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ABSTRACT

Seed development in *Arabidopsis thaliana*, has been studied at several levels. However, little has been done to study the role of sugar metabolism genes in seed pod development in this species. As the fertilized egg progresses to a mature seed, the sugars composition during different stages of the developing changes. These changes are related to metabolic processes in the developing seeds, but also to the activity of sucrose- converting and transporting genes, active at the interphase between the maternal tissue and the endosperm. Sucrose synthase (SUS) is one of these genes; it catalyses the reversible reaction of sucrose breakdown in the presence of UDP to form fructose and UDP-glucose.

In this study we looked at glucose, fructose and sucrose concentration at different time points during seed pod development. These changes in sugar concentrations were analysed in both Colombia wild type and WS (Wassilewskija) ecotypes. By comparison of the sugar composition of these ecotypes, and linking these data with phenotypic observations in both ecotypes during development, we are able to comment on the possible role of sugars in seed pod development. Also, the sugar composition of wild type seed pods were compared with those of *Atsus* mutant seed pods, and possible effects sucrose synthase mutations on the phenotype of the developing *Arabidopsis thaliana* seeds were analysed. The effect of sucrose synthase knockouts in developing seed pods were studied by comparing biochemical and phenotypic characteristics data of the *Atsus* mutants within Colombia wild type plants.

Salk line plants were screened to identify plants carrying a homozygous insertion for T-DNA in five of the sucrose synthase genes. The developing seed pods of each of the homozygous mutants were characterized biochemically via High-Performance Anion-Exchange Chromatography (HPAEC). Furthermore, seed weight, number of seed per pod, germination rate and the morphological development of the embryo were closely analysed.

The study found out that there were some biochemical effects of *Atsus* knockout mutants, and some phenotypic effects of *Atsus* knockout mutants on the developing seed pods. However, in general the effects were not as pronounced as those that were seen in maize seed, pea seed and potato tuber as a result of sucrose synthase knockout. The general pattern of glucose, fructose and sucrose were similar to the Colombia wild type, although in mature seed pods the sucrose levels in *Atsus1*, *Atsus2*, *Atsus3* and *Atsus6* were slightly, but significantly lower than in the Colombia wild type.

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Wisdom is the principal thing in all your Getting get understanding –King Solomon.

DECLARATION

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work.

Name: Benjamin Oladipo Odunlami

Signature:

Date: 19/10/2009

Abbreviations

ANOVA-Analysis of variance

AtSUS-Arabidopsis thaliana sucrose synthase

DAP- Day after pollination

DNA- Deoxyribonucleic acid

 0 C – degree centigrade

Col- Columbia

EF- Elongation factor

el- embryo length

g- gram

GC- Gas chromatography

GSP –gene specific primer

h- hour

HPAEC-High performance anion exchange chromatography

mA- milliamplitude

Min -minute

ml- millilitre

mM - millimolar

mRNA -messenger ribonucleic acid

 $M\Omega/cm$ - ohm meters per centimetre

NASC -Nottingham Arabidopsis stock centre

ng- microgram

PCR-Polymerase chain reaction

PTGS- Post transcription gene silencing

%- percentage

RAM- root apical meristem

RNAi - RNA interference

TAIR - The Arabidopsis Information Resource

T-DNA- transfer DNA

TIAG- the Arabidopsis genome initiative

TES-

s- second

sd- standard deviation

sl- seed length SUS- sucrose synthase sw- seed width T-DNA- transfer DNA TLC- thin layer chromatography T3- third generation SAM – stem apical meristem SPS- sucrose phosphate synthase UDP- Uridine diphosphate µeq - microequivalents µl- micro litre µg- micro gram µM- micro molar UNEP- United Nation Environmental Protection UTR- untranslated region UV- ultraviolet light

v/v- volume per volume

Ws- Wassilewskija