerely thank my family, especially my parents, parents-in-law, my brother and siblings-in-law for their love, support and encouragement.

Finally, I would like to thank my husband and best friend Anoop who has been the greatest positive influence and support to achieve this dream and to Edwin, Evelyn and Anna, my beautiful children.

Abbreviations

ABA	Abscisic acid
ANOVA	Analysis of Variance
AGWA	Australian Grape and Wine Authority
ADC	Arginine decarboxylase
B	Boron
Ca	Calcium
CAD	Cadaverine
CI	Coulure Index
Cl	Chloride
Cu	Copper
cv	Cultivar
cvs	Cultivars
DAO	Diamine oxidase
DAP	1, 3 Diaminopropane
dcSAM	decarboxylated S-adenosylmethionine
dSm^{-1}	deciSiemens per metre
DW	Dry weight
ECM	Extracellular matrix
ECe	Electrical conductivity
EL stages	Eichhorn-Lorenz stages
Fe	Iron
FW	Fresh weight
hr	Hour
IBA	Indole Butyric Acid
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometer
K	Potassium
kPa	Kilopascal
L	Litre
LGO	live green ovary
M	Molar
m	Metre
mg	Milligram
MI	Millerandage Index
mins	Minutes
mL	Millilitre
mM	Millimolar
mm	Millimetre
mmol	Millimol
Mn	Manganese
N	Nitrogen
Na	Sodium
ODC	Ornithine decarboxylase
P	Phosphorous
Pi	Inorganic phosphate
PA	Polyamine
PAs	Polyamines
PAO	Polyamine oxidase

PEA	Phenylethylamine
PH-PAs	Insoluble conjugated polyamines
PUT	Putrescine
P-value	Probability
RO	Reverse Osmosis
S	Sulphur
s	Second
SAM	S-adenosylmethionine
SAMDC	S-adenosylmethionine decarboxylase
SH-PAs	Soluble conjugated polyamines
Si	Silicon
S-PAs	Free polyamines
SPD	Spermidine
SPDS	Spermidine synthase
SPM	Spermine
SPMS	Spermine synthase
Spp.	Species
TYR	Tyramine
wt	Weight
Zn	Zinc
µM	Micromolar
µm	micrometer
µmol	micromol
˚C	Degree Celsius
%	Percentage