CHARACTERISATION OF TOMATO MADS-BOX GENES INVOLVED IN FLOWER AND FRUIT DEVELOPMENT

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CHARLES AMPOMAH-DWAMENA

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Abstract

MADS-box genes encode transcription factors that are involved in various aspects of plant development, by regulating target genes that control morphogenesis. Over the last decade, plant MADS-box genes have been studied extensively to reveal their control of floral development, especially in the model plants *Arabidopsis* and *Antirrhinum*. Their functions are however, not restricted to the flower but are involved in various aspects of plant development (Rounsley et al., 1995; Jack, 2001). By virtue of their extensive roles in the flower, these genes are expected to function in fruit development, which is a progression from flower morphogenesis. The aim of this study was to examine the role of MADS-box genes during flower and fruit development.

Two new members of the tomato MADS-box gene family, *TM10* and *TM29* were identified. *TM29* was isolated from a young fruit cDNA library by screening with homologous MADS-box fragments and *TM10* was amplified by polymerase chain reaction from fruit cDNA templates. These genes were characterised by sequence and RNA expression patterns and their functions examined using molecular genetic techniques. Sequence analyses confirmed that both genes belong to the MADS-box family.

TM29 shows 68% amino acid sequence identity to *Arabidopsis* SEP1 MADS-box protein. *TM29* expression pattern showed similarities as well as differences to *SEP1* (Flanagan and Ma, 1994). *TM29* is expressed in shoot, inflorescence and floral meristems unlike *SEP1*, which is expressed exclusively in floral meristems (Flanagan and Ma, 1994). *TM29* is expressed in all the four whorls of the flower. During floral

organ development, it is highly expressed at early stages of the organ primordium but decreases as the organ differentiates and matures. In the mature flower bud, *TM29* is expressed in the anther and ovary pericarp. During fruit development, *TM29* is expressed from anthesis ovary to fruit of 14 days post-anthesis with its transcript localised to the pericarp and placenta.

TM10 showed 64% amino acid identity to *Arabidopsis* AGL12, across the entire sequence. This notwithstanding, *TM10* expression differed from *AGL12*. *TM10* was expressed in shoot tissues of tomato and was not detected in roots. In contrast, the *AGL12* gene transcript was only present in the roots of *Arabidopsis* (Rounsley et al., 1995). Expression was detected in leaves, shoot growing tips, floral buds and fruit. During fruit development, *TM10* is expressed in anthesis ovary and in fruits at different growth stages.

The functions of *TM29* and *TM10* were examined by transgenic techniques and phenotypes generated were consistent with their spatial and temporal gene expression patterns. *TM29* transgenic phenotypes suggested it might be involved in the control of sympodial growth, transition to flowering, proper development of floral organs, parthenocarpic fruit development and maintenance of floral meristem identity. *TM10* affected apical dominance and flowering time, development of floral organs and parthenocarpic fruit development.

HortResearch

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Note: The genetic experimentation in this project was conducted with approval from the appropriate authority (Protocol No. GMO 00-HRA 037).

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µg	microgram
µl	microlitre
µM	micromolar
A_{260}	absorbance at 260 nm
BA	Benzyl aminopurine
bp	basepairs
CaMV	Cauliflower mosaic virus
cm	centimetres
CTAB	cethyltrimethylammonium bromide
CTP	cytidine-5-triphosphate
cv	cultivar
d.p.a	days post-anthesis
dCTP	deoxycytidine-5-triphosphate
DEPC	diethylpyrocarbonate
DIG	digoxigenin
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
dNTP	deoxy-nucleotide triphosphate
DTT	dithiothreitol
E. coli	*Escherichia coli*
EDTA	ethylene diaminetetraacetic acid
g	gram
GA	gibberellic acid
IBA	indoyl butyric acid
IPTG	isopropylthiogalactoside
kb	kilobasepairs
l	litre
LB	Luria Bertani
mg	milligram
mins	minutes

ml	millilitre
mM	millimolar
MOPS	N morpholino propane-sulfonic acid
mRNA	Messenger RNA
NAA	naphthalene acetic acid
ng	nanogram
nm	nanometer
nptII	neomycin phosphotransferase II
OD	optimal density
Oligo	Oligonucleotide
ORF	open reading frame
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pH	-logarithm $[H^+]$
RACE	rapid amplification of cDNA ends
RNase	ribonuclease
RT-PCR	Reverse transcriptase-PCR
s	seconds
SDS	sodium dodecyl sulphate
TAE	Tris acetate ethylene diaminetetraacetic acid
T-DNA	transfer-DNA
TE	Tris ethylene diaminetetraacetic acid
Tris	tris(hydroxymethyl)aminomethane
UTR	untranslated region
UV	ultraviolet
V	Volts
v	volume
X-gal	5-bromo-4-chloro-3-indoyl-ß-D-galactopyranoside
Z	zeatin