Pereira-Carvajal et al./Agro Sur 44(3): 77-87, 2016
DOI:10.4206/agrosur.2016.v44n3-08

Response of naturalized accessions of white clover to low phosphorus condition in Andisols

Respuesta de accesiones naturalizadas de trébol blanco a la condición limitante de fósforo en un Andisol

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ARTICLE INFO

Article history:
Received 05.07.2016
Accepted 14.06.2017

Keywords:
P use efficiency
P uptake efficiency
P utilization efficiency
Experimental techniques
Glasshouse condition

Original Research Article,
Special Issue: Pastures for Sustainable Productions Systems

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ABSTRACT

White clover may generate morpho-physiological adaptations to face soil phosphorus (P) deficiency. Phosphorus use efficiency (PUE) of 9 naturalized white clover accessions (WCAcc) and 2 commercial cultivars Huia and Will (controls) was evaluated under glasshouse conditions. The treatments consisted of increasing levels of P in soil mounted on PVC pots (0.025 m^2 x 1 m deep), with 3 replicates and randomly arranged inside a glasshouse. Total dry matter production (DM) and internal P concentration of the above ground materials (PIC) was determined. P uptake and components of PUE were calculated (PAE and PUtE). Additionally, the glasshouse results were compared descriptively with those obtained under field conditions (Acuña and Inostroza, 2013). Six accessions had higher DM production and were more efficient in terms of their general PUE compared to the cv. Huia control, but they did not exceed the cv. Will control. All of the accessions were more efficient in their PUE in the low-middle range of P availability showing no differences compared to the cv. Will control. This behavior was observed under glasshouse and field experimental conditions, both PAE and PUtE of the WCAcc differ in magnitude of the response, which may be associated with interspecific competition and grazing under field conditions. These results indicate that under limited availability of P in the soil the WCAcc collected have a better adaptative response. In addition, the glasshouse results were consistent with the field experiment used in the evaluations.

RESUMEN

El trébol blanco puede generar adaptaciones morfo-fisiológicas frente a una condición limitante de fósforo (P) en el suelo. El objetivo de este estudio fue evaluar la eficiencia de uso de fósforo (PUE) en nueve accesiones naturalizadas de trébol blanco en condiciones de invernadero. Se establecieron niveles crecientes de P disponible en el suelo en maceteros de PVC (0,025 m^2 x 1 m alto), con 3 repeticiones, distribuidos completamente al azar. Se determinó la producción de materia seca total (DM) y la concentración interna de P (PIC) en los tejidos aéreos, y se calculó la absorción de P y los componentes de la PUE (PAE and PUtE). Dos cultivares comerciales cv. Huia y cv. Will fueron utilizados como control. Adicionalmente, los resultados del estudio fueron comparados descriptivamente con los obtenidos en condiciones de campo (Acuña y Inostroza, 2013). Seis accesiones presentaron mayor productividad y eficiencia general de uso de P (PUE) con respecto al control cv. Huia pero no superaron al control cv. Will. Todas las accesiones fueron más eficientes en el uso de P en el rango bajo-medio (4-12 mg kg^{-1}) de P disponible siendo similares al control cv. Will. Este comportamiento fue observado en ambas condiciones experimentales, tanto PAE como PUtE en las WCAcc difieren en la magnitud de expresión de la respuesta, lo cual podría estar asociado con la competencia interespecífica y el pastoreo en condiciones de campo. Estos resultados indican que las accesiones colectadas presentan una mejor adaptación a condiciones limitantes de P en el suelo. Además, los resultados de invernadero son consistentes con las evaluaciones de campo.

Palabras clave: Eficiencia de uso de P, Eficiencia de absorción de P, técnicas experimentales, condición de invernadero.

INTRODUCTION

Soil P deficiency is considered the main limitation for white clover's growth and development (Lloyd *et al.*, 2014; Woodfield and Caradus, 1996). It has been demonstrated that these species can generate adaptation mechanisms to face low P in soil (Caradus and Snaydon, 1986; Effendy *et al.*, 2014). These adaptations may be morphological; such as greater length and root surface to increase the uptake of P from the soil (Caradus, 1981), or physiological; through an increase of the efficiency of use of the internal P for the production of biomass aerial and root (Haling *et al.*, 2016). These adaptation mechanisms are naturally generated in white clover populations growing in a native or naturalized manner under low P (Caradus and Dunn, 2000) and express themselves differently in response to the level of P deficiency in soils (Caradus and Snaydon, 1986; Effendy *et al.*, 2014). They are thus evaluated as phosphorus use efficiency (PUE) (Gourley *et al.*, 1993). PUE indicates a plant's ability to generate biomass (expressed in dry matter-DM) under different P availability conditions and results in the interaction of mechanisms which define the incorporation of P from the rhizosphere (P uptake efficiency, PAE) and the use of P uptake in biomass production (P utilization efficiency, PUtE) (Gourley *et al.*, 1993; Manschadi *et al.*, 2014).

Differences in P responses of a naturalized population of white clover have been reported under field and glasshouse conditions (Blair and Godwin, 1991; Effendy *et al.*, 2014; Godwin and Blair, 1991; Trolove *et al.*, 1996). It has also been reported that the evaluation of these species under glasshouse conditions may not be related to the P response under field conditions (Richardson *et al.*, 2011). Glasshouse experiments could be an important tool for determining the potential use of nutrients in white clover populations, on studies that seeks to select populations for future improvements. However, for this it is very important that the conditions for root exploration per plant (volume of pot) to be similar to those found in the field.

At present, fertilization P presents the great challenge of reducing external input, for which it is indispensable to improve the efficiency of P use in agricultural systems (Simpson *et al.*, 2011). The eutrophication of surface waters, and the finite sources of P for agriculture, encourage researchers to seek strategies to become more efficient in the use of P to agricultural systems (Scholz *et al.*, 2013). In pasture systems, legumes are highly relevant to reduce N external inputs to the soil. However, species such as white clover, are characterized by low efficiency of use of P with respect to grasses (Woodfield and Caradus, 1996). However, the phenotypic plasticity of species such as white clover, give the possibility of selecting native populations adapted to limiting conditions of P (Effendy *et al.*, 2014; Lloyd *et al.*, 2014; Simpson *et al.*, 2014).

Southern Chile is dominated by soils derived from volcanic ash (mainly Andisols), which are naturally characterized by a high total P content, but a low to very low availability of the same for plants. This condition has allowed naturalized populations of white clover, (introduced more than 100 years ago), have developed morphophysiological mechanisms of subsidence to these conditions. This naturalized populations of white clover were collected, from Andisols with naturally P deficient soils (Ortega *et al.*, 1994; Zappe *et al.*, 1994). Nine of these populations were evaluated in the field under grazing conditions (Acuña and Inostroza, 2013) and inside under glasshouse conditions (present study) to quantify their adaptability to highly P deficient soils, and in this way, to be able to select populations better adapted to the natural condition of P in Andisols

The aims of this study were to evaluate the differences of PUE and their components (PAE and PUtE) in 9 naturalized accessions of white clover under different levels of P availability in glasshouse conditions. This study also aimed to validate the evaluation technique of the accessions under glasshouse conditions by comparing this technique's PAE and PUtE results with those presented by Acuña and Inostroza (2013), corresponding to field conditions.

MATERIALS AND METHODS

Plant material

We evaluated nine naturalized accessions of white clover (WCAcc), collected in soils with P deficiency in the south of Chile (Ortega *et al.*, 1994). Two commercial cultivars, cv. Huia and cv. Will, were used as control (Table 1). Both the naturalized accessions of white clover and the commercial cultivars were evaluated under glasshouse conditions. Control cv. Huia has presented a medium to high P response in previous studies (Caradus and Chapman, 1996; Crush and Caradus, 1995) and is the most common cultivar used in Chile due to its good adaptability to grazing. Whereas, control cv. Will is a medium-leaved clover introduced from the USA and is a new popular cultivar because has presented a high DM productivity and P response, but lower adaptation to grazing due to the morphological characteristics.

Establishment and treatment characteristics

The study was conducted in a glasshouse at the Austral University of Chile in the Agricultural Sciences Faculty, between April 2009 and February 2010. Each pot (PVC cylinders) was 18 cm in diameter (0.025 m^2) and 100 cm tall. Pots were filled with soil collected from a natural grassland. The soil was placed in the pots in a similar layers as it was collected under field conditions. The soil is classified as Duric Hapludand (CIREN, 2003), and showed a natural P deficiency throughout the profile (4 mg P-Olsen kg^{-1}). Five treatments of P availability were established 35 days prior to sowing in the uppermost 20 cm and were corroborated by soil analysis 5 days prior to planting (Table 2), with three replicates per P level. In each pot, were sown 9 seeds (360 seed m^{-2}) of each WCAcc or control cultivar respectively and was done on April 28, 2009. The experiment was managed under non-limiting conditions of water (70-100% of field capacity) for white clover growth. Each pot was randomly distributed inside the glasshouse. The average temperature inside the glasshouse was 17-21° C from April to October and 27-35° C between November and February.

Measurements and analysis

During the experiment, there were three sampling dates (cuts) between November and February (200, 230 and 270 days after sowing). Plant cuts left 4 cm of residue height. Above ground dry matter production (DM), expressed in g DM m^{-2} was evaluated on each sampling date. The P concentration (PIC) was also evaluated according to the Saavedra (1975) through calcination of biomass cut. Regarding direct evaluations of DM and PIC, we calculated the P uptake (g P m^{-2}), P uptake efficiency PAE (g P uptake m^{-2} mg P Olsen kg^{-1}), P utilization efficiency (PUtE) (g DM g P uptake^{-1}), and P use efficiency (PUE) which is the product of PAE and PUtE.

Data analysis

Regression analyses were used to evaluate the association among the variables. For each regression was used the total accumulated biomass during the season. Analyses of variance (ANOVA) for PUE, PAE, PUtE and DM production were also performed. Both variables, PAE and PUtE, corresponds to the slope of the linear model adjusted for each variable. While PUE corresponds to the product among PAE and PUtE. Means were separate by a Fisher LSD test ($p< 0.05$). Furthermore, to validate the evaluation methodology of the accessions, correlation analysis was performed for PUE, PAE and PUtE, between the results obtained in the glasshouse and those presented by Acuña and Inostroza (2013) (data of second growth season). To compare results, we analyzed the PUE, PAE and PUtE between two ranges of P availability, low-middle (4-15 mg P-Olsen kg^{-1}) and middle-high (15-27 mg P-Olsen kg^{-1}).

RESULTS

Dry matter response

DM production was affected by P availability ($p< 0.01$), accessions ($p< 0.01$) and the interaction between these two factors ($p< 0.01$). When P availability increased the WCAcc presented two distinct positive responses in terms of DM production (Figure 1). The first response was characteristic of the control cv. Huia, which when evaluated with a quadratic adjustment

Table 1. Identification key of white clover accessions (WCAcc) in the glasshouse condition with respect to the nomenclature shown in Ortega *et al.* (1994) and Acuña and Inostroza (2013).

Cuadro 1. Identificación de accesiones de trebol blanco (WCAcc) en condiciones de invernadero con respecto a la nomenclatura mostrada en Ortega *et al.* (1994) and Acuña y Inostroza (2013).

Experiment	Identification key of white clover accessions								
Ortega *et al.* (1994) Acuña and Inostroza (2013)	2-3-X	7-1-X	9-1-X	5-2-X	8-2-X	12-2-X	8-1-X	9-2-X	6-1-X
Glasshouse experiment	Acc1	Acc2	Acc3	Acc4	Acc5	Acc6	Acc7	Acc8	Acc9

Table 2. Available P in soil for each treatment.

Cuadro 2. Fósforo disponible en el suelo para cada tratamiento.

Treatment identification	P1	P2	P3	P4	P5
Available P in soil (mg P-Olsen kg^{-1})	4	8.7	15.2	20.4	27
Rate of KH_2PO_4 (kg ha^{-1})	0	219	585	804	1170

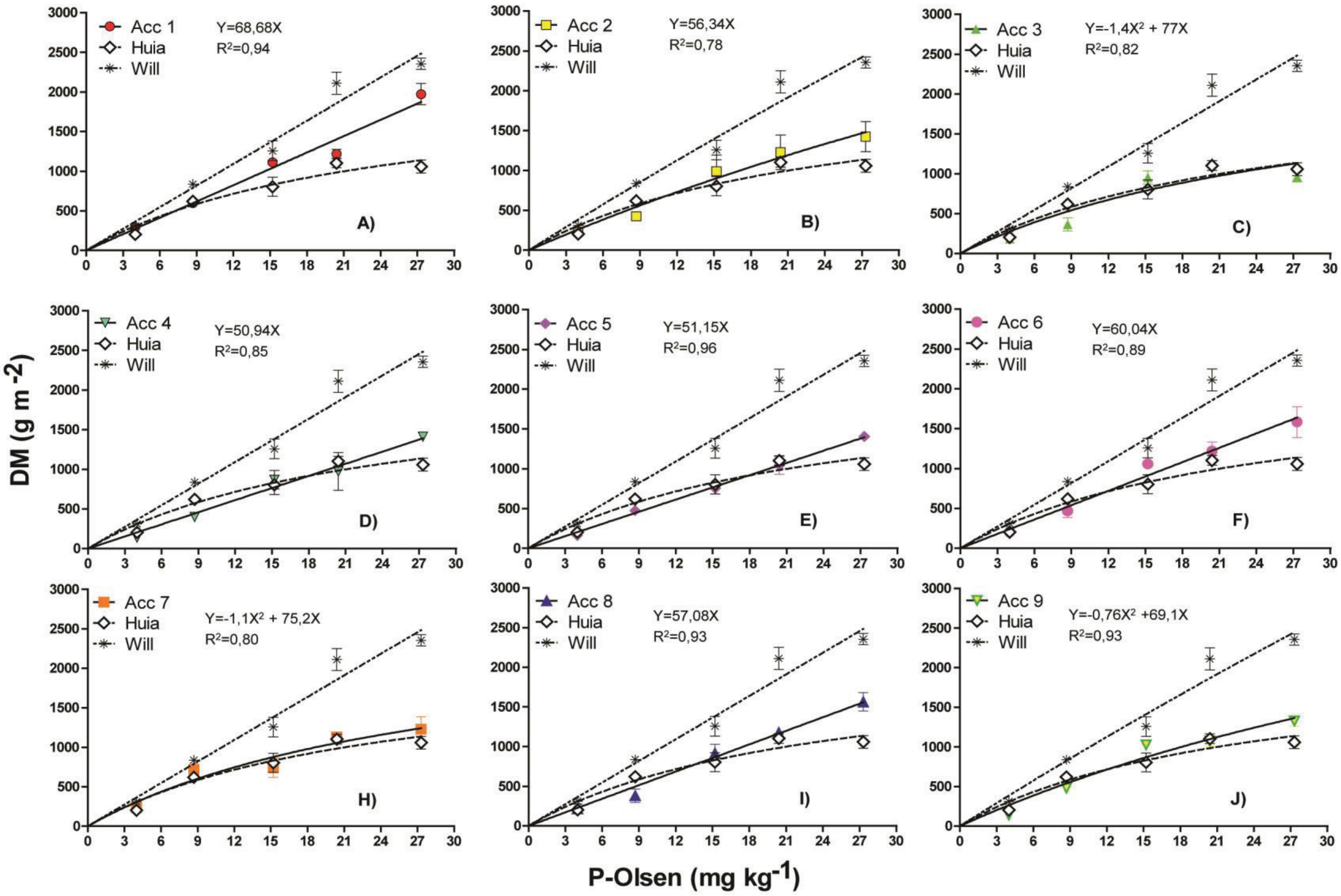

Figure 1. Response of each white clover accession (continues line) in relation to their total dry matter (DM) in function of the P availability of the soil compared to control cv. Huia (dashed line) and cv. Will (dotted line). Vertical bars indicate standard error of the mean. The significant equation adjustments ($p< 0.05$) for each WCAcc are shown in the graph.

Figura 1. Respuesta de la producción de material seca total (DM) de cada una de las accesiones de trébol blanco (línea continua) en función de la disponibilidad de P comparado con el control cv. Huia (línea segmentada) y cv. Will (línea punteada). Barras verticales indican error estándar de la media. Ajuste significativo de la ecuación ($p< 0,05$) para cada WCAcc.

(WCAcc 2, 3, 7 and 9) showed its P response decreasing above 15 mg P-Olsen kg^{-1}. The second response presented a lineal adjustment characteristic of the control cv. Will (WCAcc 1, 4, 5, 6, 8). WCAcc1 showed the highest response ($p < 0.05$) (Figure 1A). However, this did not exceed the P response of the control cv. Will.

P concentration and uptake

Internal P concentration (PIC) for the average of three sampling dates fluctuated between 0.9 and 2.4 % (Figure 2A), but did not show a general trend related to the increase of P availability (data not shown). However, all accessions increased their PIC between the lowest and highest P availabilities. When P availability increased, P uptake also increased in all of the accessions evaluated ($p < 0.05$). Thus, a significant ($p < 0.05$) interaction between P availability and accessions was also found. Total DM production showed a positive relationship with the PIC (Figure 2). In the adjusted model between DM and PIC, the Will cultivar is excluded, because this type of clover (medium-leaved) presents contrasting productive characteristics with respect to small-leaf cultivars such as Huia and WCAcc. The stronger positive relationship was found for P uptake (R^2= 0.98) (Figure 2B). Thus, on average, the DM increased by 575 g DM g P uptake^{-1} in the accessions evaluated.

Phosphorus uptake and utilization efficiency

The PAE varied between the WCAcc evaluated ($p < 0.05$). Only 3 accessions (3, 7 and 9), showed similar PAE than the control cv. Huia (Figure 3A), whereas the remaining WCAcc showed major PAE than cv. Huia ($p > 0.05$). WCAcc 5, was intermediate between cv. Huia and cv. Will. While, WCAcc 1, 2, 4, 6 and 8 showed no differences ($p > 0.05$) compared to their control cv. Will (0.13 mg P mg P-Olsen kg^{-1}). Moreover, these parameters showed no clear responses in relation to the evaluated increases in P availability.

The PUtE varied less than PAE among WCAcc (Figure 3B). Only 3 WCAcc (1, 6 and 8) showed significantly ($p < 0.05$) more efficiency in the internal utilization of P uptake (PUtE) than the control cv. Huia. WCAcc 1 was the most efficient accession, but it still did not exceed the control cv. Will (697 g DM g^{-1} P uptake). Furthermore, contrary to PAE, the PUtE decreased or remained the same with increasing levels of P availability (Table 3).

P Use efficiency (PUE) and its relationship to its components (PAE and PUtE)

WCAcc presented significant differences ($p < 0.05$) in terms of P use efficiency (PAE*PUtE). Accessions 1, 6 and 8 were the most efficient (68, 64 and 63 g DM mg P-Olsen kg^{-1} respectively), whereas, WCAcc 3 and 7 were the least efficient (34, 38 g DM mg P-Olsen kg^{-1} respectively). Seven of the evaluated accessions were more efficient (32%) than the control cv. Huia (the least efficient), but none exceeded the control cv. Will (Figure 4). Furthermore, the PUE tended to decrease or remain the same with increasing levels of P availability over 15 mg P-Olsen kg^{-1} (Figure 6).

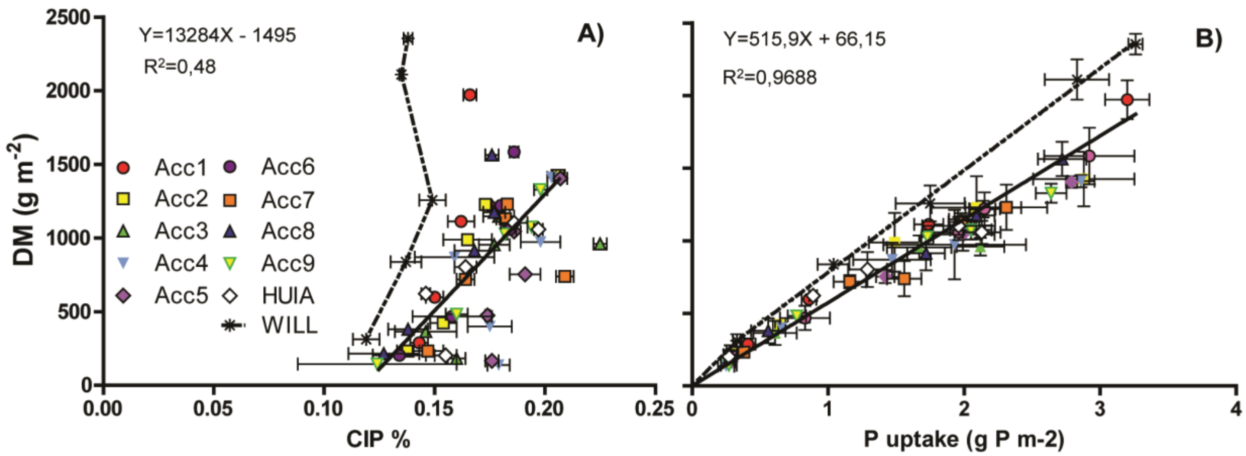

Figure 2. Relationship between the total dry matter (DM) and internal P in tissue (A) and P uptake of WCAcc (B). Equation and R^2 in each graph indicate the average response of WCAcc (dotted line) ($p < 0.05$). Control cv. Will was not considered in the equation adjustments. Vertical and horizontal bars indicate standard error of the mean at each point.

Figura 2. Relación entre la producción de material seca (DM) y el contenido interno de P en los tejidos aéreos (A) y la absorción de P (B). Ecuación y R^2 en cada gráfica indican la respuesta promedio de la WCAcc (línea punteada) ($p < 0{,}05$). El control cv. Will no se considera para el ajuste de la ecuación. Barras verticales y horizontales indican el error estándar de la media para cada punto.

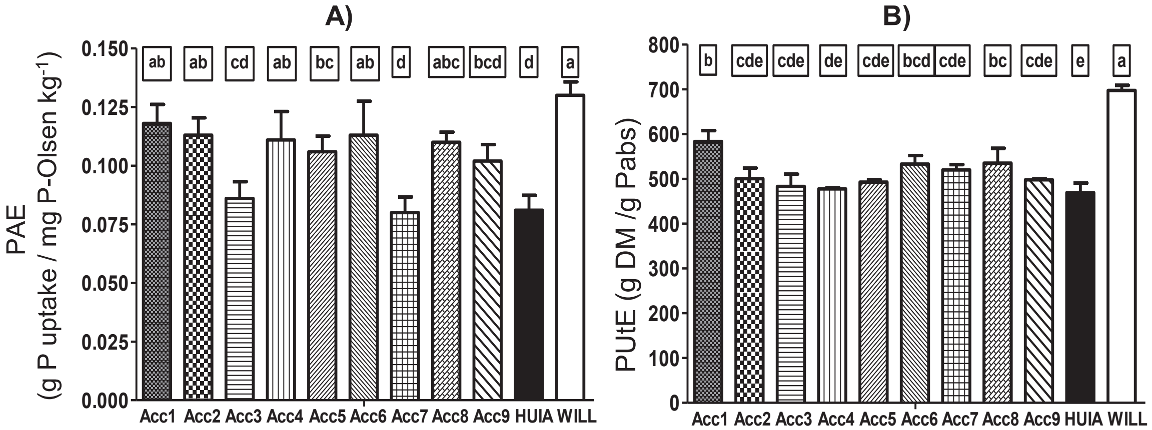

Figure 3. P uptake efficiency (A) and P utilization efficiency (B) of the WCAcc. Vertical bars indicate standard error of the mean, different letters indicate significant differences LSD ($p < 0.05$) between WCAcc.

Figura 3. Eficiencia de absorción de P (A) y eficiencia de utilización de P (B) de las WCAcc. Barras verticales indican error estándar de la media, letras diferentes indican diferencias LSD ($p < 0,05$) entre las WCAcc.

Table 3. Statistical parameters for comparison of experimental condition Glasshouse (GH) and Field (F). Pearson correlation for PUE, PAE and PUtE ($p < 0.05$) between same level of P in GH and F. Mean for each experimental condition was calculate as average of nine WCAcc (not include both controls). Different letters indicate significant differences LSD ($p < 0.05$) between experimental condition. Coefficient of variation was calculated as average of nine WCAcc (without including both controls).

Cuadro 3. Parámetros estadísticos para la comparación de condiciones experimentales invernadero (GH) y campo (F). Correlaciones de Pearson para PUE, PAE y PUtE ($p < 0,05$) entre el mismo nivel de P en GH y F. El valor de la media de cada condición experimental fue calculado como el promedio de las 9 WCAcc (no se incluyeron los controles). Letras diferentes indican diferencias significativas mediante LSD ($p < 0,05$) entre condiciones experimentales. El coeficiente de variación fue calculado como el promedio de las 9 WCAcc (no se incluyeron los controles).

	PUE		**PAE**		**PUtE**	
	F Low P	F High P	F Low P	F High P	F Low P	F High P
GH Low P	0.61*		0.72*		ns	
GH High P		0.63*		0.88*		ns

	GH Low P	GH High P	F Low P	F High P
PUE				
Mean (g DM (mg $kg^{-1})^{-1}$)	55.0	61.5	50.4	27.9
SEM	4.36	2.84	1.77	0.77
Group (LSD)	ab	a	b	c
CV WCAcc (%)	23.80	13.83	10.54	8.34
PAE				
Mean (g P uptake (mg $kg^{-1})^{-1}$)	0.09	0.09	0.15	0.10
SEM	0.007	0.003	0.006	0.005
Group (LSD)	b	b	a	b
CV WCAcc (%)	23.62	10.88	13.35	14.11
PUtE				
Mean (g DM (g P uptake)$^{-1}$)	628.7	571.8	354.2	297.4
SEM	14.60	19.62	3.14	4.28
Group (LSD)	a	b	c	d
CV WCAcc (%)	6.97	10.29	2.66	4.32

PUE was only associated with PAE ($p < 0.05$) (Figure 5B) and not with PUtE. This relationship was represented by a bilinear adjustment ($p < 0.05$). Below 0.1 PAE, PUE increased by 393 g of DM (mg P-Olsen kg^{-1})$^{-1}$ and above this value, PUE increased by 1179 g of DM (mg P-Olsen kg^{-1})$^{-1}$ for each additional unit of PAE.

PUE assessment in contrasting ranges of P availability

The WCAcc presented significant variation ($p < 0.05$) in terms of PUE, as did its components (PAE and PUtE) (Figure 6). The PUE tended to decrease or remain the same with increasing levels of P availability over 15 mg P-Olsen kg^{-1}. Evaluated accessions were more efficient in the low-middle range (4-15 mg P-Olsen kg^{-1}) than in the upper range (15-27 mg P-Olsen kg^{-1}). Moreover, WCAcc 5 and 7 were the least efficient in the low-midd-

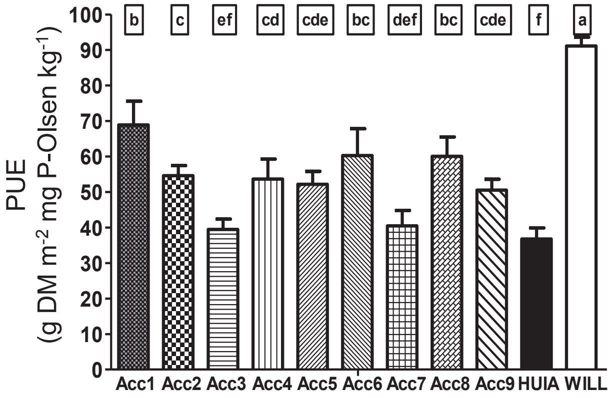

Figure 4. P use efficiency of the WCAcc. Vertical bars indicate standard error of the mean. Different letters indicate significant differences LSD ($p < 0.05$) between the WCAcc.

Figura 4. Eficiencia de uso de P de las WCAcc. Letras diferentes indican diferencias LSD ($p < 0,05$) entre las WCAcc.

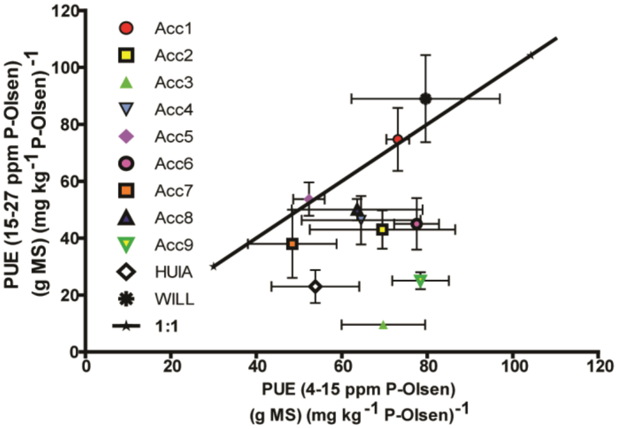

Figure 6. Relationship between the P use efficiency in the low-middle and middle-high ranges of P available in the soil. Relationship 1:1 (continuous line) indicates a similar efficiency for both ranges of P availability evaluated. Vertical and horizontal bars indicate standard error at each point.

Figura 6. Relación entre la eficiencia de uso de P en los rangos bajo-medio y medio-alto de P disponible en el suelo. Relación 1:1 (línea continua) indica similar eficiencia en ambos rangos de P disponible evaluado. Barras verticales y horizontales indican el error estándar de cada punto.

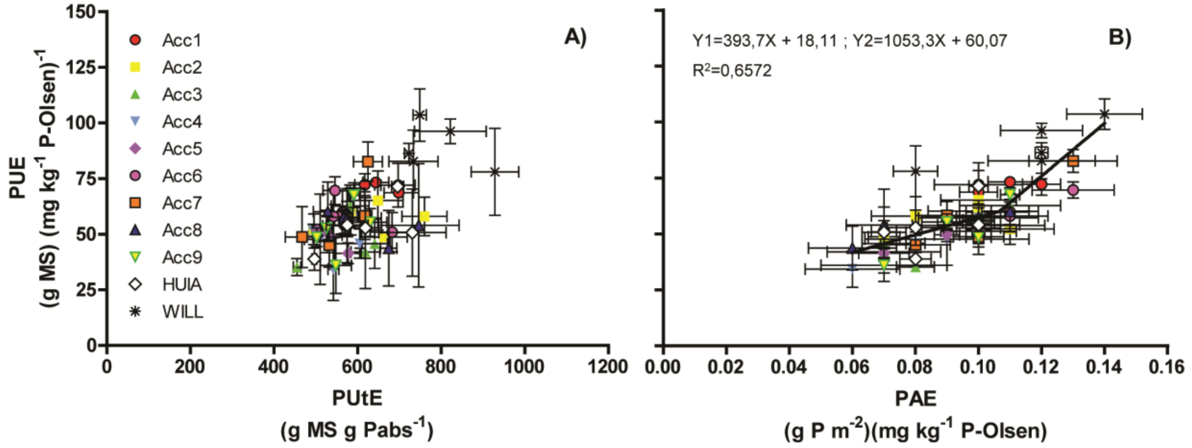

Figure 5. Relationship between P use efficiency, P uptake efficiency (A), and P utilization efficiency (B). The significant adjust ($p < 0.05$) for the average response of WCAcc is indicated by a continuous line. Vertical and horizontal bars indicate standard error of the mean.

Figure 5. Relación entre la eficiencia de uso de P y la eficiencia de absorción (A) y utilización de P (B). El ajuste significativo ($p < 0,05$) promedio de la respuesta de las WCAcc se indica con la línea continua. Barras verticales y horizontales indican error estándar de la media.

le range, whereas, WCAcc1 was the most efficient in both of the evaluated P availabilities, showing no difference compared to the control cv. Will.

Comparison of PUE under glasshouse and field conditions

The result show that the value of PUE under both evaluation conditions, presented significant correlation in both level of P availability in soil (r 0.61 and r 0.67) (Table 3). For its part, PUE presented significant correlation between both experimental conditions, in both P ranges evaluated (r 0.72 and r 0.88) (Table 3). Meanwhile, PUtE showed no significant correlation between experimental conditions (Table 3). The differences in PUE between both experiments (glasshouse and field) were of grater magnitude on high levels of P in soil (Figure 7a, b). Meanwhile, on glasshouse con-

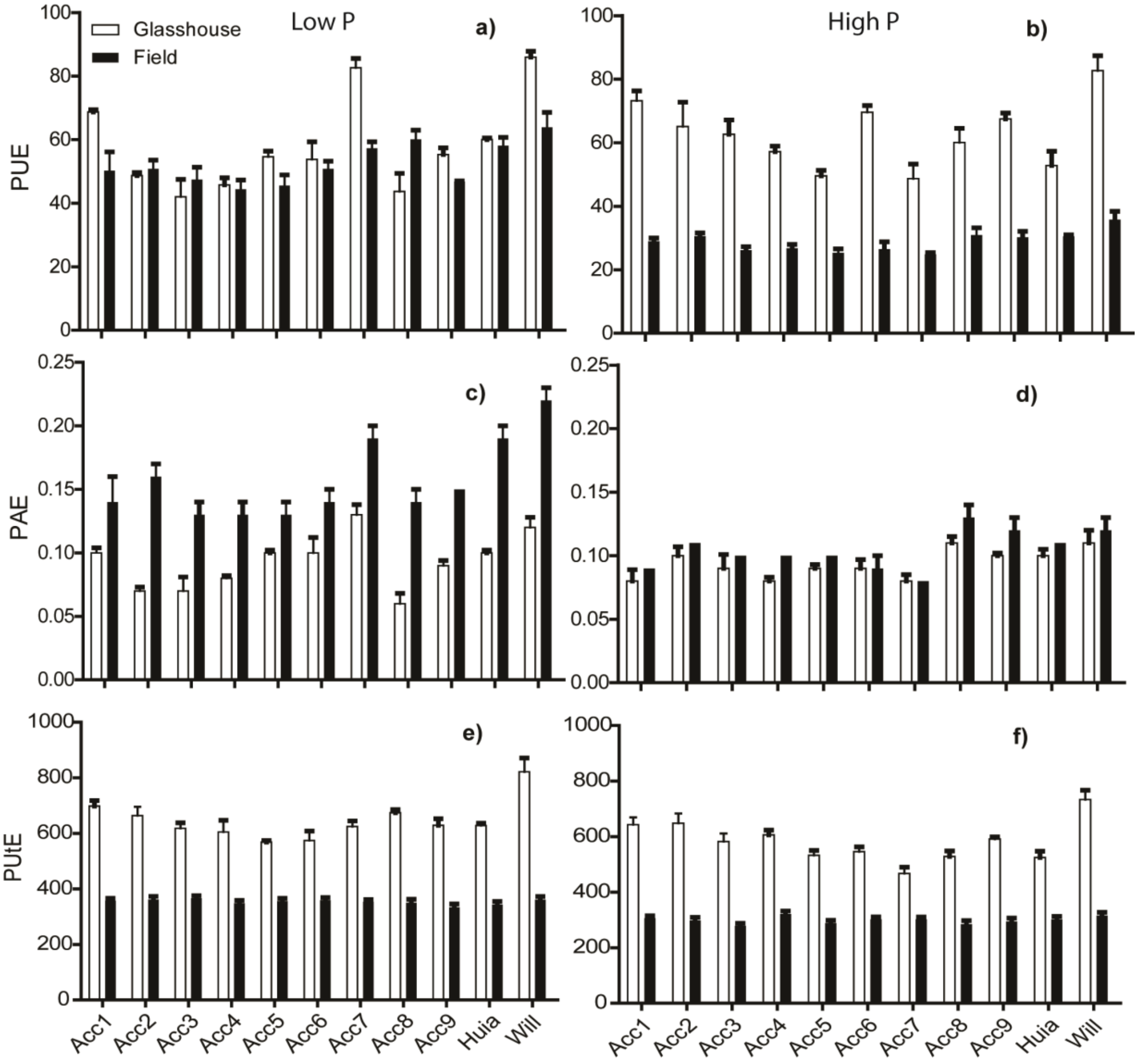

Figure 7. Comparison of the results found in glasshouse versus field conditions. PUE in Low P (a), PUE in High P (b), PAE in Low P (c), PAE in High P (d), PUtE in Low P (e) and PUtE in High P (f). White bars represent results under glasshouse and black bars represent adapted results in field. Low P in glasshouse correspond to results in the range of 4-15 mg P-Olsen kg^{-1} and High P correspond to result in the range of 15-27 mg P-Olsen kg^{-1}. The field experiment corresponds to the adapted graph made by Acuña and Inostroza (2013).

Figura 7. Comparación de los resultados obtenidos bajo invernadero versus en condiciones de campo. PUE en bajo P (a), PUE en alto P (b), PAE en bajo P (c), PAE en alto P (d), PUtE en bajo P (e) y PUtE en alto P (f). Las barras blancas representan los resultados bajo invernadero y las barras negras representan los resultados de campo. Los valores de bajo P en invernadero corresponde a resultados en el intervalo de 4-15 mg de P-Olsen kg^{-1} y los de Alto P corresponden al resultado en el intervalo de 15-27 mg de P-Olsen kg^{-1}. El experimento de campo corresponde los resultados adaptados de Acuña y Inostroza (2013).

dition the WCAcc were similar or more efficient in PUE than the control cv. Huia, but only WCAcc 7 were not different in PUE in low P, with respect to control cv. Will. On the other hand, the component of PUE (PAE and PUtE), presented different response between experimental condition (Fig. 7 c, d ,e ,f). PAE in low P, was higher in field conditions, but did not present differences between experiments, in high level of P (Figure 7 c, d). Moreover, the PAE in soils with high P availability only decreased significantly ($p < 0.05$) under field conditions. While, PUtE, in average was higher in glasshouse conditions ($p < 0.05$), in both ranges of P in soil (Figure 7 e, f).

By its side, the PUtE under field conditions presented fewer differences among the accessions compared to the glasshouse conditions (Table 3 and Figure 7 e, f). In both conditions and ranges of P availability, the accessions were similar or more efficient to the control cv. Huia ($p > 0.05$). However, in both experimental conditions the P utilization efficiency decreased with the increasing P availability (Table 3 and Fig. 7 e, f).

DISCUSSION

Under glasshouse conditions, control cv. Will presented the greatest P response to the P availabilities evaluated (4-27 mg P-Olsen kg^{-1}). Of the 9 naturalized white clover accessions assessed, five of them (WCAcc 1, 4, 5, 6 and 8) responded to the increase in P availability similarly to the control cv. Will. Furthermore, this variation was strongly explained by the WCAcc *x* P interaction, which further supports our hypothesis that the accessions will generate adaptation mechanisms to face the limiting P conditions, as well as in other germplasm collections (Caradus and Snaydon, 1986; Lloyd *et al.*, 2014). In addition, based in the hypothetical P response proposed by Gourley *et al.* (1994), all accessions would be moderately to highly efficient in using P (Figure 1 and Figure 4).

The variation in P response supports the differences found in the evaluated accessions PUE. Based on the yield potential definition of Evans and Fischer (1999), PUE under glasshouse conditions could be considered as a potential assessment for white clover due to weather (temperature, radiation and humidity) and soil (root exploration) conditions, since these conditions were not a limiting factor in terms of the expression of a mechanistic response to P availability. Regarding this, the mechanism expressed by the accessions seems to relate to acquisition and P uptake, which was observed in the strong association between PUE and PAE, compared to PUtE (Figure 5). More specifically, the adaptative morphophysiological mechanisms, such as rhizospheric effects (Hinsinger, 2001), increased of root/root hairs density (Caradus, 1981) and mycorrhizal association (Smith and Read, 2008) would be most important under low P availability, however, the radical components were not measured in this study.

Though there was no association between PUE and PUtE, the low PIC in all of the accessions (0.9 and 2.4 %) could be considered an internal physiological mechanism (PUtE) of adaptation (Lambers *et al.*, 2010). This could be based on the fact that plants maintain the minimum PIC necessary for the metabolic process that occur in the leaf (Hart and Jessop, 1983) by promoting internal mobilization of P from inactive or reserve tissues (internal P pool) to growing tissues (Akhtar *et al.*, 2008; Veneklaas *et al.*, 2012) post defoliation (biomass cuts). However, this could cause problems in terms of the forage's nutritional quality.

All of the evaluated accessions were more efficient in the low-middle range of P availability (Figure 6) compared to the middle-high range. This behavior could indicate the adaptative plasticity of the accessions in response to the different P availabilities in the soil. This adaptative plasticity is a result of the morpho-physiological mechanisms (Mackay, 1991) which have developed from the interaction between a plant and its environment over time (Baligar and Fageria, 2015; Caradus and Snaydon, 1986; Crush and Caradus, 1995; Gourley *et al.*, 1993; Gourley *et al.*, 1994). In this study the main adaptation would be physiological, being associated to a greater efficiency of internal use of P of the WCAcc, generating greater biomass per unit of P absorbed in conditions of low P.

Comparison between glasshouse and field conditions

The magnitude of expression in the parameters evaluated (PAE and PUtE) was the principal difference among experimental evaluation techniques (Figure 7).

In contrast with other studies (Caradus and Dunn, 2000), in this comparison, PUE under glasshouse conditions was confirmed by the response found under field conditions. However, of the PUE components (PAE and PUtE) only PAE presented correlation between both experiments, this is could be attributed, in general terms, to: (i) the large volume of soil (0.025 m^3 per pots) provided for the exploration and development of axial roots and root hairs under glasshouse conditions, which promotes P uptake (Lynch, 2007), (ii) the utilization of natural grassland soils for the treatments, which could stimulate the contribution of microorganisms to the indirect rhizospheric activity (Manschadi *et al.*, 2014) and (iii) evaluating the parameters for a full season of growth, allowing the expression of the adaptative mechanisms after each biomass cut. By it side, the non-correlation for PUtE between the two experiments would be mainly attributable to the competition between white clover and perennial ryegrass, which existed under field conditions, which affects the interception capacity of radiation causing a lower production of biomass for each unit of P that was uptaked (Woodfield and Caradus, 1996).

It is possible to think that when an experimental glasshouse design properly simulates field conditions, the evaluation in the glasshouse could allow for greater advances in the selection and evaluation of germplasm P efficient. However, it is necessary to determine the main factors that prevent the expression of the potential use efficiency in field conditions, either the absorption or the utilization of the evaluated nutrient, since this way there would be more consistency in the results when comparing between glasshouse and field conditions.

CONCLUSIONS

PUE and their components, PAE and PUtE, were different among the WCAcc evaluated; furthermore, all of the accessions presented a positive response to increasing P levels in the soil. Moreover, PUE was mainly explained by the absorption efficiency of P (PAE) in the range of P evaluated. Finally, the results obtained in the glasshouse were validated by those obtained under field conditions; however, the magnitude of expression was different among experimental techniques.

ACKNOWLEDGEMENTS

We thank the "Fondo Regional de Tecnología Agropecuaria (FONTAGRO)" for its financial contribution to this research through Project N° 787 2005. We greatly thank to Ruth Espinoza for technical assistance in this thesis.

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