Investigating factors that affect grapevine fruit set during abiotic stress

By

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Abstract

In angiosperms pollen tubes play a crucial role in sexual reproduction in delivering male gametes to female tissue for fertilization. Any type of impairment in pollen tube growth (PTG) might lead to the poor fruit set. Poor fruit set leads to partially developed berries and poor yields in grapevine. Salinity is a major environmental factor that constrains optimal fruit set. However, whether PTG is restricted in the style during saline conditions is still unknown.

PTG relies upon many co- ordinated processes including cytoskeletal rearrangements, vesicle trafficking, signal transduction pathways and pollen–pistil interactions. Various chemical factors are known to affect PTG, including gamma-aminobutyric acid (GABA) which at low concentrations guides the pollen tube to the ovary but at high concentrations inhibit pollen tube growth. GABA concentration also increases in plant tissues under stress, including salinized conditions.

The transport of ions across various pollen tube membranes is crucial for PTG; the proteins responsible for the ion movement across grapevine pollen are unknown. ALMTs/QUAC (Aluminum activated malate transporters/Quick activating anion channels) were found in Arabidopsis pollen tubes and are candidates for the movement of Cl^-. Recently it was discovered that anion currents through ALMT are gated by GABA. Here, the link between ALMTs and GABA and its role in controlling PTG under stress is explored.

Pollen performance and its potential role in poor fruit set under saline conditions was explored using Shiraz (BVRC17) vines. Pollen tube length and growth rate for salt treated vines was found to be significantly less in first 4 hours as compared to untreated pollen grains when grown in *in vitro* conditions on pollen germination media (PGM). Pollen grains were treated with exogenously-applied GABA (1-100 mM) using the *in vitro* pollen assay; length increased after 1-5 mM GABA and then decreased after 20-100 mM GABA treatment. An analogue of GABA (Muscimol) was inhibitory to PTG and an antagonist of GABA binding in mammalian $GABA_A$ receptors (Bicuculline) was stimulatory adding more evidence for the role of GABA in regulating PTG.

In order to check the GABA levels GABase assay was done and GABA levels were found to be nearly 2 fold increase in salt stressed flowers as compared to control flowers which may be contributing to the reduced fruit set through stunted pollen tube growth.

GABA concentration in tissue was examined using a GABase assay and found to be nearly 2 fold increased in salt stressed flowers as compared to control flowers which was hypothesized to be contributing to the reduced fruit set through stunted pollen tube growth. Gene

expression levels were examined for GABA shunt enzymes in control and salt treated flowers but were not significantly different.

ALMT expression in flowers, pollen grains and pollen tubes was examined. Five *ALMT* were found in flowers (*Vv ALMT 7, 9-like, 9-like_2, 10* and *13*), two in pollen tubes (*Vv ALMT 9-like* and *10*) and one in pollen grains (*Vv ALMT 10*). Ion transport by Vv ALMT 9-like was found to be GABA sensitive and therefore is a prime candidate for transducing GABA signals in pollen tubes which regulate PTG under standard and salinized conditions.

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. One of the figures in the thesis from Chapter 4 is included in an article published in a high impact journal. Other than this it contains no material previously published or written by another person, except where due references has been made in the text. I certify that no part of this work will, in the future, be used in a submission 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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Satwinder Kaur Date

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Abbreviations

ACA- Auto inhibited Ca^{2+} ATPases

ADF- Actin Depolymerizing Factor

ALMTs- Aluminum Activated Malate Transporters

ATP- Adenosine triphosphate

Ca^{2+}- Calcium ion

Ca^{2+}-CAM- Ca^{2+}- Calmodulin

CaCA- Ca^{2+} cation Antiporter

$CaNO_3$- Calcium Nitrate

$CaCl_2$- Calcium Chloride

CER- Controlled Environment Room

C:N- Carbon: Nitrogen

˚C- Degree celsius

CHX- Cation Hydrogen exchanger

CNGC- Cyclic Nucleotide Gated ion channel

CNS- Central Nervous System

DAO- Diamine Oxidase

ETC- Electron Transport Chain

FAO- Food and Agriculture Organization

GABA- γ- Amino butyric Acid

GABA-T- GABA-Aminotransferase

GAD-Glutamate Decarboxylase

GAT-GABA transporter

GHB- γ- hydroxybutyric acid

GLR- Glutamate receptor channel

H_2O_2- Hydrogen Peroxide

IBA- Indole Butyric acid

LGOs- Live Green ovaries

LN-Liquid Nitrogen

mm- Millimeter

mM- Milli Molar

MES- 4- Morpholineethanesulphonic acid

meq l^{-1}- milliequivalent per litre

Mg^{2+}- Magnesium

$MgSO_4$- Magnesium Sulphate

NA- niflumic acid

Na- Sodium

NAD- Nicotinamide adenine dinucleotide

NG- non germinated

NO_3^-- Nitrate ion

NPPB- 5-nitro-2-(3-phenylpropylamine)-benzoic acid

PAs- Polyamines

PAO- Polyamine Oxidase

%- Percent

PG- pollen germination

PTG- pollen tube growth

PTL- pollen tube length

PGM- pollen germination media

PO_4^{-}Phosphate ion

Pop 2- Pollen pistil 2

Put -Putrescine

QUAC- Quickly activating anion channel

R-type- Rapid type

ROS- Reactive Oxygen species

SEM- standard error mean

SLAC/ SLAH- Slowly activating anion channel

SO_4^{-}Sulphate

SSADH- Succinic semialdehyde dehydrogenase

SSR- Succinic semialdehyde reductase

Spm- Spermine

Spd- Spermidine

TRIS- Tris- (hydroxymethyle) aminomethane

µm- micro meter

µM- micro Molar