Characterization of an AtPAP26-like protein (TrPAP26) from white clover (*Trifolium repens* L.)

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Abstract

Phosphate levels in soils are often in deficit in New Zealand agriculture systems, resulting in the need for phosphate supplements in the form of fertilizers. Plants are able to adapt to many environmental stresses and display a wide range of responses designed to cope with phosphate-deficiency, and the study of these may lead to the production of crop and pasture plants that can utilize added P more efficiently. One adaptive mechanism is to express purple acid phosphatase (PAP) genes, the protein products of which are able to generate, transport, and recycle inorganic phosphates from phosphate-rich compounds both intracellularly and extracellularly. Their general mechanism of action is to hydrolyze phosphate-rich esters that are found within cells, the cell wall or in the rhizosphere. One PAP, AtPAP26, has been extensively characterized in *Arabidopsis thaliana* and displays high levels of acid phosphatase activity during phosphate-starvation. *AtPAP26* has been found to be the predominantly expressed PAP during phosphate-starvation and the enzyme plays a key role in supplying inorganic phosphate to the plant by hydrolyzing the organic phosphates present in the rhizosphere. An *AtPAP26*-like sequence has been identified previously in white clover and so this project firstly cloned the full-length *TrPAP26* and then examined expression in response to phosphate-starvation. The protein product (TrPAP26) was also characterized and compared to AtPAP26 in terms of its putative biochemical functions.

TrPAP26 was predicted to be a 55 kDa protein with three N-glycosylation sites, a signal peptide of 21 amino acid residues, and a metal-ligating motif typical of PAPs. Its observed mass was closer to 45 kDa, and preliminary experiments, using recombinant TrPAP26 partially purified from transgenic tobacco, suggested that it hydrolyzed a wide range of phosphate-rich esters including adenosine triphosphate (ATP), phosphoenolpyruvate (PEP), and pyrophosphate (PPi), but not inositol hexakisphosphate (phytate). *TrPAP26* transcript levels were found to be constitutive in the roots of white clover, but correlated positively with phosphate supply in other tissues. The protein and activity levels were not directly correlated with the transcript levels suggesting other methods of regulation such as post-

translational modifications, including N-glycosylation. TrPAP26 accumulated more in the mature leaves of white clover plants grown with a full supply of phosphates. Taken together, these results suggest that TrPAP26 may play a role in internal P remobilization, rather than P scavenging directly.

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Abbreviations

-Pi	inorganic phosphate-deficient
+Pi	inorganic phosphate-sufficient
6xHis	six histidine residues
amp	ampicillin
APS	ammonium persulfate
ATP	adenosine triphosphate
AtPAP12	*Arabidopsis thaliana* purple acid phosphatase 12
AtPAP26	*Arabidopsis thaliana* purple acid phosphatase 26
BAP	6-benzyl aminopurine
BCIP	5-bromo-4-chloro-3'-indolyphosphate
BLAST	basic local alignment search tool
bp	base pairs
BSA	bovine serum albumen
cDNA	complementary deoxyribonucleic acid
cm	centimetre
Da	Dalton
DEPC	diethylpyrocarbonate
DNA	deoxyribonucleic acid
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
EST	expressed sequence tag
FFE	first fully expanded leaf
g	gram
g	gravity
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GC	guanidine and cytosine
GDP	gross domestic product
GS4B	glutathione sepharose 4B

GST	glutathione S-transferase
GUS	β-glucuronidase
ha	hectare
I	internodes
IPTG	isopropyl thiogalactoside
kan	kanamycin
kb	kilobase pairs
kDa	kiloDalton
kg	kilogram
KOH	potassium hydroxide
L	litre
LB	Luria-Bertani
M	molar
m	metre
MALDI-TOF/TOF	mass spectrometer for amino acid sequence of peptides
mg	milligram
min	minute
mL	millilitre
mM	millimolar
mRNA	messenger ribonucleic acid
NAA	1-napthaleneacetic acid
ng	nanogram
nm	nanometre
NZ	New Zealand
P	phosphorus
PAGE	polyacrylamide gel electrophoresis
PAP	purple acid phosphatase
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PEP	phosphoenolpyruvate
Pi	inorganic phosphate

pNPP	*p*-nitrophenol phosphate
pNP	*p*-nitrophenol
Po	organic phosphate
PUE	phosphate use efficiency
PVDF	polyvinylidene difluoride
qPCR	quantitative polymerase chain reaction
R	roots
RACE	rapid amplification of cDNA ends
rpm	rotations per minute
SDS	sodium dodecyl sulfate
SEM	standard error of the mean
spec	spectinomycin
TAE	Tris-acetate-EDTA
TEMED	tetramethylethylenediamine
Tm	melting temperature
TrPAP26	*Trifolium repens* purple acid phosphatase 26
uL	microlitre
ug	microgram
V	volt
v/v	volume by volume
w/v	weight by volume
X-gal	5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside