

**The Association of Homeotic Gene Expression
with Stem Cell Formation and Morphogenesis in
Cultured *Medicago truncatula***

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STATEMENT OF ORIGINALITY

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PUBLICATIONS

PUBLICATIONS

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Conference Proceedings

Chen S-K, Kurdyukov S, Rose RJ (2005) Investigation of the expression of *WUSCHEL*, *WOX* and *CLAVATA* genes in the highly embryogenic *Medicago truncatula* genotype Jemalong 2HA. Abstract CILR Symposium, 2005, Sunshine Coast Queensland.

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ABSTRACT

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Somatic embryogenesis (SE) can be induced *in vitro* in *Medicago truncatula* 2HA by auxin and cytokinin but not in wild type Jemalong. Wild-type Jemalong will only form callus in the presence of auxin and cytokinin and both Jemalong and 2HA will form roots in response to auxin alone. The F2 analysis of 2HA X Jemalong crosses suggest that a single gene may open the way to SE but additional genes are required to maximise the process. Auxin and cytokinin are required for 2-3 weeks for SE and for *de novo* root formation auxin is essential for about one week. Absciscic acid (ABA) and ethylene, both stress related hormones, enhance SE induction but inhibit callus and *de novo* root formation.

The *WUSCHEL* (*WUS*) gene was investigated in *M. truncatula* (*Mt*) and identified by the similarity with *Arabidopsis WUS* in amino acid sequence, phylogeny, promoter element patterns, and expression patterns *in planta*. *MtWUS* is induced by cytokinin after 24-48 h in embryogenic cultures and maximum expression occurs after 1 week which coincides with totipotent stem cell induction. *MtWUS* expression, as illustrated by promoter-GUS studies, subsequently localises to the embryo and corresponds to the onset of *MtCLV3* expression. RNAi studies show that *MtWUS* expression is essential for callus and somatic embryo production. There is evidence based on the presence of *MtWUS* promoter binding sites that *MtWUS* is required for the induction of *MtSERF1* which appears to have a key role in the signalling required for SE induced in 2HA.

MtWOX5, as for *MtWUS*, was identified by similarity to *Arabidopsis WOX5* based on amino acid sequence, phylogeny, promoter element patterns, and expression patterns *in planta*. *MtWOX5* expresses in the auxin induced root primordium and root meristem and appears to be involved in pluripotent stem cell induction. GA suppresses the *MtWOX5* expression in the root apex and suppresses the root primordium induction, consistent with the importance of *MtWOX5* in *in vitro* root formation.

The evidence is discussed that the homeotic genes *MtWUS* and *MtWOX5* are “hijacked” for stem cell induction which is key to somatic embryo and *de novo* root induction. In relation to SE, a key role for *WUS* in the signalling involved in induction is discussed and a model developed.

ABBREVIATIONS

2,4-D	2,4-dichlorophenoxyacetic acid
2HA	<i>Medicago truncatula</i> cv. Jemalong 2HA
ABA	Absciscic Acid
ABAR	ABSCISIC ACID RECEPTOR
ABI	ABA INSENSITIVE
ABF	Auxin Binding Factor
ACC	1-aminocyclopropane-1-carboxylic acid
AGL15	AGAMOUS-LIKE 15
AHK	<i>Arabidopsis</i> Histidine Kinase
AHP	<i>Arabidopsis</i> Histidine-Phosphotransfer
APRT	Adenine phosphoribosyltransferase
ARF	Auxin Response Factor
ARR	<i>Arabidopsis</i> response regulator
AVG	Aminoethoxyvinylglycine
BA	Benzyladenine
BBM	BABY BOOM
CHASE	Cyclases/histidine-kinases-associated sensory extracellular
CKX	Cytokinin oxidase/dehydrogenase
CLV	CLAVATA
cZ	<i>cis</i> -zeatin
DELLA	DELLA-domain, named after the first five amino acids
DMAPP	Dimethylallyl diphosphate
DPI	Diphenyleneiodonium sulfate
dZ	Dihydrozeatin
FUS3	FUSCA3
GA	Gibberellin
GA ₃	Gibberellic Acid 3
GA2ox	GA 2 β -hydroxylase
GA3ox	GA 3 β -hydroxylase
GA20ox	GA 20-oxidase
GPA1	G protein <i>Arabidopsis</i> α subunit
HMBDP	Hydroxymethylbutenyl diphosphate
IAA	Indole-3-acetic acid
iP	N ⁶ -(Δ 2-isopentenyl)-adenine
IPA	Indole-3-pyruvic acid
IPT	Adenosine phosphate-isopentenyltransferase (ISOPENTYL TRANSFERASE)
IPOx	Indole-3-acetaldoxime
JA	Jasmonic acid

APPENDIX 8 Cell proliferation in 1 week cultured leaf explants

LEC	LEAFY COTYLEDON
LOG	LONELY GUY
LRR	Leucine Rich Repeat
MADS	Domain found in <u>M</u> CM1, <u>A</u> GAMOUS, <u>D</u> EFICIENS and <u>S</u> RF
MemT	<i>meta</i> -methoxytopolin
MeoT	<i>ortho</i> -methoxytopolin
MEP	Methylerythritol phosphate
mT	<i>meta</i> -topolin
MtSK1	<i>Medicago truncatula</i> serine/threonine kinase 1
NAA	α -Naphthalene Acetic Acid
OC	Organising Centre
oT	<i>ortho</i> -topolin
PEM	Proembryogenic masses
PIN	PIN-FORMED
PKL	PICKLE
QC	Quiescent Centre
QHB	QUIESCENT-CENTER-SPECIFIC HOMEBOX
RAM	Root Apical Meristem
RDP	Riboside 5'-diphosphate
RMP	Riboside 5'-monophosphate
ROS	Reactive oxygen species
RTP	Riboside 5'-triphosphate
SA	Salicylic acid
SAM	Shoot Apical Meristem
SCF	Complex named for its components; SKP1, cullin and F-Box
SCR	SCARECROW
SE	Somatic embryogenesis
SEs	Somatic embryos
SERK	SOMATIC EMBRYOGENESIS RECEPTOR KINASE
SERF	SOMATIC EMBRYO RELATED FACTOR
SPY	SPINDLY
STM	SHOOTMERISTEMLESS
TF	Transcription factor
TIR1	Transport Inhibitor Response 1
Trp	Tryptophan
tZ	trans-zeatin
WOX	WUSCHEL-related homeobox
WUS	WUSCHEL