ISSN 1858-4322

JURNAL BUDIDAYA PERTANIAN

Volume 6, Nomor 1, Juli 2010

Fakultas Pertanian, Universitas Pattimura

EVALUATION OF PHOSPHORUS USE EFFICIENCY IN FOUR BREEDING LINES OF WHITE CLOVER (*Trifolium repens* L.)

J. Effendy

Faculty of Agriculture, Pattimura University
Jl. Ir. M. Putuhena Kampus Poka Ambon, 97233

ABSTRACT

Effendy, J. 2010. Evaluation of Phosphorus Use Efficiency in Four Breeding Lines of White Clover (*Trifolium repens* L.). Jurnal Budidaya Pertanian 6: 6-10.

Phosphorus deficiency is one of the most growth-limiting factors in acid soils in various parts of the world. The objective of this study was to screen 4 white clover breeding lines (*Trifolium repens* L.) at 0, 0.25, and 0.5 mM P. Pi content in shoot and acid phosphatase (APase) activity in root were related to tissue P concentrations and P-use efficiency. Shoot FW and root FW were found to be the plant growth parameter most sensitive to P deficiency. Significant breeding line differences in P use efficiency were found. BL 45 was found to be the most superior to all BLs in P uptake under P deficient condition. Phosphorus use efficiency was higher in shoot and decreased with increasing levels of P application. These results indicate selection of white clover breeding lines for satisfactory performance under low P availability can be carried out using shoot and root weight as criteria.

Key words: White clover, acid phosphatase, breeding lines, Pi content.

INTRODUCTION

Phosphorus deficiency has been identified as one of the major limiting factors for crop production in highly weathered soils such as the Oxisols and Utisols in many parts of the world (Haynes, 1984; Sanchez and Salinas, 1981). The high capacity of these soils to fix P in forms largely unavailable to plants presents serious agronomic and economic constraints. Several soil properties, especially clay, Fe, and Al contents are closely related to the P sorption capacity of these soils. Due to low natural phosphorus and high fixation capacity, a heavy dose of P is needed to achieve high production on these soils (Fageria et al. 1982, 1988; Yost et al. 1979).

Phosphorus deficiency in high P sorbing soils can be corrected by an initial application of a large quantity of P, repeated band application of a large quantity of P, or combination of an initial broadcast application and repeated band applications (Yost et al. 1979). However, farmers are facing difficulties with increasing costs of fertilizers, especially in developing countries. An integrated fertilization-plant breeding approach seems likely to give more economically viable and practical results in the future. The possibility of exploiting cultivar differences in absorption and utilization of P to improve efficiency of P fertilizer use or to obtain higher productivity on P-deficient soils has received considerable attention in recent years (Baligar and Barber, 1979; Clark and Brown, 1975; Fageria and Barbosa Fillio, 1982; Nielsen and Barber, 1978). The objective of this study was to evaluate white clover breeding lines for phosphorus use efficiency. Phosphorus efficiency defined as mg dry weight (DW) produced per mg of P absorbed by roots and shoots. Efficient breeding lines with other desirable characteristics can be use directly in advance field trials or in breeding program.

MATERIALS AND METHODS

Plant Materials

Four breeding lines of white clover (*Trifolium repens* L.) breeding line (BL) 43 (accession number C23143: poor performance in low fertility and high performance in high fertility), BL 45 (accession number C23145: low performance in low fertility and low performance in high fertility), BL 47 (accession number C23147: high performance in low fertility and high performance in high fertility) , and BL 49 (accession number C23149: low performance in high fertility and high performance in low fertility (Table 1) were kindly gifted by Dr. Derek Woodfield, AgResearch Grasslands, Palmerston North, New Zealand.

Plant Growth Conditions and Growth Measurements

Apical cuttings of each breeding line, cut to include the first three nodes, were planted in trays of moistened vermiculite and watered with half-strength Hoagland nutrient solution (Hoagland & Arnon, 1950). Plants were maintained in a controlled environment chamber in the National Climate Laboratory, Horticulture and Food Research Institute of New Zealand, Palmerston North, and after two weeks in vermiculite (by which roots had developed), were

transferred to 2 L dark colour plastic trays covered with polythene to exclude light. The roots were placed through the hole and submerged in liquid media. After growth in liquid medium for 6 weeks, the plants were sufficient size for use in experiments. Plants from each breeding line were maintained in half-strength Hoagland nutrient solution (P-containing). At 14 days, a subset of plants of each breeding line was harvested for use in experiments.

Leaf Phosphate Measurement

At day 14, the first mature leaf from a single stolon from each six plants of each breeding line maintained in P-containing condition was weight, frozen in liquid nitrogen, ground to a fine powder, and 100 µL of 5.0 M of sulphuric acid (H_2SO_4) added to each sample. Each sample was then vortexed at room temperature for 1 min, 1.0 mL of water was added and after further mixing, the samples were centrifuged at 13,000 × *g* for 5 min. Triplicate (50 µL) aliquots of the supernatant was removed, diluted five-fold with water, and 50 µL of each pippetted into wells of a microtitre plate (A/S Nunc, Rothkilde, Denmark). Fifty µL of a series of P standards, containing 0, 40, 80, 120, 160, and 200 µL KH_2PO_4, were also pipetted in triplicate, into separate wells of the plate. To each sample or standard, 200 µL of the phosphate assay reagent [1.75% (w/v) L-ascorbic acid in 13.5% (v/v) concentrated assay reagent (16 mM ammonium molybdate, 2.25 mM H_2SO_4, 0.15 mM antimony potassium oxide (+) tartrate)], was added, mixed and the absorbance of each well measured at 620 nm using an Anthos HTII plate reader (Anthos Labtec Instruments, Salzburg, Austria). The percentage P per gram FW was calculated using the P standard curve.

Extraction of Cell Wall Protein

Root tissues were excised from plants of four breeding lines maintained in P-containing media, washed with water, blot dried, weighed, and then frozen in liquid nitrogen. The frozen tissue was powdered and extracted with 25 mM 2-mercaptoethanol for 30 min on ice, at a 3:1 extractant:tissue fresh weigh ratio. The slurry was centrifuged at 12 000× *g* for 10 min at 4 °C, the supernatant removed and designated as the water-soluble whole tissue fraction. The pellet was resuspended with three volumes of 25 mM 2-mercaptoethanol, and after incubation on ice, the slurry was centrifuged as before, the supernatant again removed and discarded and the pellet resuspended with three volumes of 2-mercaptoethanol. This extraction procedure was repeated once more with 2-mercaptoethanol, and then a further 10 times with water. The final water wash extraction was removed from the pellet with two successive centrifugations and the pellet was then extracted with one volume of 1.0 M NaCl at 37 °C for 1 h. This slurry was then resuspended and extracted with one volume of 1.0 M NaCl on ice for 30 min and then one volume of 1.0 M NaCl at 4 °C for 18 h. After centrifugation, the 1.0 M NaCl extracts were pooled, designated the ionically-bound (1 M salt-extractable) cell wall extract, and used in APase assays.

Acid Phosphatase Assay

To measure activity in the root and leaf wall extracts, typically 5 to 20 µL of each fraction was made up to 50 µL with 50 mM sodium citrate buffer (pH 5.8) and 200 µL of substrate [2.5 % (w/v) ρ-nitrophenyl phosphate in 50 mM sodium citrate buffer (pH 5.8) at 20 °C. At specific time intervals, 50 µL was removed, added to 50 µL of 1.0 M NaOH and the absorbance read at 405 nm.

Statistical Analysis

Breeding lines were evaluated regarding leaf Pi content, APase activity, growth parameters, P-uptake in shoot, and ER-P in shoot. Results were submitted to statistical analyses using the SAS – System for Windows 6.11 (SAS Inc., 1996) program, through the GLM procedure. Analysis of variance of the treatment degrees of freedom were performed, allowing comparisons among four breeding lines. When test F was significant, the Duncan's test (P= 0.05) for multiple mean comparisons was applied to identify differences between breeding lines.

RESULTS AND DISCUSSION

Analysis of variance (Table 2) revealed a highly significant (P = 0.01) difference between breeding lines for Pi content, shoot DW, shoot FW, root FW, total biomass (BM) FW, P-uptake in shoot, and ER-P in shoot and was significant for APase activity. Similarly, the effects of P treatments were highly significant for Pi content, APase activity, P-uptake in shoot, and ER-P in shoot, and were not significant for shoot DW, shoot FW, root FW, and total BM FW. Except for P-uptake in shoot, all interactions among breeding lines and P levels were not significant.

Pi content, APase activity, growth parameters, and P uptake response to P levels are presented in Table 3. Except for Pi content in shoot and P-uptake in shoot, APase activity in root, P-uptake in shoot, and ER-P in shoot decreased significantly with increasing levels of P. This means that the P used in the experiment was appropriate for screening purposes. One of the prerequisites of varietal screening for mineral stress is that the growth medium should be deficient and/or toxic in the nutrient under study.

Table 1. Description of white clover breeding lines based on breeding lines evaluated for responsiveness to applied P in a field study in moist hill country. Descriptions include breeding line number and P-response category from Caradus and Dunn (2000).

Breeding Line (BL)	Accession Number	P-responsive category based on breeding line classification
BL 43	LLHH C23143	Poor performance in low fertility and high performance in high fertility
BL 45	LLHC C23145	Low performance in low fertility and low performance in high fertility
BL 47	LHHH C23147	High performance in low fertility and high performance in high fertility
BL 49	LHHL C23149	Low performance in high fertility and high performance in low fertility.

Table 2. F values for analysis of variance of growth parameters, P concentration, P-uptake, and P-efficiency ratio of four white clover breeding lines (BL)

Source of Variance	Pi content	APase activity	Shoot DW	Shoot FW	Root FW	Total BM FW	P-uptake in shoot	ER-P in shoot
BL	6.98 **	3.23*	10.70**	8.24**	6.71**	7.90**	27.67**	30.49**
Phosphorus	69.29**	33.02**	0.10NS	0.12NS	0.09NS	0.05NS	19.41**	19.78**
G x P	1.38NS	0.20NS	0.42NS	0.31NS	0.18NS	0.29NS	2.20*	1.60NS

NS = Not significant; *, ** = Significant at 0.05 and 0.01 probability levels, respectively.
P-uptake in shoot = P concentration x shoot DW
P-efficiency ratio (ER-P) in shoot = mg shoot DW/mg P in shoot

Table 3. Influence of P levels on Pi content, APase activity, growth parameters, and P uptake of four white clover breeding lines

Plant P status and Growth parameter	P level		
	Low (0 mM P)	Medium (0.25 mM P)	High (0.5 mM P)
Pi content in shoot ($mg.g^{-1}$)	0.0088c	0.0102b	0.0117a
APase in root ($min^{-1}g^{-1}FW$)	2.2451a	1.7996b	1.3541c
Shoot DW (g/leaf)	1.7053a	1.6791a	1.6422a
Shoot FW (g/plant)	9.5288a	9.7243a	9.9197a
Root FW (g/plant)	2.4319a	2.3935a	2.3552a
Total BM FW (g/plant)	12.2748a	12.1178a	11.9607a
P-uptake in shoot (mg/leaf)	0.0144c	0.0165b	0.0194a
ER-P in shoot (mg/mg P per leaf)	226.4100a	174.6800b	166.0000b

Values for each growth parameter and P uptake under different levels of P followed by the same letter are not significantly different at the 0.05 level by Duncan's Multiple Range Test.
Data are averaged over breeding lines.
P-uptake in shoot = P content in shoot x shoot DW.
ER-P in shoot = mg shoot DW/mg P in shoot.

To identify which of the growth parameters is most sensitive to P deficiency, increases in shoot DW, shoot FW, and root FW at medium and high P levels as compared to zero P level were calculated (Table 4). Shoot FW exhibited the maximum increase in growth with P addition and was followed by root FW and shoot DW. This means that shoot weight was the most sensitive response parameter to P deficiency. Root FW was the second most sensitive. These two growth parameters can, therefore, be used for white clover breeding line screening experiments. Shoot weight is much more easily determined as compared to root weight and is recommended for P-screening studies of legumes under greenhouse conditions.

Table 4. Increase (%) in white clover growth parameters at medium and high P levels as compared to a zero P level of four white clover breeding lines

Growth parameters	P levels	
	Medium (0.25 mM P)	High (0.5 mM P)
Shoot DW	-3.6950	-1.5249
Shoot FW	2.0464	4.1033
Root FW	-1.5625	-3.1661

Growth increase = [(growth at medium or high P-growth at low P)/(growth at low P)] x 100

White clover breeding lines responded differently to P application in terms of root and shoot weight. Root FW at low, medium and high P levels varied from 0.75 to 8.04, 0.89 to 7.01, and 0.32 to 8.39 g/plant, respectively (data not presented). Similarly, shoot FW varied from 2.59 to 30.18 g at the low P level, 2.69 to 24.25 g at the medium P level, and 0.92 to 42.29 g at the high P level. From a practical point of view the breeding lines which produced well under a low level of P and responded well to added P are the most desirable. Breeding line such as BL 45 falls into this category. High shoot growth of this BL is also associated with high root growth. This means that P uptake efficiency may be related to root growth. Extensive root growth under condition of low P availability in soils might be a genetic characteristic of considerable importance in areas in which soils are low in available P and in which economic constraints limit rates of fertilizer application.

Phosphorus uptake efficiency of the shoots is presented in Table 5. Phosphorus uptake efficiency was highest at the low level of P and decreased with increasing P levels. This means that, in the classical mode of plant response to increasing nutrient supply, the greatest amounts of dry matter produced per unit of absorbed P were at the lowest level of P supply. As the concentration of P in the growth medium was increased, P uptake increased (Table 3), but less dry matter was produced for each additional unit of absorbed P (Table 4). Similar results were obtained by Fageria (1988) for P uptake efficiency of rice grown in solution culture. This may be related to P uptake rate.

Overall, the results showed that BL 45 produced a considerably high shoot DW and shoot FW in response to P-deficiency. BL 45 has also been proven to be excellent performer in liquid media due to its ability to grow more roots in response to P-deprivation. This is because BL 45 has the longest tap root, more dispersed and longer lateral roots and more dispersed shallower basal roots (data not shown). The ability to grow more shoots and roots in BL 45 is due to its ability to mobilize intracellular APase which function to release P from senescent tissue for remobilizing and in bypassing the P-requiring steps in C metabolism (Plaxton and Carswell, 1999). BL 45 was also superior to all breeding lines in P uptake under P deficiency as well as under P sufficiency, suggesting that higher affinity of transporters and/or low P minimum P concentration at which uptake starts may exist in BL 45.

Table 5. Phosphorus use efficiency (mg dm/mg P absorbed) in shoots of 4 white clover breeding lines

Breeding line (BL)	P levels		
	Low	Medium	High
BL 43	154.86b	114.93c	104.93c
BL 45	280.78a	206.13a	177.56b
BL 47	228.51a	165.86b	156.15b
BL 49	241.50a	211.81a	225.36a

Means in the same column followed by the same letter are not significantly different at P = 0.05 by Duncan's Multiple Range Test

CONCLUSIONS

With the rising cost of P fertilizer, the potential of using nutrient efficient breeding lines to increase and/or stabilize crop production becomes increasingly important. Results of this study indicate that white clover breeding lines differ significantly in their P requirements. However, it should be noted that changes in the levels of Pi in the media were not measured. From a practical point of view, the breeding lines which produced well under a low level of P and responded well to added P are the most desirable. BL 45 falls into this category. Shoot as well as root weight can be used as a criterion for P-screening studies of white clover under greenhouse conditions. In conclusion, screening of breeding lines for efficiency of P uptake and utilization can effectively performed on a large number of breeding lines in nutrient solution. Selected breeding lines can then be re-screened in soil, with only a relatively small number of most promising breeding lines to proceed to the field testing.

REFERENCES

Baligar,V.C. & S.A. Barber. 1979. Genotypic differences of corn for ion uptake. Agron. J. 71: 870-873.

Clark, R.B. & J.C. Brown. 1975. Corn lines differ in mineral efficiency. *Ohio Rep.* 60: 83-86.

Fageria, N.K. 1976. Effect of P, Ca, and Mg concentration in solution culture on growth and uptake of these ions by rice. *Agron. J.* 68: 726-732.

Fageria, N.K. & M.P. Barbosa Filho. 1982. Screening rice cultivars for low level of phosphorus tolerance. *R. Bras. Ci. solo.* 6:146-151.

Fageria, N.K., M.P. Barbosa Filho & J.R.P. Carvalho. 1982a. Response of upland rice to phosphorus fertilization on an oxisol of central Brazil. *Agron. J.* 74: 51-56.

Fageria, N.K., M.P. Barbosa Filho & M.J. Garber. 1982b. Adequate nutrient levels and plant density for the rice experiments under greenhouse conditions. *Pesq. Gropec. Bras. Brasilia.* 17: 1279-1284.

Fageria, N.K., R.J. Wright & V.C Baligar. 1988. Rice cultivar evaluation for phosphorus use efficiency. *Plant Soil* 111: 105-109.

Haynes, R.J. 1984. Lime and phosphate in the soil-plant system. *Adv. Agron.* 37: 249-315.

Nielsen, N.E. & S.A. Barber. 1978. Differences among genotypes of corn in the kinetics of P uptake. *Agron. J.* 70: 695-698.

Plaxton, W.C. & M.C. Carswell. 1999. Metabolic aspects of the phosphate starvation response in plants. *In:* Lerner HR, ed. *Plant responses to environmental stress: from phytohormones to genome reorganization*. New York, NY, USA: Marcel-Dekker, 350–372.

Sanchez, P.A. & J.G. Salinas. 1981. Low input technology for managing oxisols and ultisols in tropical America. *Adv. Agron.* 34: 279-398.

Yost, R.S., E.J. Kamprath., E. Loboto & G.C. Naderman. 1979. Phosphorus response of corn on an oxisol as influenced by rates and placement. *Soil. Sci. Soc. Am. J.* 43: 338-343.