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LEAF VEIN DENSITY AND PHOTOSYNTHETIC RATE IN *ROSA*: IS THERE A CORRELATION?

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Summary: A thorough knowledge of the leaf vascular network and its evolution is essential for understanding the fluid dynamics of water movement in leaves. Recent studies have shown how these networks can be involved in the performance of photosynthesis, which is linked to the density of the vascular network per unit of leaf area. The aim of this study is to highlight the correlation between leaf vein density (V_D) and physiological parameters. The hypothesis proposed was tested on the leaves of the "Isabel Renaissance" (IR) rose cultivar. The main result concerned the V_D, which was significantly correlated with the maximum leaf net photosynthetic rate (P_N). This finding supports the hypothesis that the vascular system of IR leaves can be correlated with leaf photosynthesis performance.

Key words: Bundle sheath extensions, leaves, net photosynthetic rate, vein density, leaf temperature.

Resumen: Densidad de venas foliares y la tasa de fotosíntesis en *Rosa*: ¿Existe una correlación? Un conocimiento profundo de la red vascular de la hoja y su evolución es esencial para la comprensión de la dinámica de fluidos del movimiento del agua en las hojas. Estudios recientes han demostrado cómo estas redes pueden estar implicadas en el rendimiento de la fotosíntesis, que está vinculada con la densidad de la red vascular por unidad de área de la hoja. El objetivo de este estudio es poner de relieve la correlación entre la densidad de venas de la hoja (V_D) y parámetros fisiológicos. La hipótesis propuesta fue probada en las hojas del cultivar de rosa "Isabel Renaissance" (IR). El resultado principal referido a V_D, se correlacionó significativamente con la tasa de fotosíntesis neta máxima de la hoja (P_N). Este hallazgo apoya la hipótesis de que el sistema vascular de las hojas de IR se puede correlacionar con el rendimiento de la fotosíntesis de la hoja.

Palabras clave: Extensiones de la vaina del haz, hojas, tasa de fotosíntesis neta, densidad de venas, temperatura foliar.

INTRODUCTION

A fundamental physiological aspect, which is shared among living organisms, is a high branching and structural complexity of the vascular architecture of leaves (Parsons-Wingerter, & Wickerman, 2011; Sack *et al*., 2012). The structure of the xylem, which is able to increase hydraulic efficiency and maintain water column continuity, is fundamental for the survival of plant species. However, the factors that have driven this improvement are still under study. The vascular architecture of a leaf has two main functions: to transport nutritive substances and to provide mechanical support. Water, solutes and hormones pass through the xylem, while carbohydrates (which are produced in the leaf) are conducted via the phloem (Roth-Nebelisck *et al*. 2001). The vein network plays a fundamental role in defining the maximum limit of photosynthetic efficiency (Brodribb *et al*., 2007; Sack & Sccofoni, 2013). Recent studies have highlighted how vein density (V_D) per unit area and mesophyll thickness determine the efficiency of the hydraulic system (Brodribb *et al*., 2007). As observed by Brodribb *et al*. (2007), V_D (defined as the total length of veins per unit area, Price *et al*., 2014) is closely associated with the rate of photosynthesis. V_D can be correlated with the distance that the water must travel through the mesophyll. Furthermore, a novel study highlighted how the V_D may be involved in the efficiency of leaf cooling (Pagano & Storchi,

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2015). However, the factors that have led to these results are still under study. The aim of this study is to highlight the correlation between the leaf vein density (V_D) and net photosynthetic rate (P_N).

Materials and Methods

Abbreviations used: *E* – transpiration rate; g_s – stomatal conductance; LA – leaf area; LED – light emitting diode; P_N – net photosynthetic rate; ppm – parts per million; RH – relative humidity; V_D – leaf vein density.

The research was carried out on one cultivar of *Rosa* L. (*R.* x *anglais* cv. Isabel Renaissance). Three plants were used for the analyses conducted in the laboratories of the CREA research centre located in Arezzo - Italy (N 43° 48', E 11° 87'), which were exposed to the following environmental condition: 23.50 °C, 51.10 % of relative humidity. Ten leaves from each plant were randomly selected from the main shoots for the gas exchange measurements listed in Table 1. Leaf gas exchange measurements were performed with a CIRAS-I portable photosynthesis system (PP Systems, Hitchin), when the leaves were still attached to the plant. CIRAS determinations were made between 10:00 and 11:00 AM. The reference CO_2 concentration and the relative humidity (RH) in the CIRAS device were set at 400 ppm and 70 %, respectively. Furthermore, the same leaves were temporarily placed in a refrigerated chamber at 4°C for 24 h before image acquisition in order to keep them fresh for the image analysis. The images were obtained using an EOS 700D camera (Canon) with an EF 100 mm f/2.8 Macro USM objective (Canon) and a Dinolite digital microscope (AM-313T5, AnMo Electronics Corporation, Hsinchu) placed over a LED illumination panel. The leaves were flattened in order to achieve maximum focus during image acquisition. After capturing the shape of the leaf on camera, the vein image was acquired with a Dinolite microscope at 230x magnification. DinoXcope 1.9.9 (AnMo Electronics Corporation) software was used to identify the V_D. The microscope was set vertically to the sample and each image was acquired with the best focus obtainable with DinoXcope software. Image acquisition was performed by three rectangles of 1 mm^2 areas into a virtual grid. The rectangles were drawn to include at least two 2° order of veins. We subsampled three rectangular boxes for each leaf (according to Sack *et al.*, 2012). The boxes represented typical fragments and were located between the midvein and margin. The images were processed with Fiji 1.50i software (ImageJ, National Institutes of Health) which enables the user to measure the physical parameters that characterize the leaf network. V_D data on the major veins were collected from the images manually. The image analysis was performed on the same leaves used for the gas-exchange measurement. The V_D (measured from veins visible from their bundle sheath extensions) was measured using ImageJ segmented line tool on the images collected.

Table 1. Physiological parameters measured in "Isabel Renaissance" cultivar of *Rosa*

Parameters	Mean ± SD
P_N (µmol CO_2 m^{-2} $s^{-1)}$	5.69 ± 1.04
g_s (mmol H_2O m^{-2} s^{-1})	156.75 ± 36.70
E (mmol H_2O m^{-2} s^{-1})	2.18 ± 0.21
PAR (µmol m^{-2} s^{-1})	393.17 ± 13.76
LA (cm^2)	13.37 ± 3.89
V_D (mm mm^{-2})	3.08 ± 0.63

Statistical analysis

Statistical analyses were performed with Prism v. 5.0 (GraphPad software San Diego, California). In particular, we tested for differences in correlation between net photosynthesis and V_D (Fig. 1) and net photosynthesis vs leaf temperature (Fig. 2) in order to determine the best fit. The best agreement was observed using a nonlinear regression (second order polynomial; GraphPad Prism 5.0). The principal component analysis (PCA) was carried out with PAST software (http://folk.uio.no/ohammer/past).

Results

The plants were characterized by the following density/area of veins per unit: 3.08 ± 0.63 mm

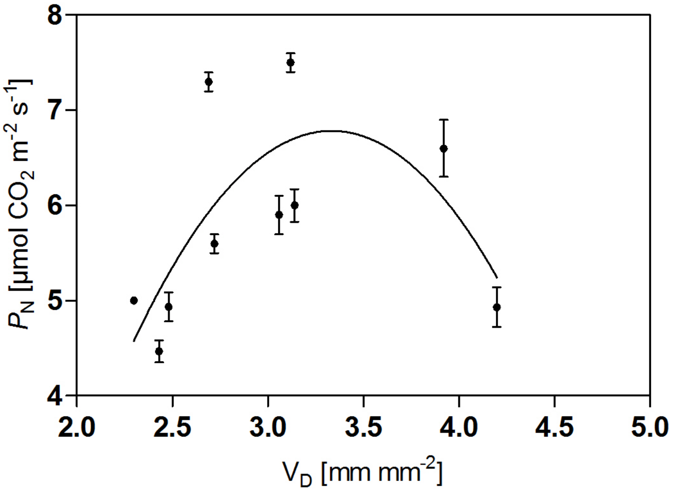

Fig. 1. Relationship between V_D and P_N. Each point into the graph contains an average of three crops and three CIRAS measurement (± Standard Deviation, $r^2 = 0.50$, $p<0.05$).

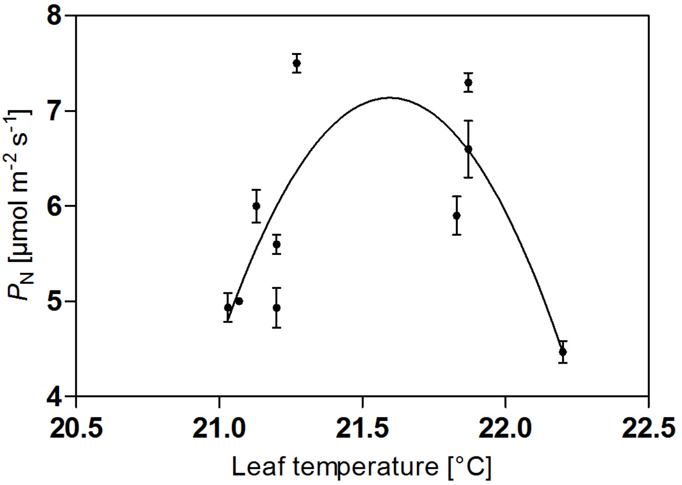

Fig. 2. Relationship between leaf temperature and P_N. Each point into the graph contains an average of three crops and three CIRAS measurement (± Standard Deviation, $r^2 = 0.58$, $p<0.05$).

mm^{-2}. Furthermore, maximum P_N correlated with the V_D (mm mm^{-2}), thus confirming its positive link with the density (Fig. 1). Each point in the correlation graph contains an average of three crops (30 measurements) and three CIRAS measurements. The IR showed the following statistical mean values ± SD: P_N [5.69 ± 1.04 µmol m^{-2} s^{-1}], stomatal conductance (g_s) [156.75 ± 36.70 mmol (H_2O) m^{-2} s^{-1}] and transpiration (*E*) [2.18 ± 0.21 mmol (H_2O) m^{-2} s^{-1}], photosynthetic active radiation (PAR) [393.17 ± 13.76 µmol (photon) m^{-2} s^{-1}] and leaf area (LA) of [13.37 ± 3.89 cm^2] (see Table 1). Finally, the PCA graph shows a correlation between leaf temperature, V_D and the P_N maximum (Fig. 3) in mature leaves of the IR cv.

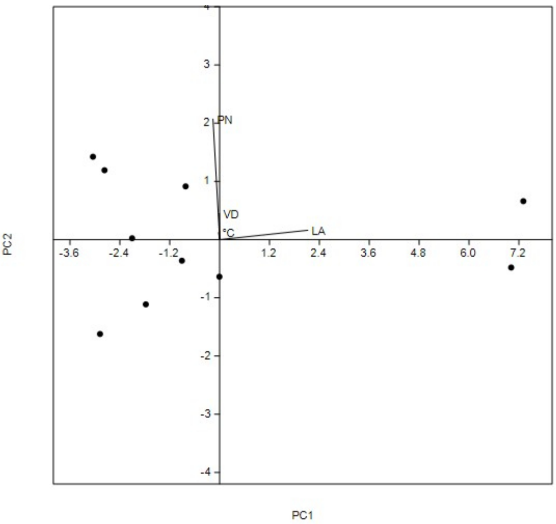

Fig. 3. PCA analysis of P_N, V_D, leaf temperature (°C) and LA. Each point into the graph contains an average of three crops and one CIRAS measurement (PC1 91.54%; PC2 6.24%).

DISCUSSION

The aim of the study is to highlight the possible correlations between leaf anatomic architecture with physiological parameters. The vein network of the IR cultivar analysed was characterized by a lower mean density/area of vein per unit when compared to other species such as *Vitis vinifera* L.: Sangiovese cv (4.98 ± 0.51 mm mm^{-2}, $P<0.05$) and Montepulciano cv (5.70 ± 0.40 mm mm^{-2}, $P<0.05$) (Pagano *et al.*, 2016). A higher density of the vascular system plays an important role in water conduction (Sack & Holbrook, 2006; Zwieniecki *et al.*, 2007) and in biomechanical support of the leaf. A significantly positive correlation was observed between V_D and maximum P_N. The relationship shown in figure 1 is not linear: after one point of the V_D the curve of the P_N no longer increases. In fact, the nonlinear relationship probably means that the saturation point has reached its maximum rate beyond which the P_N decreases. This result supports the hypothesis that the vascular system is directly linked to P_N performance. We are aware that the photosynthesis can be influenced by leaf age and various environmental factors such as temperature, CO_2, light, drought stress, cultivar genotype (Crafts-Brandner & Salvucci, 2000; Palliotti *et al.*, 2009), but we also believe that V_D may play a fundamental role in P_N performance. As regards to the effect of temperature, Lieth observed that temperatures (Cara Mia cv) above 30 °C caused a sharp decline in net photosynthesis and that the photosynthetic process was totally interrupted in very high temperatures (Lieth & Pasian, 1990). Similarly, the decline in net photosynthesis became very pronounced above 21.5 °C (Fig. 2). The PCA graph (Fig. 3) shows that the leaf area was not correlated with the other parameters. In fact, the PCA supports the hypothesis that only P_N, V_D and leaf temperature (°C) are associated. This finding supports the hypothesis that the vascular system in IR cv leaves can be correlated with leaf photosynthesis performance. Other studies should be carried out to determine which correlation exists between stomata density per unit and vein density and how they are linked to transpiration (Brodribb, 2009). The image analysis could also be used for the characterization of the V_D in order to determine the variety. In conclusion, the main goal of this study was to determine that the P_N maximum correlated with the V_D in IR cv.

BIBLIOGRAPHY

BRODRIBB, T. J. 2009. Xylem hydraulic physiology: The functional backbone of terrestrial plant productivity. *Plant Sci*. 177: 245-251.

BRODRIBB, T. J., FIELD, T. S. & G. J. JORDAN. 2007. Leaf Maximum Photosynthetic Rate and Venation Are Linked by Hydraulics. *Plant Physiol.* 144: 1890-1898.

CRAFTS-BRANDNER, S. J. & M. E. SALVUCCI. 2000. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO2. *Proc. Natl. Acad. Sci. USA* 97: 13430-13435.

LIETH, J. H. & C. C. PASIAN. 1990. A Model for Net Photosynthesis of Rose Leaves as a Function of Photosynthetically Active Radiation, Leaf Temperature, and Leaf Age. *J. Amer. Soc. Hort. Sci.*115: 486-49.

McCLENDON, J. H. 1992. Photographic survey of the occurrence of bundle-sheath extensions in deciduous dicots. *Plant Physiol.* 99: 1677-1679.

PANTIN, F., MONNET, F., JANNAUD, D., COSTA, J. M., RENAUD, J., MULLER, B., SIMMONNEAU, T. & B. GENTY. 2013. The dual effect of abscisic acid on stomata. *New Phytol.* 197: 65-72.

PAGANO, M., CORONA, P. & P. STORCHI. 2016. Image analysis of the leaf vascular network: physiological considerations. *Photosynthetica*. DOI: 10.1007/s11099-016-0238-2.

PAGANO, M. & P. STORCHI. 2015. Leaf vein density: a possible role as cooling system. *J. Life Sci.* 9: 299-303.

PALLIOTTI, A., SILVESTRONI, O. & D. PETOUMENOU. 2009. Photosynthetic and Photoinhibition Behavior of Two Field-Grown Grapevine Cultivars under Multiple Summer Stresses. *Amer. J. Enol. Vitic*. 60:189-198.

PARSONS-WINGERTER, P. & M. B. VICKERMAN. 2011. Informative Mapping by VESGEN Analysis of Venation Branching Pattern in Plant Leaves Such as Arabidopsis thaliana. *Gravitat. Space Biol*. 25: 69-71.

PRICE, C., MUNRO, P. R. T. & J. S. WEITZ. 2014. Estimates of leaf vein density area scale dependent. *Plant Physiol*. 164: 173-180.

ROTH-NEBELISCK, A., UHL, D., MOSBRUGGER, V. & H. KERP. 2001. Evolution and function of leaf venation architecture: a review. *Ann. Bot. (Oxford, U. K.)*. 87: 553-566.

SACK, L. & N. M. HOLBROOK. 2006. Leaf hydraulics. *Annu. Rev. Plant Biol*. 57: 361-381.

SACK, L., SCOFFONI, C., MCKOWN, A. D., FROLE, K., RAWLS, M., HAVRAN, J. C., TRAN, H. & T. TRAN. 2012. Developmentally based scaling of leaf venation architecture explains global ecological patterns. *Nat. Commun.*, DOI: 10.1038/ncomms1835.

SACK, L. & C. SCOFFONI. 2013: Leaf venation: structure, function, development, evolution, ecology and applications in the past, present and future. *New Phytol.* 198: 983-1000.

ZWIENIECKI, M. A., BRODRIBB, T. J. & N. M. HOLBROOK. 2007: Hydraulic design of leaves: insights from rehydration kinetics. *Plant Cell Environ*. 30: 910-9210.

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