Evaluation of the efficacy of the inducible over-expression of 9-*cis*-*epoxycarotenoid dioxygenase1* (*NCED1*) to confer improved water use efficiency in transgenic plants

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Abstract

In order to trial the concept of inducible overproduction of the plant hormone abscisic acid (ABA) to confer increased water use efficiency, the forage legume *Trifolium repens* (L.) (white clover) and the model plant species *Nicotiana tabaccum* (L.) (tobacco) were transformed with the construct *9-cis-epoxycarotenoid dioxgenase 1* (*NCED1*) gene from *Solanum lycopersicon* (*SlNCED1*) driven by the RUBISCO small subunit promoter (*SSUp*). For white clover, a total of 18 putatively genetically-independent transgenic lines were obtained through selection in tissue culture, and these were further cloned by vegetative propagation to give 56 plants in total. Ten of these tested positive for *NCED1* insertion using polymerase chain reaction (PCR) with genomic DNA. Establishment of transgenic clover on soil was problematic, but seven putatively transformed lines were established. Of these, only one line potentially expressed *SlNCED1*, but the transcriptional levels were too low, as determined by semi-quantitative reverse-transcriptase dependent PCR (sqRT-PCR), for any further analysis. This low expression, and the fact that only one line was identified, led to the decision to discontinue investigations with white clover.

In parallel, the more amenable tobacco transformation system was also used with the *SlSSUp::NCED1* construct to act as proof-of-concept. Fourteen putatively genetically independent transgenic lines of tobacco were obtained through tissue culture, six of which were successfully established onto soil. A range of integrated gene copy numbers and *NCED1* expression levels were identified in the T_o lines using genomic PCR and sqRT-PCR. Self-fertilised seed was collected from each transgenic line, but the germination rate from all of the transgenic lines was significantly lower than wild-type. Those lines that did germinate often displayed a range of aberrant growth phenotypes. After trialing methods to evaluate water use efficiency, a total of 47 T_1 seedlings

displaying a normal seedling phenotype were established on soil. A range of water use efficiencies were observed as determined by analysis of plant growth rates against water use, followed by a transpiration assay of plants deemed ‘efficient’ and ‘poor’ users of available water. No correlation of *SlNCED1* expression with the more efficient users of available water was observed, and so ultimately it was concluded that the transgene was ineffective at raising the water use efficiency of tobacco as determined by the parameters measured in thesis.

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Abbreviations

^{o}C	*degrees* Celsius
%	percent
µg	microgram
µL	microlitre
µM	microMolar
AAO	abscisic aldehyde oxidase
ABA	abscisic acid
ABA-GE	abscisic acid glucose ester
BAP	benzyl aminopurine
bp	base pair
CaMV 35S	cauliflower mosaic virus 35S promoter
Cef	cefotaxime
CR	clover regeneration (media)
DEPC	diethylpyrocarbonate
DIG	digoxigenin
DNA	deoxyribonucleic acid
DNAase	deoxyribonuclease
DTT	dithiothreitol
CTAB	cetyl trimethylammonium bromide
dNTP	deoxynucleotide triphosphate
DPA	dihydrophaseic acid
EDTA	ethylene diaminetetraacetic acid
EGTA	ethylene glycol tetraacetic acid
FAA	formalin acetic acid alcohol

FC	field capacity
FCW	field capacity weight
g	gram
GA	gibberellic acid
hr	hour
Kan	kanamycin sulfate
L	litre
LEA	late embryogenesis active
M	Molar (moles per litre)
mg	milligram
min	minute
mL	millilitre
mM	milliMolar
mRNA	messenger ribose nucleic acid
mQ	milliQ grade water
MS	Murashige and Skoog salt mix
NAA	1-napthaleneacetic acid
ng	nanogram
nM	nanoMolar
Nic	Nicotiana transformation (media)
NtGAPDH	*Nicotiana tabacum* glyceraldehyde-3-phosphate dehydrogenase
PA	phaseic acid
PCR	polymerase chain reaction
PVP	polyvinylpyrrolidone
rbcS3Cp	RUBISCO small subunit promoter

RNA	ribose nucleic acid
RT-PCR	reverse-transcriptase-dependent polymerase chain reaction
RUBISCO	ribulose-1,5-bisphosphate carboxylase/oxidase enzyme,
SDS	sodium dodecylsulfate
Sln1	slender-1
SlNCED1	*Solanum lycopersicum* 9-*cis*-epoxycarotenoid dioxygenase
sqPCR	semi-quantitative polymerase chain reaction
SSC	saline sodium chloride (buffer)
SSUp	RUBISCO small subunit promoter
t-ABA	*trans*-abscisic acid
T_0	transgenic generation zero
T_1	transgenic generation 1
Tbsp	tablespoon
Tm	Melting temperature at which DNA strands separate in preparation for annealing
TR	relative transpiration rate.
Tris	tris(hydroxymethyl)aminomethane
Tween 20	polyoxyethylenesorbitan monolaurate
V	volts
v/v	volume per volume
Wt	wild type
WUE	water use efficiency
w/v	weight/volume
x *g*	acceleration due to gravity (9.8m s^{-2})
YEB	yeast extract broth

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