Characterization of the *Arabidopsis* MADS-box transcription factor, *AGL104*

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ABSTRACT

AGL104 is an *Arabidopsis* MADS-box transcription factor belonging to the MIKC* clade. The exclusive expression of MIKC* genes in the gametophyte generation of both mosses and angiosperms has fueled questions regarding the function of these genes in both these taxa and the notion that the developmental program of the gametophyte generation in both these taxa may be fundamentally similar even though the structures themselves differ greatly in their phenotype. Since transcription factors control development and changes in the developmental control genes is thought to be a major source of evolutionary changes in morphology, characterization of MIKC* genes is expected to provide clues to the evolutionary changes in land plant body form. In angiosperms, *AGL104* is reported to be expressed late and exclusively in the male (pollen) and female (embryo sac) gametophyte. Since late pollen development, such as pollen germination and pollen tube elongation, is thought to occur independently of transcription, the exclusive and high level of expression of a transcription factor is thus intriguing.

We report the expression of *AGL104* in developing anthers, mature pollen, pollen tubes and the egg apparatus of the embryo sac. Our study is the first report of *AGL104* expression in the pollen tubes. Our data showing spatial expression of *AGL104* in the different developmental stages of pollen, with weak expression in the uninucleate microspore that increases and culminates in the mature pollen, is also novel since spatial expression of this gene during pollen development had not been previously reported.

Functional characterization through gain-of-function and loss-of-function analyses shows that *AGL104* promotes pollen germination and an increased pollen tube length when measured 4 hours after pollination. The implication of this data is that, despite popular notions, active gene regulation is taking place during pollen germination and tube elongation. Further functional analysis in the pollen and the embryo sac is required to establish the precise role of *AGL104* in the angiosperms. This information will then lay the groundwork for future comparisons of MIKC* activity in the basal and higher plants and determine if changes in MIKC* gene function were responsible for evolutionary changes in land plant body form.

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Abbreviations

µ	microns
µg	microgram(s)
µl	microlitre(s)
35S	35S promoter from the cauliflower mosaic virus, CaMV
A.tumefaciens	*Agrobacterium tumefaciens*
Amp	Ampicillin
ATP	Adenosine triphosphate
bp	base pairs
C.elegans	*Caenorhabditis elegans*, a nematode
cDNA	complementary deoxyribonucleic acid
d	day(s)
DNA	deoxyribonucleic acid
dNTP	deoxy-nucleotide-triphosphate
E.coli	*Escherichia coli*; a bacterium
EDTA	ethylene diamine tetra acetate
g	gram(s)
GUS	ß-glucuronidase
hap	hours after pollination
HCl	Hydrochloric acid
hr(s)	hour(s)
Kan	Kanamycin
kb	kilobase(s)
KOH	Potassium hydroxide
l	litre(s)
LB	Luria-Bertani; used as bacterial growth media
M	molar; moles per litre
mg	milligram(s)
milliQ	water purified using the Milli-Q Ultrapure System
min	minute(s)

ml	millilitre(s)
mM	millimolar
MOPS	3-(N-morpholino) propanesulfonic acid
MS	Murashige Skoog, media
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ng	nanogram(s)
o/n	over night
°C	degrees Celsius
ORF	open reading frame
PCR	polymerase chain reaction
psi	unit for pressure
Rif	Rifampicin
RNA	ribonucleic acid
rpm	revolutions per minute
RT	reverse transcription/transcriptase
rt	room temperature
SC	sperm cell
sec	second(s)
Spec	Spectinomycin
TBE	Tris borate EDTA
T-DNA	transposon DNA
TE	Tris-EDTA buffer
Tris	tris(hydroxymethyl) aminomethane
U	enzyme units
UV	ultra violet
V	volts
VC	vegetative cell
VN	vegetative nuclei
w/v	weight/volume
wt	wildtype

X unit for buffer concentration

- Gene names (and loci) are italicised eg. *AGL104*
- Proteins are capitalised eg. AGL104

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