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# Root architecture and morphology of advanced synthetic lines developed by the red clover breeding program of INIA-Chile

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## Introduction

Red clover (*Trifolium pratense* L.) is one of the most important forage legumes in temperate regions of the world. In Chile, it is the only forage species with a breeding program that has been continue over decades and produced commercial cultivars. Because of the good seed and forage production potential, red clover cultivars released by the INIA breeding program (INIA-BP) are commercialized both in Chile and abroad. The main limitation of the species worldwide is its low persistence, determined by the high plant mortality due to the deterioration of the main root and crown (Ortega *et al*., 2014). In the last 26 years, the red clover INIA-BP has improved the productivity and persistence of the species, achieving high genetic gains (Ortega *et al*., 2014). However, selection methodology until now has not considered consistently phenotyping of roots, even though root architecture and morphology could be related to plant adaptation as described for other species. Therefore, the objective of this work was to characterize the root architecture and morphology of seven advanced synthetic lines (ASLs) of red clover developed by INIA-BP and four commercial cultivars.

## Materials and Methods

The experiment was carried out under glasshouse conditions at the Instituto de Investigaciones Agropecuarias (INIA) in Chillán, Chile (36°34´S; 72°06´W), between October-2013 and May-2014. A total of 11 populations were studied, considering four commercial cultivars and seven ASLs. The commercial cultivars were: Quiñequeli INIA and Redqueli INIA (bred in Chile), Starfire (bred in USA) and Tuscan (bred in NZ). The ASL were: Si4G2, SIIi4, SIIi5, SIIi6, SIIP1, SP3G2 y SP3G3. Individual plants of each population were established on mesocosms of PVC tubes of 11 cm of diameter and 100 cm depth, containing as substrate a mixture (v/v) of fine sand (50%), vermiculite (35%), soil (10%) and perlite (5%). The trial was organized in a complete block design with four replicates, each replicate consisting of 5 mesocosms per population and one plant per mesocosm. Before sowing, each mesocosm was fertilized with 1.6 L of nutritive solution described by Zhu *et al.* (2010); during the trial they were fertilized periodically with a solution of 10 g $L^{-1}$ of Phostrogen (Bayer, Newbury, UK). Irrigation water was delivered daily using 2.0 L $h^{-1}$ drippers. Environmental temperature and relative moisture were registered hourly with a sensor Hobo (H08-032-IS, Onset). The temperature and volumetric moisture content of the substrate were registered with capacitance sensors (5TE, Decagon). Evaluations were: 1- Shoot (leaves+stems) yield (ShootDM) in three oportunities (93, 147 and 193 days after sowing, DAS); 2- Chlorophyll content, evaluated weekly from emergency up to 93 days in four leaflets per mesocosm with a SPAD meter (Minolta); 3- Leaf area (Larea), leaf dry weight (LDW) and specific leaf area (SLA=Larea/LDW) before each shoot DM evaluation, sampling 5 fully expanded leaves (PEL) from the upper third of each plant; 4- Root characterization, after the last shoot sampling, roots were carefully washed and maintained in individual sealed bags at 4°C with a solution of ethanol 70% v/v a 4°C for digitalization (Canon EOS Rebel T5i) and imagine analysis of crown diameter (Dcro), average tap root diameter (Dtap), structural roots diameter (Dstr=primary+secondary), external path length (EPL=number of link or segment of whole root system), and relative length density (RLD=cm root/$cm^3$ of soil), all of them in the first 20 cm of the mesocosm. All the morphological and structural root traits were estimated with WinRhizo Pro software (Regent Instrument Inc.). Dry matter yield of shoots and roots was obtained at 65° until sample weigh was at equilibrium. Results were analyzed by ANOVA and means were separated by LSD test (p=5%); also, correlations were estimated between variables and principal component analysis to study the relationship between variables and populations. All statistical analyses were performed in R software (http://www.r-project.org/).

## Results and Discussion

The red clover populations showed morphological differences in its aerial parameters. The Larea and SLA varied significantly between populations ($P<0.05$). The ASL SIIi5 and the cv Tuscan showed the lowest values of Larea (120 $cm^2$) and the highest values of SLA (490 $cm^2 g^{-1}$). The Chilean cultivars and the rest of the ASLs showed statistically similar values of Larea and SLA (Larea=170 $cm^2$ y SLA=420 $cm^2 g^{-1}$ on average). Chlorophyll content increased lineally in all the populations up to 93 DAS. The ASLs SIIP1 and SP3G3 showed the highest chlorophyll increase (0.42 SPAD $day^{-1}$) and were superior to the cultivars Quiñequeli INIA and Redqueli INIA. Dry matter yield and biomass partition was statistically different between populations. The ASL SIIi5 and Tuscan showed the lowest ShootDM, rootDM and DMroot: DMshoot relationship. All the characters describing root morphology and architecture were also different between populations ($P<0.05$). For instance, the RLD varied between 0.10 (Tuscan) and 0.21 cm $cm^{-3}$ (SIIi6); the EPL varied between 503.2 (Tuscan) and 1173.8 link per root (SIIi4). The PCA showed that Dtap, RLD, EPL and RootDM were significantly correlated with ShootDM (Figure 1). The SLA was negatively correlated with ShootDM and is a trait that has been modified through breeding. The oldest Chilean cultivar Quiñequeli INIA showed the highest value of SLA and the new germplasm (from Redqueli INIA to the ASLs) has been lowing SLA (Figure 1). The ASL SIIi4, SIIi6 and SP3G2 showed the highest value of shootDM joint to the highest value of EPL, RLD and Dtap. On the other hand, the ASL SP3G3 also showed a high value of shootDM but with the highest value of chlorophyll content (SPAD) and root dry mater production and partitioning (Fig. 1).

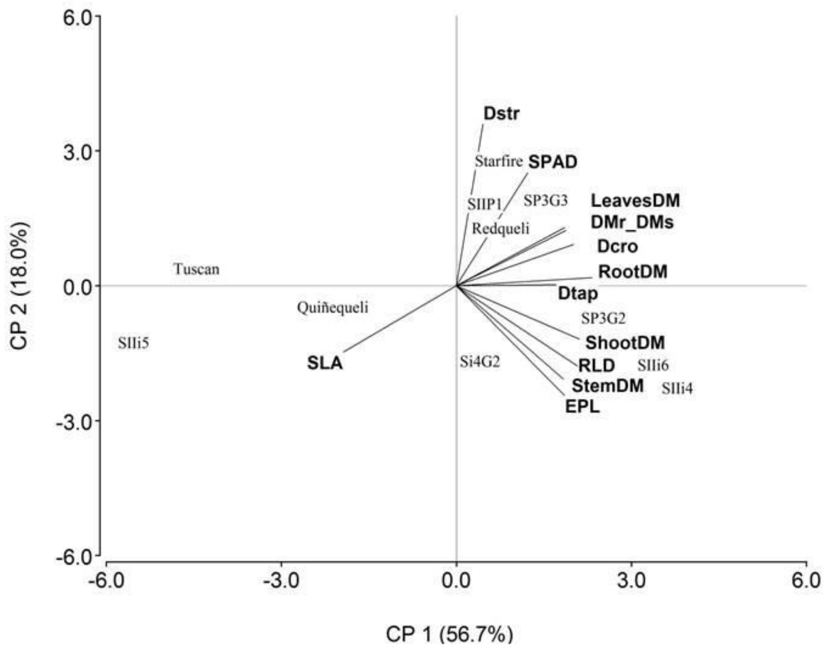


**Fig 1:** Biplot of the first two principal components (PC1 and PC2) for the principal component analysis of 12 traits evaluated in 11 red clover populations. The traits are: specific leaf area (SLA), external path length (EPL), stem dry mater (StemDM), relative length density (RLD), shoot dry mater (Shoot DM), tap root diameter (Dtap), root dry mater (RootDM), crown diameter (Dcro), DMroot:DMshoot relationship (DMr_DMs), leaves dry mater (LeavesDM), chlorophyll content (SPAD) and structural roots diameter.

## Conclusion

The red clover populations studied showed broad difference in the morphology and architecture of its root system. The RLD and EPL are architecture root traits highly associated with forage dry mater production in red clover.

## References


Ortega, F., L. Parra and A. Quiroz. 2014. Breeding red clover for improved persistence in Chile: a review. *Crop and Pasture Science* 65: 1138-1146.

Zhu, J., K. M. Brown and J. P. Lynch. 2010. Root cortical aerenchyma improves the drought tolerance of maize (*Zea mays* L.). *Plant, Cell and Environment* 33: 740-749.


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