RESPONSES TO PHOSPHATE DEPRIVATION IN WHITE CLOVER (*TRIFOLIUM REPENS* L.)

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Abstract

Four breeding lines of white clover (*Trifolium repens* L.) were obtained from AgResearch Grasslands, Palmerston North, New Zealand, that had been shown previously to differ in terms of specific growth responses to added phosphate (P) in the field. These were designated Breeding Line (BL) 43 (low performer on low P; low performer on high P), BL 45 (low performer on low P; high performer on high P), BL 47 (high performer on low P; high performer on high P), and BL 49 (high performer on low P; low performer on high P). These breeding lines and five selected genotypes that were propagated from each line (designated 43-7, 43-8, 45-14, 45-4 and 47-9) were rooted in half-strength Hoagland solution in vermiculite for two weeks and then transferred to half-strength Hoagland liquid media for five weeks prior to the initiation of the experiments. For the breeding line screening, plants were acclimatized in a constant temperature environment for one week prior to treatments, while for the genotypic screening, plants were maintained in a temperature-controlled glasshouse. These lines and genotypes were characterized in relation to P uptake and utilization efficiency by growing in P-sufficient media (+P; 0.5 mM KH_2PO_4) and P-deficient media (-P; 0 mM KH_2PO_4) for 3, 5, 7 and 14 days (for the breeding line screening) and 7, 14 and 21 days (for the genotype screening). Over the time course, inorganic phosphate (Pi) content in leaves, non-specific acid phosphatase (APase) activity in intact roots (both as a total soluble activity and a cell-wall-associated activity), isoenzyme analyses, shoot dry weight (DW) and fresh weight (FW), leaf area, weight of an individual leaf (designated as the weight of the first fully expanded leaf), root FW, and the root:shoot (R:S) ratio were determined.

Pi deprivation enhanced the induction of one major low mobility cell wall acidic isoform, two minor high mobility cell wall acidic isoforms and one major low mobility cell wall basic isoform in all genotypes. Furthermore, the activity of one major low mobility cell wall basic isoform was more higher in genotype 45-14 and one minor high mobility cell wall basic isoform was induced only in genotype 45-14 in response to Pi deprivation.

In terms of individual BLs and genotypes, the screening results showed that BL 49 and genotype 45-14 displayed a constant Pi content and a slow induction of APase activity in the –P media, and had the highest total biomass FW in both +P and –P media.

Overall (in both treatments) BL 49 and genotype 45-14 are the most efficient at utilizing available P as they produced the largest biomass FW, produced more roots in P-deprived media when compared with the other BLs and genotypes, and were more efficient in utilizing the P for the synthesis of biomass. BLs 43 and 45 and genotypes 43-7 and 43-8 are less efficient at utilizing available P, while under P deprivation, BL 45 and genotype 45-14 are the most efficient at utilizing P compared to the other BLs and genotypes. The study also showed that the Pi content in leaves and APase activity in roots was found to be the plant parameter most sensitive to Pi deprivation, and the results suggest that the selection of white clover germplasm for satisfactory performance under low P availability can be carried out using these two parameters as criteria.

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Abbreviations

$A_{405\ nm}$	absorbance [log(*Io/I*)] in a 1 cm light path at 405 nm
3-PGA	3-phosphoglyceric acid
APase	acid phosphatase
APS	ammonium persulphate
AtACP5	*Arabidopsis thaliana* acid phosphatase type 5
AtPAP12	*Arabidopsis thaliana* purple acid phosphatase type 12
AVG	aminoetoxyvinylglycine
BL	breeding line
BM	biomass
cDNA	complementary deoxyribonucleic acid
cv	cultivar
d	day
DNA	deoxyribonucleic acid
DOT	days of treatment
DTT	dithiothreitol
DW	dry weight
FW	fresh weight
G	genotype
g	gram
g	acceleration due to gravity (9.81 m s^{-2})
h	hour
kD	kiloDalton (unit of molecular mass)
kPa	kiloPascal
L	litre
LePS2	*Lycopersicon esculentum* phosphate starvation-induced gene type 2
M	molar, moles per litre
mg	milligram
min	minute
MilliQ water	water that has been purified by passing through a MilliQ ion exchange column

µg	microgram
µM	micromolar
miR	microRNA
mL	milliliter
mm	millimeter
mM	millimolar
NIL	near isogenic line
nm	nanometer
NS	not significant
°C	degree Celsius
OD	optical density at x nm in a 1 cm light path
PAE	phosphorus acquisition efficiency
PAGE	polyacrylamide gel electrophoresis
PEP	phospho(enol) pyruvate
PGA	phosphoglycerate
Pht	phosphate transporter
Pi	inorganic phosphate
psr1	phosphate starvation response type 1
PUE	phosphorus utilization efficiency
pup1	phosphate under-producer mutant type 1
R:S	root per shoot
RNA	ribonucleic acid
RNase	ribonuclease
RO	reverse osmosis
s	second
S-APase	secreted acid phosphatase
SPT2	*Saccharomyces cerevisiae* phosphate transporter type 2
TEMED	N,N,N',N'-Tetramethylethylenediamine
Tris	tris (hydroxymethyl) methylamine
V	Volt (kg m^2 s^{-3} A^{-1})
v/v	volume per volume
W	Watt (kg m^2 s^{-3})
w/v	weight per volume
ρNPP	ρ-nitrophenyl phosphate