



BRIEF COMMUNICATION

Variations in the photoprotective responses of trees and seedlings of the endangered *Pitavia punctata* (Ruiz & Pav.) Molina

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Abstract

Pitavia punctata (Ruiz & Pav.) Molina ('Pitao') is an endangered species that regenerates under the shade. We monitored the photochemical efficiency of photosystem II in four localities spanning the geographical distribution of the species in Central Chile. Technical fluorescence parameters and JIP-test-derived parameters were measured in trees (up to 15 m) and seedlings (< 50 cm). We observed significant effects of the type of plant and locality on the performance index (PI_{ABS}) and maximum fluorescence (F_M). PI_{ABS} was higher in trees from the Los Barros locality ($PI_{ABS} = 41$), whereas seedlings located in Colcura locality had $PI_{ABS} = 26$. Seedlings exhibited higher F_M compared to trees ($F_M = 940$ and 895 for seedlings and trees, respectively), particularly in locality Ramadillas ($F_M = 1,025$). Our results provide information on a topic largely understudied in native species in Chile and are useful for designing and monitoring restoration processes for *P. punctata*.

Keywords: chlorophyll *a* fluorescence; performance index; photochemical efficiency of photosystem II; Pitao.

Pitavia punctata (Ruiz & Pav.) Molina ('Pitao') is an endemic and endangered tree species considered a Natural Monument in Chilean legislation (Ministerio de Agricultura 1995, Rivera Caniulao 2021). *P. punctata* is found growing in wet shady sites and ravines and is part of a sclerophyllous vegetation where *Peumus boldus* Molina, *Lithrea caustica* Hook. et Arn., and *Cryptocarya alba* (Molina) Looser are the dominant species. It is also present in coastal temperate forests dominated by

Nothofagus glauca (Phil.) Krasser (Rodríguez *et al.* 1983, Le Quesne and Medina 1998). The range of distribution of the species spans from 35° to 37°S in Central Chile in an area considered a biodiversity hotspot of global importance (Arroyo *et al.* 2008). This area is characterized by its Mediterranean-type climate in which newly established seedlings must cope with different types of environmental stresses, including summer water stress, combined with heat and light stress (Martínez-Ferri *et al.*

Highlights

- Trees and seedlings of *P. punctata* were monitored for chlorophyll *a* fluorescence
- Photoprotective responses were measured in the natural habitat of the species
- PI_{ABS} was higher in trees, and F_M was higher in seedlings

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Abbreviations: ABS/RC – effective antenna size of an active reaction centre (RC); F_0 – minimal fluorescence from a dark-adapted leaf; F_M – maximal fluorescence from a dark-adapted leaf; F_V – maximal variable fluorescence from a dark-adapted leaf; F_V/F_0 – ratio between variable and minimal fluorescence; F_V/F_M – maximum quantum yield of primary PSII photochemistry; PI_{ABS} – performance index on absorption basis; S_m – normalized area above the OJIP transient; ΔV_{IP} – efficiency with which a PSII trapped electron is transferred to final PSI acceptors; ψE_0 – the probability that a photon trapped by the PSII reaction center enters the electron transport chain.

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2000). Light stress affects the photosynthetic capacity of plants by exposing them to an excess of absorbed energy that cannot be fully utilized in photochemical reactions, *i.e.*, an imbalance between the generation of electrons and their utilization (Tausz *et al.* 2004), which causes considerable changes in the quantum yield of PSII. The chlorophyll *a* fluorescence emission analysis of PSII has become a widely used and suitable technique to assess stress conditions on a photosynthetic basis (Swoczyna *et al.* 2022).

The establishment of *P. punctata* seedlings occurs naturally in shaded sites. However, as they grow and reach height, older stages gradually receive more light. The photosynthetic response of this transition has been frequently studied in species of the genus *Nothofagus* (*e.g.*, Coopman *et al.* 2008, 2010), which are present in the ecosystem of *P. punctata*, and in other species (Niinemets 2010). In contrast, many aspects of the photosynthetic apparatus involved in the physiological adaptations of *P. punctata* to Mediterranean-type climates are largely unknown. In this observational study, we aimed to describe the main physiological variables involved in the photochemical efficiency of PSII in trees (up to 15 m of height) and seedlings (< 50 cm of height) of *P. punctata* naturally growing in different sites within the species' natural range in Central Chile.

This study was implemented in naturally grown trees and seedlings of *P. punctata* distributed across four localities in Central Chile. A total of 61 individuals were sampled across localities (32 trees and 29 seedlings). The localities represented the natural geographic distribution of the species and were Ramadillas (36°S, 72°W), Los Barros (37°S, 72°W), San Ramón (35°S, 72°W), and Colcura (37°S, 73°W). San Ramón has a mean annual precipitation (MAP) and temperature (MAT) of 956 mm and 12.7°C, respectively. The microsite corresponds to a flat and swampy area with high disturbance (assessed by visual inspection of the general state of natural vegetation, presence and development of the forest, invasive species, signs of fires, and damage to vegetation); we sampled 8 trees and 9 seedlings. In Ramadillas (9 trees and 6 seedlings sampled), MAP and MAT are 1,106 mm and 14.5°C. The microsite is close to a stream and has low disturbance and the presence of adult individuals of *Nothofagus glauca* (Phil.) Krasser. In Colcura, MAP and MAT were 1,414 mm and 13.2°C. The individuals (5 trees and 11 seedlings) were sampled in a deep ravine with a stream, and the microsite had medium disturbance. In Los Barros, MAP and MAT were 788 mm and 13.7°C. The microsite had low disturbance and the presence of adult individuals of *Nothofagus dombeyi* (Mirb.) Oerst. The individuals (7 trees and 9 seedlings) were sampled in a flat area close to a stream.

Chl *a* fluorescence transient of intact, healthy, and fully expanded leaves of trees and seedlings of *P. punctata* were measured in the four localities at 10:00 h local time with a modulated fluorimeter (*OSp30+*, Opti-Sciences, USA) set for the JIP-test protocol. The measurements were performed on three leaves per individual (and then averaged for analyses) from 9 to 12 January 2024

(summertime in the Southern Hemisphere). The leaves of each plant were dark-adapted in clips for 30 min before measurements, and later, Chl *a* fluorescence transients of dark-adapted leaves were measured. The transients were induced by 1-s illumination providing a maximum light intensity of 3,500 $\mu\text{mol}(\text{photon})\text{ m}^{-2}\text{ s}^{-1}$. This light intensity was safe and allowed us to reach F_M , and the OJIP steps were revealed. The *OSp30+* fluorimeter measured the O (20 μs), J (2 ms), and I (30 ms) as the intermediate stage, and P (300 ms) as the peak. The data obtained were used in the JIP-test (Strasser *et al.* 2004) to calculate the parameters of PSII photochemistry. Concerning the whole list of JIP-test parameters (Strasser *et al.* 2000, 2004), we addressed our attention to the key parameters that are the major plant stress indicators (Kalaji *et al.* 2014, 2017), *i.e.*, the maximum quantum yield of primary PSII photochemistry (F_V/F_M), the normalized area above the OJIP transient (S_m), the apparent antenna size of an active PSII (ABS/RC), the ratio between variable and minimal fluorescence (F_V/F_0), the probability that a photon trapped by the PSII reaction center enters the electron transport chain (ψE_0), the efficiency with which a PSII trapped electron is transferred to final PSI acceptors (ΔV_{IP}), and the potential for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors (PI_{ABS}). Data were evaluated through variance analysis comparing the type of plant (trees and seedlings), and locality (San Ramón, Ramadillas, Los Barros, and Colcura), and the interaction between both factors. All factors were considered fixed, and significant mean values were separated by the Tukey's test ($p < 0.05$). All statistical analyses were performed with SPSS version 22.0 software (SPSS Inc., Chicago, Illinois, USA).

We detected significant variations at the level of plant type and locality for PI_{ABS} and F_M (Table 1). F_M was 4.8% higher in seedlings compared to trees, whereas at the locality level, the highest F_M were found in Colcura and Los Barros localities. The interaction between plant type and locality was mostly explained by the highest F_M of seedlings in Ramadillas locality (Fig. 1A). In the case of PI_{ABS} , the interaction between plant type and locality was associated with the responses observed in trees located in Los Barros, which consistently had the highest PI_{ABS} . On the other hand, seedlings located in Ramadillas had the lowest PI_{ABS} (Fig. 1B). S_m , ψE_0 , and ΔV_{IP} were higher in Los Barros, Ramadillas, and San Ramón localities (Table 1).

For the species *P. punctata*, there is no evidence indicating how the photoprotective system of trees or seedlings adapts to cope with the stresses typically experienced in Mediterranean-type climate areas. Most of the physiological parameters under consideration in this study were similar between trees and seedlings, which partially agrees with our expectations because seedlings were growing under the shaded understory, whereas trees received medium levels of light intensity. The ABS/RC, which is a measure of the apparent antenna size of an active PSII, was similar between trees and seedlings, suggesting a similar absorption of photons per reaction center. Likewise, the lack of differences in F_V/F_0 , considered

Table 1. Mean values and significance for the parameters of JIP-test in the study. F_0 – minimal fluorescence from a dark-adapted leaf; F_M – maximal fluorescence from a dark-adapted leaf; F_V/F_M – maximum quantum yield of primary PSII photochemistry; S_m – normalized area above the OJIP transient; ABS/RC – apparent antenna size of an active PSII; F_V/F_0 – ratio between variable and minimal fluorescence; PI_{ABS} – potential for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors; ψE_0 – probability that the energy of a trapped excitation is used for electron transport beyond Q_A ; ΔV_{IP} – the efficiency with which a PSII trapped electron is transferred to final PSI acceptors. *Different lowercase letters indicate statistically significant differences among localities or type of plant. Values in bold indicate significant p -values.*

| | F_0 | F_M | F_V/F_M | S_m | ABS/RC | F_V/F_0 | PI_{ABS} | ψE_0 | ΔV_{IP} |
|--------------------|-------|-------------------|-----------|-------------------|----------|-----------|-------------------|-------------------|--------------------|
| Locality | | | | | | | | | |
| Colcura | 184 | 944 ^a | 0.80 | 9.7 ^b | 2.1 | 4.23 | 27.1 ^b | 0.55 ^b | 0.07 ^c |
| Los Barros | 178 | 948 ^a | 0.81 | 13.1 ^a | 2.0 | 4.43 | 35.8 ^a | 0.61 ^a | 0.12 ^{ab} |
| Ramadillas | 182 | 898 ^{ab} | 0.79 | 11.9 ^a | 2.1 | 4.03 | 27.1 ^b | 0.57 ^b | 0.10 ^b |
| San Ramón | 177 | 862 ^b | 0.81 | 13.2 ^a | 1.9 | 4.32 | 35.0 ^a | 0.60 ^a | 0.12 ^a |
| Type of plant | | | | | | | | | |
| Trees | 174 | 895 ^b | 0.81 | 12.3 | 2.0 | 4.31 | 32.1 | 0.61 | 0.11 |
| Seedlings | 187 | 940 ^a | 0.82 | 11.4 | 2.1 | 4.22 | 30.1 | 0.62 | 0.12 |
| Significance level | | | | | | | | | |
| Locality (L) | 0.824 | 0.002 | 0.689 | 0.000 | 0.216 | 0.289 | 0.001 | 0.000 | 0.000 |
| Type of plant (P) | 0.135 | 0.008 | 0.239 | 0.123 | 0.074 | 0.213 | 0.239 | 0.467 | 0.113 |
| L × P | 0.837 | 0.000 | 0.322 | 0.676 | 0.074 | 0.158 | 0.025 | 0.089 | 0.145 |

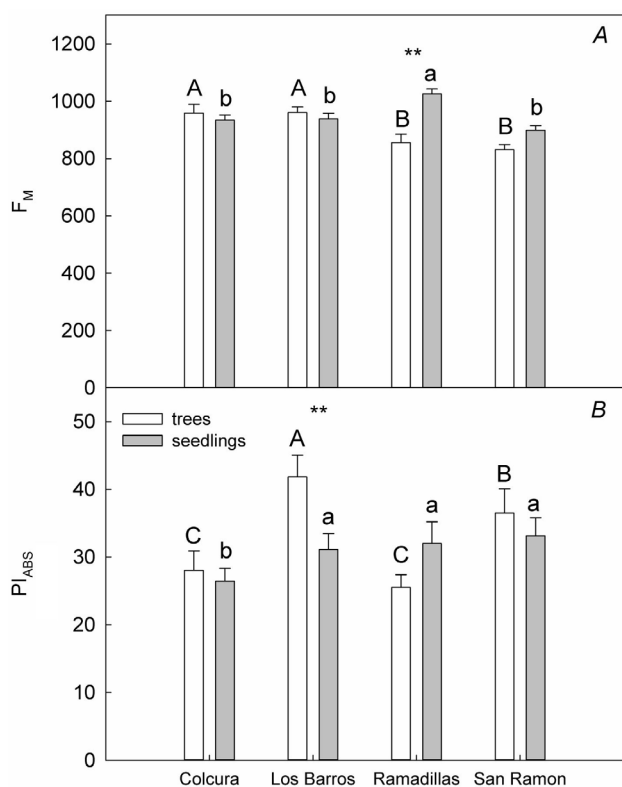


Fig. 1. Responses in Chl emissions of the different types of plants according to the locality. (A) F_M – maximum quantum yield of primary PSII photochemistry, (B) PI_{ABS} – potential for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors. Asterisks indicate significant differences for the type of plant within a locality ($p < 0.05$ for **). Capital and lowercase letters indicate differences among trees or seedlings across localities, respectively.

a proxy of heat dissipation (Tsimilli-Michael 2020), was similar in trees compared to seedlings. Moreover, F_0 was similar between trees and seedlings, but it was observed that trees had low levels of F_M . This suggests that seedlings had more reaction centers closed than trees, particularly in localities with low disturbance levels and the presence of trees higher than 30 m, which provide protection (*i.e.*, Ramadillas locality). This may indicate a low interruption of electron transport in the reaction centers in the seedlings evaluated (Yamada *et al.* 1996). Lower F_M values in trees suggest the presence of photoinhibition (Maxwell and Johnson 2000) and a possible disruption of the electron transport in the reaction centers (Yamada *et al.* 1996) due to exposure to higher levels of light in comparison to seedlings. Despite that branch sampling in adult trees was done at a low height (< 2 m), their leaves were exposed to more light than those in seedlings. As plants grow and move through the canopy layers, they are exposed to increasingly higher irradiances. Several species change their light requirements throughout ontogeny (Lusk *et al.* 2008), and it is common to find transitions within a species from shade to sun as they grow (Poorter *et al.* 2005). *P. punctata* may experience changes in its tolerance to light according to changes in the ontogenetic phase. Light requirements may be lower at early stages (seedlings), while the opposite might be true for trees. However, this hypothesis needs to be tested with longer experiments, sampling at different canopy levels, and plants at different developmental stages.

The performance index (PI_{ABS}), which is related to plant vitality (Mehta *et al.* 2010), reflects the functionality of PSII and provides quantitative information on the state of plants under light, water, or heat stress conditions (Strasser *et al.* 2004, Strauss *et al.* 2006) and is more sensitive to stress than F_V/F_M when considered

individually (Gonçalves *et al.* 2007). In our study, PI_{ABS} was higher in trees, particularly in those located in Los Barros, characterized by the presence of tall trees. This corroborates that the magnitude of changes in quantum yield of PSII in our study was age- but also site-dependent. The lower PI_{ABS} observed in seedlings under shade may be due to reduced light and hence lower light absorption as well as reduced photochemical efficiency. Conversely, the higher PI_{ABS} of trees exposed to different levels of light might be attributed to higher light absorption. This indicates higher photochemical efficiency of PSII (Krause and Somersalo 1989), thereby allowing more efficient energy transfer from chlorophyll to PSII (Groninger *et al.* 1996). Higher PI_{ABS} in trees also suggests that light trapping and electron transport beyond Q_A function better than in seedlings, which, despite having lower PI_{ABS} , showed no symptoms of photoinhibition (Öquist *et al.* 1992). However, this superiority of trees in PI_{ABS} must be interpreted with caution because the PI_{ABS} of trees had a relative deviation of +2.7% with respect to the average PI_{ABS} of 31.2, whereas seedlings exhibited a deviation of -3.0%.

Our results suggest that the establishment of *P. punctata* seedlings requires shade conditions, but at later developmental stages, the individuals gain access to the well-lit canopy layer. Thus, excess light might be an important factor affecting the initial establishment of this species. A successful restoration will therefore depend on managing light intensity at early stages of establishment. In high-light environments, the use of tree shelters plays an important role because they increase humidity, protect from excessive irradiance, and reduce extreme temperatures (Padilla *et al.* 2011). It has been observed that shelters improve the outplanting performance of species that regenerate under canopy cover (e.g., Acevedo *et al.* 2020, Quiroz *et al.* 2021) as seedlings are unable to increase carbon uptake when they are suddenly exposed to high irradiances. However, in extensive restoration projects, the implementation of shelters may be cost-prohibitive. A complementary and less expensive technique to facilitate the establishment and survival of *P. punctata* seedlings could be the use of nurse plants (Padilla and Pugnaire 2006). The use of nurse plants has been proven to facilitate the establishment of Mediterranean species in dry sites, reducing the adverse effects of water scarcity and excessive irradiation (Gómez-Aparicio *et al.* 2004).

To summarize, most of the parameters related to photochemical efficiency of PSII were similar between seedlings of *P. punctata* growing under the shade and adult trees with intermediate levels of light intensity. However, the PI_{ABS} was higher in trees, indicating a superior photosynthetic performance compared to seedlings.

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