



**HAL**  
open science

## Ecotype effects on photosynthesis performance using A/PFFD among *Pinus nigra* Arn.

Sondes Fkiri, Touhami Rzigui, Hanene Ghazghazi, Larbi M. Khouja, Abelhamid Khaldi, Frédéric Guibal, Zouheir Nasr

### ► To cite this version:

Sondes Fkiri, Touhami Rzigui, Hanene Ghazghazi, Larbi M. Khouja, Abelhamid Khaldi, et al.. Ecotype effects on photosynthesis performance using A/PFFD among *Pinus nigra* Arn.. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 2023, 51 (3), 10.15835/nbha51312599 . hal-04211049

**HAL Id: hal-04211049**

<https://amu.hal.science/hal-04211049v1>

Submitted on 15 Jan 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Ecotype effects on photosynthesis performance using A/PFFD among *Pinus nigra* Arn.

Sondes FKIRI<sup>1\*</sup>, Touhami RZIGUI<sup>1</sup>, Hanene GHAZGHAZI<sup>1</sup>, Larbi M. KHOUJA<sup>1</sup>, Abelhamid KHALDI<sup>1</sup>, Frédéric GUIBAL<sup>2</sup>, Zouheir NASR<sup>1</sup>

<sup>1</sup>National Research Institute of Rural Engineering, Water and Forestry (INRGREF), University of Carthage, BP 10, Ariana 2080, Tunisie; [sondesfkiri@gmail.com](mailto:sondesfkiri@gmail.com) (\*corresponding author); [rzigitouhami@gmail.com](mailto:rzigitouhami@gmail.com); [hanene8116@yahoo.fr](mailto:hanene8116@yahoo.fr); [Khoujalarbi15@gmail.com](mailto:Khoujalarbi15@gmail.com); [khalditn@yahoo.fr](mailto:khalditn@yahoo.fr); [zouheirnasr84@gmail.com](mailto:zouheirnasr84@gmail.com)

<sup>2</sup>Institut Méditerranéen de Biodiversité et d'Ecologie (IMBE), Aix Marseille Univ, Univ Avignon, CNRS, IRD, Europôle méditerranéen de l'Arbois, BP 80, 13 545 Aix en Provence Cedex 4, France; [frederic.guibal@imbe.fr](mailto:frederic.guibal@imbe.fr)

## Abstract

This study aimed to provide insights on intraspecific variability of photosynthesis performance of 19 provenances of black pine planted in a common garden. The experiment was conducted in an experimental trial located at Souiniet (the southern limit of its range) Photosynthetic capacity characterized by light-saturated net photosynthetic rates, associated light compensation points and apparent quantum yield was monitored by measuring the response of leaf gas exchange to light levels. Amax was the highest in provenances Puget Theniers (*nigra* ssp), Saint-Guilhem (*salzmanni* ssp), Marghese and les barres (*laricio* ssp). The lowest value was recorded in Olette of the subspecies *salzmanni*. Needles from two provenances Crimea (*pallasiana* ssp) and Les Barres (ssp *laricio*) revealed the highest apparent quantum yield ( $\Phi$ ), followed by Brougatlès Ales (*salzmanni* ssp), Trenta Coste (ssp *laricio*), Les barres and Puget Theniers (*nigra* ssp). The lowest apparent quantum Yield was recorded in *Laricio* subspecies (Cosenza; Les barres and Bois frerot). The highest value of dark respiration (Rd) was shown in provenance Les barres (*laricio* ssp), while Grancia and Tavola of the *laricio* subspecies reported the lowest one. Provenances Tavola (*laricio* ssp) and Puget Theniers (*nigra* ssp) exhibited the highest LCP. The provenance Crimée (*pallasiana* ssp) and Aspromonto (*laricio* ssp) recorded the lowest values. The maximum values of photosynthesis are positively correlated with the total chlorophyll contents and Leaf Mass area. Our study illustrates that Photosynthesis performance showed a difference between 19 provenances of black pine; it seems that black pine photosynthetic performance is eco-typical independent.

**Keywords:** light curves; net photosynthesis; *P. nigra* Arn; provenance

## Introduction

Adaptation and phenotypic plasticity are two natural processes that trees and forests have at their disposal to cope with climate change (Sow *et al.*, 2018; Diamond *et al.*, 2021). The short-term response of

forests is based on phenotypic plasticity and the exploitation of existing adaptive diversity, whereas the long-term response is based on the genetic evolution of adaptive traits through selection (Lefèvre *et al.*, 2010).

The process of adaptation to novel environmental conditions can occur via two mechanisms: (1) the expression of phenotypic plasticity, and (2) evolution via selection for particular phenotypes, resulting in the modification of genetic variation in the population (Fox *et al.*, 2019)

The ability of forest trees to adapt to climate change is highly dependent on their genetic diversity and geographical origins (Schaberg *et al.*, 2008; Soto *et al.*, 2010). Genetic diversity is essential to the adaptive capacity of tree populations and is at the midst of current concerns on the future of forest species because of the large and rapid selection pressures related to climate change are expected (Kremer *et al.*, 2002). Adaptive genetic diversity is very high between forest species, between geographical origins of the same species and within each population (Lefèvre, 2015). As compared to other groups, conifers have a very high genetic diversity (Hamrick, 1992), but the genetic diversity of pines is considered to be more "adaptive" than other groups.

Among pines, *Pinus nigra* is one of the most diverse species (Lee *et al.*, 2002; Nkongolo *et al.*, 2002). This collective species has been the object of several studies that have investigated the intraspecific variability of growth performance and responses to climate in order to select the best adapted provenances to future climate conditions (Strumia *et al.*, 1997; Köse *et al.*, 2012; Levanic *et al.*, 2013; Shishkova *et al.*, 2013). The species *P. nigra* is known for its relatively high phenotypic and genetic diversity (Jagielska *et al.*, 2007; Lucic *et al.*, 2010; Seho *et al.*, 2010; Giovannelli *et al.*, 2017). Varelides *et al.* (2001) had shown differences in growth between seventeen provenances of *P. nigra* from Greece, Turkey, Corsica and Calabria with significant interactions with various soil types in three sites in northern Greece. Furthermore, according to Kreyling *et al.* (2012), the cold resistance of *P. nigra* was found to be different between the provenances of cold regions that have shown a higher level of resilience. A study conducted on anatomical variables of the wood interested in the origins of *Pinus nigra* Arn. subsp. *salzmanni* has highlighted the adaptive nature of the wood anatomy of this species. (Esteban *et al.*, 2011). Seedlings from six provenances of *P. nigra* that were exposed to the drought and warming over two years have shown that the origins of *P. nigra* differ greatly in their performance, while exhibiting a uniform sensitivity to the drought and local average temperature (Thiel *et al.*, 2012). Growth analyses from four black pine provenances (Austria, Bosnia, France (Corsica) and Italy) on dry sites of south-western Germany revealed that the behaviour of the Corsican provenance differs from the three other sources (Seho *et al.*, 2010).

This species is distinguished by high genetic diversity and high anatomical, morphological, and physiological variability (Isajev *et al.*, 2004; Giovanelli *et al.*, 2018). As such, it provides a suitable model for studying intraspecific diversity in responses to environmental change.

Genetic adaptation may be assessed in comparative plantations that are experimental sites in which species or several genetic units of the same species (geographical origins, families, and clones) grown under the same environmental conditions. This study was carried out in a comparative plantation in the southern limit of its range, which allows in particular, testing the existence of genetic differences between geographical origins. As environmental variations are controlled in a common garden, the differences observed in the expression of a given trait (the phenotype) are due to the genetic variability that exists between the different genotypes being compared. Some phenotypic traits can be well differentiated while others exhibit little difference.

The main objective of this study was to evaluate the in situ intraspecific variability of photosynthesis performance across black pine populations from different localities in the world. In another way, we investigated whether habitat of origin has any effect on photosynthetic acclimation. For this purpose, 19 provenances of black pine planted on the common garden in the southern limit of its range were used.

## Materials and Methods

### Study site

The Souiniet common garden (36°48'N, 8°48'E, 255 m, 492 m) was established in 1964 and characterised by a cold and humid climate, The mean annual temperature is 15.6 °C, the hottest month is July (31 °C) and the coldest one is January (4.4 °C). The soil is a balanced combination of loam, sand and clay.

Each provenance is represented by 21 trees, and the trees are 3 m apart. The understory vegetation is dominated by *Erica scoparia* L. and *Halimium halimifolium* (L.) Willk. Nineteen provenances (399 seedlings) of black pine (*Pinus nigra* Arn.) from France, Spain, Italy, Bulgaria, Russia and Turkey were planted in experimental trials under the same ecological conditions. The geographical origin of the provenances is shown in Table 1.

**Table 1.** The Geographical origin for the sample populations of black pine (*Pinus nigra* Arn.)

| Provenances | Sub-species                   | Provenance     | Country of origin |
|-------------|-------------------------------|----------------|-------------------|
| P1          | <i>Pinus nigra salzmanni</i>  | Brougates Ales | France            |
| P2          | <i>Pinus nigra calabrica</i>  | Trenta Coste   | Italy             |
| P3          | <i>Pinus nigra calabrica</i>  | Les Barres     | France            |
| P4          | <i>Pinus nigra calabrica</i>  | Cosenza        | Italy             |
| P5          | <i>Pinus nigra corsicana</i>  | Bois frerot    | France            |
| P6          | <i>Pinus nigra austriaca</i>  | Puget Theniers | France            |
| P8          | <i>Pinus nigra nigricana</i>  | Kustentil      | Bulgaria          |
| P9          | <i>Pinus nigra pallasiana</i> | Alaçam         | Turkey            |
| P10         | <i>pinus nigra calabrica</i>  | Catanzaro      | Italy             |
| P11         | <i>Pinus nigra corsicana</i>  | Les Barres     | France            |
| P12         | <i>Pinus nigra salzmanni</i>  | Saint-Guilhem  | France            |
| P13         | <i>Pinus nigra pallasiana</i> | Crimée         | Russia            |
| P14         | <i>Pinus nigra calabrica</i>  | Grancia        | Italy             |
| P15         | <i>Pinus nigra calabrica</i>  | Aspromonto     | Italy             |
| P16         | <i>Pinus nigra salzmanni</i>  | Cazorla        | Spain             |
| P17         | <i>Pinus nigra calabrica</i>  | Tavola         | Italy             |
| P18         | <i>Pinus nigra salzmonni</i>  | Olette         | France            |
| P19         | <i>Pinus nigra calabrica</i>  | les Barres     | France            |
| P20         | <i>Pinus nigra corsicana</i>  | Marghese       | Corse             |

### Gas exchange measurements

The photosynthetic response of leaf needles to different levels of photosynthetic photon flux density (PPFD) was measured at ambient CO<sub>2</sub> concentration (400 ppm) with an open infrared gas analysis system (Li-Cor 6400-40 equipped with a red-blue LED source; Li-Cor Inc., Lincoln, NE, USA). Light response curves were measured for attached leaves at 25 °C at an airflow rate of 300 cm<sup>3</sup> min<sup>-1</sup>. The vapor pressure deficit was kept at 1.2 ± 0.2 kPa. Needles were allowed to reach a steady-state at an incident light level of 1200 μmol m<sup>-2</sup> s<sup>-1</sup> PAR. We then changed the PPFD, and net photosynthesis (An) was recorded at each PPFD level once it became stable. Stomatal conductance (gs) and transpiration (E) were also recorded concurrently. Instantaneous water use-efficiency (WUE) was calculated as An/E (μmol CO<sub>2</sub> μmol<sup>-1</sup> H<sub>2</sub>O). Photosynthetic capacity was estimated by light-saturated net photosynthetic rate (Amax) as determined by fitting An/PPFD curves using a three component exponential function (see Meng *et al.*, 2014 - eqn. 1):

$$An = A_{max} (1 - e^{-b \cdot PPFD}) + c$$

Where  $A_n$  is the net photosynthetic rate, and  $A_{max}$  is the maximum photosynthetic rate. For each light curve, the apparent quantum yield based on the incident light ( $\Phi$ ) was calculated as the initial slope at the 3 lowest PPFD values (between 0 and  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The light compensation point (LCP) was estimated from the x-axis intercepts.

#### *Photosynthetic pigments*

The same leaves used for the measurement of gas exchange were sampled for the assessment of the total content of chlorophyll and carotenoids. Leaf tissue of known area (measured using a leaf area meter, Portable Laser, Model CI-202) was incubated in 80% acetone until all of the chlorophyll was visibly extracted. Spectrophotometer readings of the extracts were obtained at 750, 663, 645 and 453 nm to determine the chlorophyll a and b and total carotenoid contents using the equations of Porra *et al.* (1989) and Robbelen (1957).

#### *Leaf mass area*

Leaf mass per area (LMA) is an important leaf trait widely used for relating light capture to plant growth and carbon gain, it is calculated as the leaf dry mass per unit leaf area (Kattge *et al.*, 2011).

#### *Statistics*

All experiments were repeated at least 3 times, and the mean values and standard errors of the data are shown. All ANOVA analyses were performed using the software package SigmaPlot® (Systat Software Inc., San Jose, CA, USA). Statistically significant differences at the 5% level were calculated with the Student's t-test.

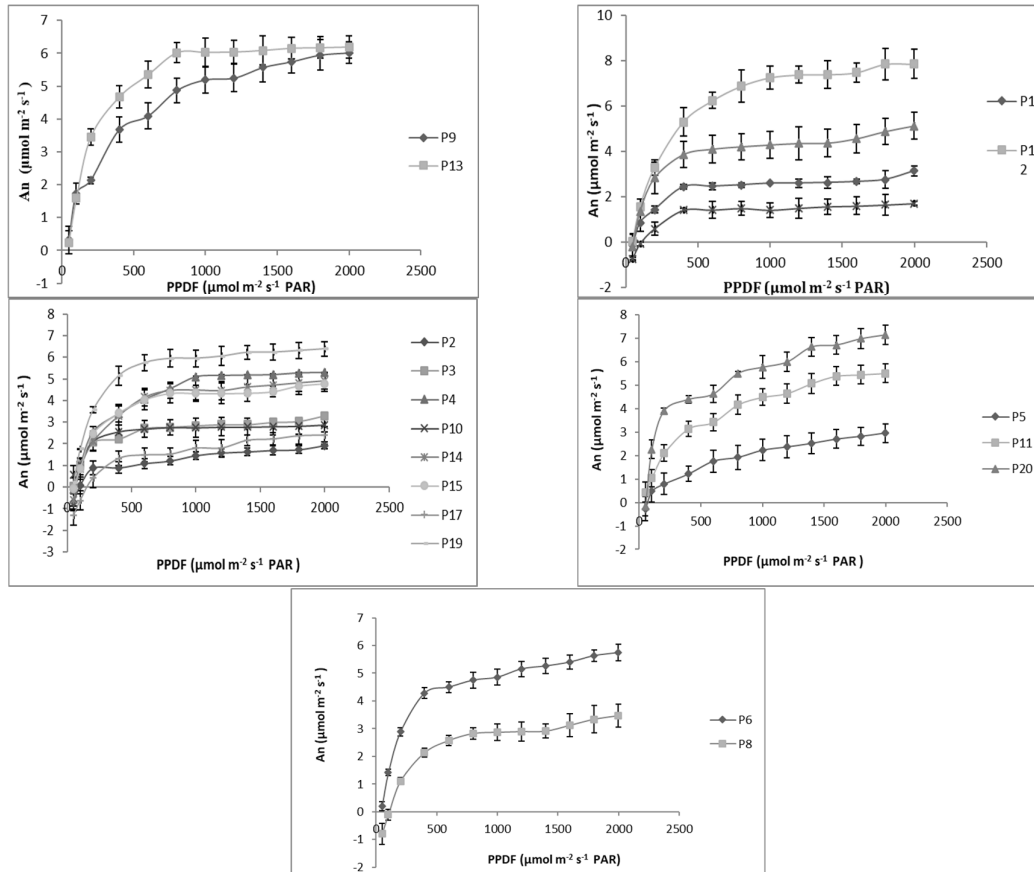
## **Results**

#### *Light response curve of photosynthesis*

The light response curves of photosynthesis were similar in all provenances (Figure 1). The rate of net photosynthesis ( $A_n$ ) of *P. nigra* increased rapidly with increasing light intensity (0 to 400).

Beyond  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $A_n$  becomes stable and therefore saturated. Gas exchange parameters are estimated from light intensity response curves (Table 2). The plateaus of these curves correspond to the maximum photosynthetic capacity (photosynthetic assimilation in saturating light,  $A_{max}$ ). The apparent quantum yield ( $\Phi$ ) estimated at the base of the incident light ( $\text{PAR} < 200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) provides information on the number of photons necessary for the fixation of a  $\text{CO}_2$  molecule. The light compensation point (LCP) corresponds to the light in which photosynthesis exactly compensates for respiration (zero balance).

The results obtained revealed that  $A_{max}$ ,  $R_d$ ,  $\Phi$  and LCP are significantly different between provenances ( $p < 0.05$ ) (Table 2).  $A_{max}$  varied between 7.925 and  $1.9 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ .  $A_{max}$  is highest in provenances P6 (*nigra* ssp), P12 (*salzmanni* ssp), P20 and P19 (*laricio* ssp). The lowest value was recorded in P18 of the subspecies *salzmanni*. Needles from two provenances Crimea (P13) (*pallasiana* ssp) and Les Barres (P3) (ssp *laricio*) revealed the highest quantum yield ( $\Phi$ ), followed by P1 (*salzmanni* ssp), P2, P19 (*laricio* ssp) and P6 (*nigra* ssp). On the other hand, the lowest  $\Phi$  was recorded in provenances P4, P11 and P5 (ssp *laricio*). Respiration ( $R_d$ ) is highest in provenance P11 of the subspecies *laricio* var. *corsicana*, on the other hand P14 and P17 of the *laricio* subspecies recorded the weakest respiration. Provenances P17 (ssp *laricio*) and P6 (*nigra* ssp) exhibited the highest light compensation point (LCP). P13 (*pallasiana* ssp) and P15 (*laricio* ssp) recorded the lowest values.



**Figure 1.** Light response curves: Net carbon assimilation ( $A_n$ ) of attached needles measured at different PPFD levels in an atmosphere of 400 ppm  $\text{CO}_2$  and 25 °C from 4 sub-species. Values are means  $\pm$  SE of at least three independent measurements.

#### Variations of $g_s$ , $E$ and $WUE_i$ in response to PPFD

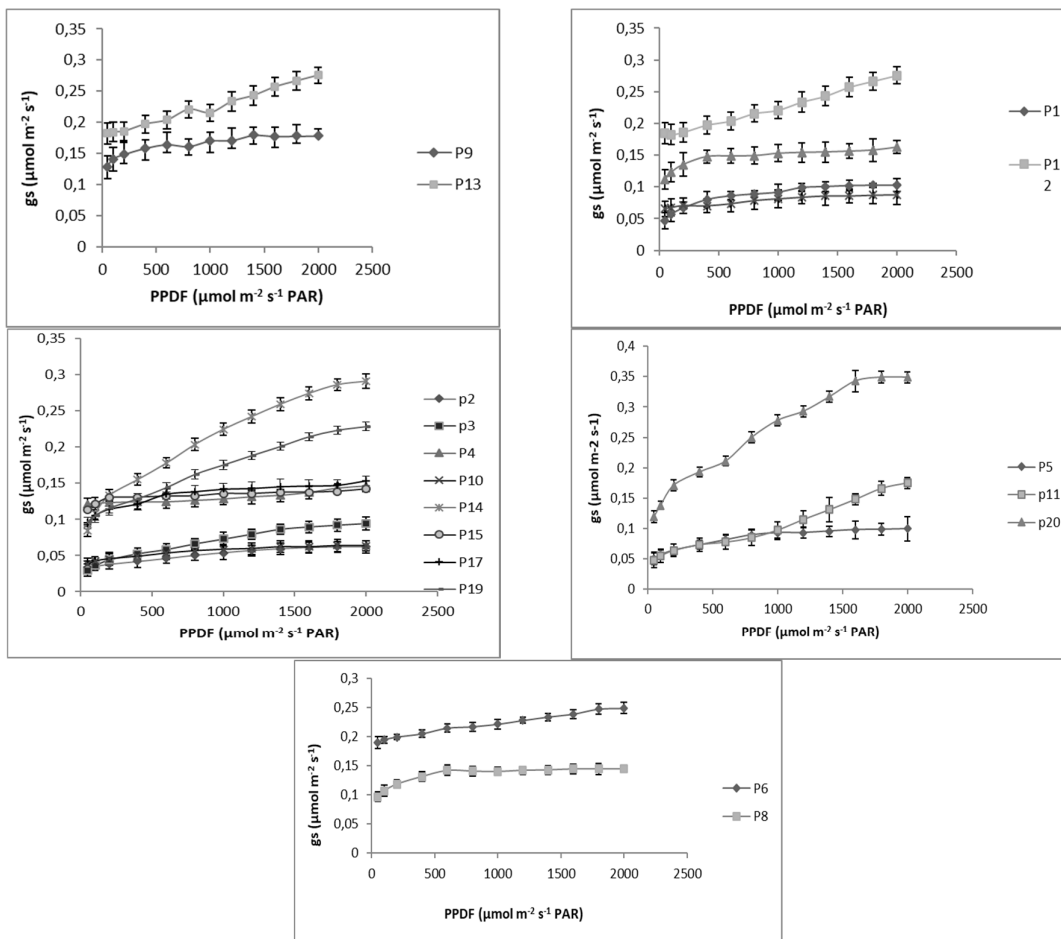
Transpiration ( $E$ ) (Figure 2) and Stomatal conductance ( $g_s$ ) (Figure 2) increased as light intensity. However, a significant difference in  $g_s$  and  $E$  responses as a function of PPFD was recorded between subspecies and provenances. Provenances P6 of the subspecies *nigra*, P14, P17 and P20 of the subspecies *laricio* and P12 of the subspecies *salzmanni* recorded the highest stomatal conductance and needle transpiration at light intensities above 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The lowest  $g_s$  and  $E$  values for all applied light intensities were recorded in provenances P10, P15 and P3 of the *laricio* subspecies.

**Table 2.** Light-saturated photosynthesis ( $A_{\text{max}}$ ), light compensation point (LCP) and apparent quantum yield ( $\Phi$ ) in needles of *Pinus nigra* provenances grown under three light regimes

| Provenances | $A_{\text{max}}$<br>( $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ ) | $\Phi$ (mol $\text{CO}_2$ mol $^{-1}$<br>photon) | $R_d$<br>( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) | LCP<br>( $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) |
|-------------|--|--|---|---|
| <b>P1</b>   | 3,046g   | 0,009c   | -0,841d   | 85efg   |
| <b>P2</b>   | 2,730g   | 0,009c   | -1,883ih  | 94,67e  |
| <b>P3</b>   | 3,417g   | 0,0114b  | -1,742gh  | 80fgh   |
| <b>P4</b>   | 4,960efd   | 0,0017g  | -1,953ij  | 76fgh   |
| <b>P5</b>   | 2,908g   | 0,0018g  | -1,485f   | 154,68b   |
| <b>P6</b>   | 7,424a   | 0,0084c  | -0,413b   | 66,68ij   |
| <b>P8</b>   | 6,218bc  | 0,0033fg   | -1,892ih  | 107,33d   |

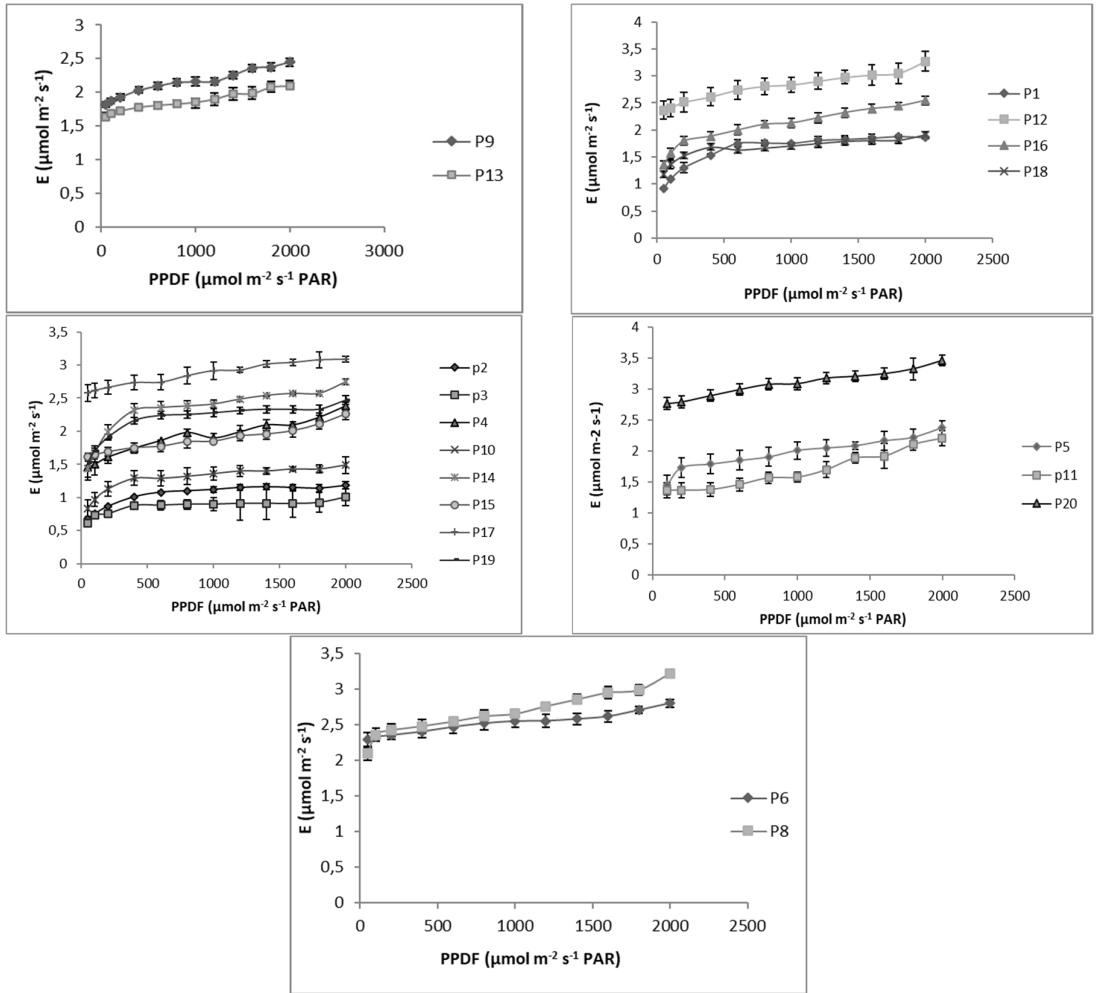
|            |          |                     |          |                    |
|------------|----------|---------------------|----------|--------------------|
| <b>P9</b>  | 5,840bcd | 0,0058 <sup>c</sup> | -1,61fg  | 60jk               |
| <b>P10</b> | 3,215g   | 0,00721d            | -0,551bc | 70hij              |
| <b>P11</b> | 5,556cde | 0,0016g             | 0,25a    | 57jk               |
| <b>P12</b> | 7,322a   | 0,0044f             | -0,761d  | 95,68 <sup>e</sup> |
| <b>P13</b> | 5,227bc  | 0,013a              | -2,141k  | 51,33k             |
| <b>P14</b> | 4,800ef  | 0,0045f             | -2,371l  | 89,5ef             |
| <b>P15</b> | 4,507f   | 0,0063ed            | -0,613c  | 52,33k             |
| <b>P16</b> | 5,01efd  | 0,033fg             | -1,598fg | 94,33 <sup>e</sup> |
| <b>P17</b> | 3,095g   | 0,0039fg            | -2,442l  | 208,33a            |
| <b>P18</b> | 1,907h   | 0,0062ed            | -1,464f  | 126,67c            |
| <b>P19</b> | 6,666b   | 0,0089c             | -2,1kj   | 70hij              |
| <b>P20</b> | 7,925a   | 0,006ed             | -1,262e  | 66,33ij            |

Different letters indicate significant differences between provenance ( $p \leq 0.05$ ,  $n=57$ ; Student's t-test). Amax: capacity photosynthesis; AQY: apparent quantum yield; Rd: dark respiration and LCP: light compensation point.



**Figure 2.** Response of stomatal conductance ( $g_s$ ), as a function of PPFD in an atmosphere of 400 ppm  $\text{CO}_2$  and 25 °C

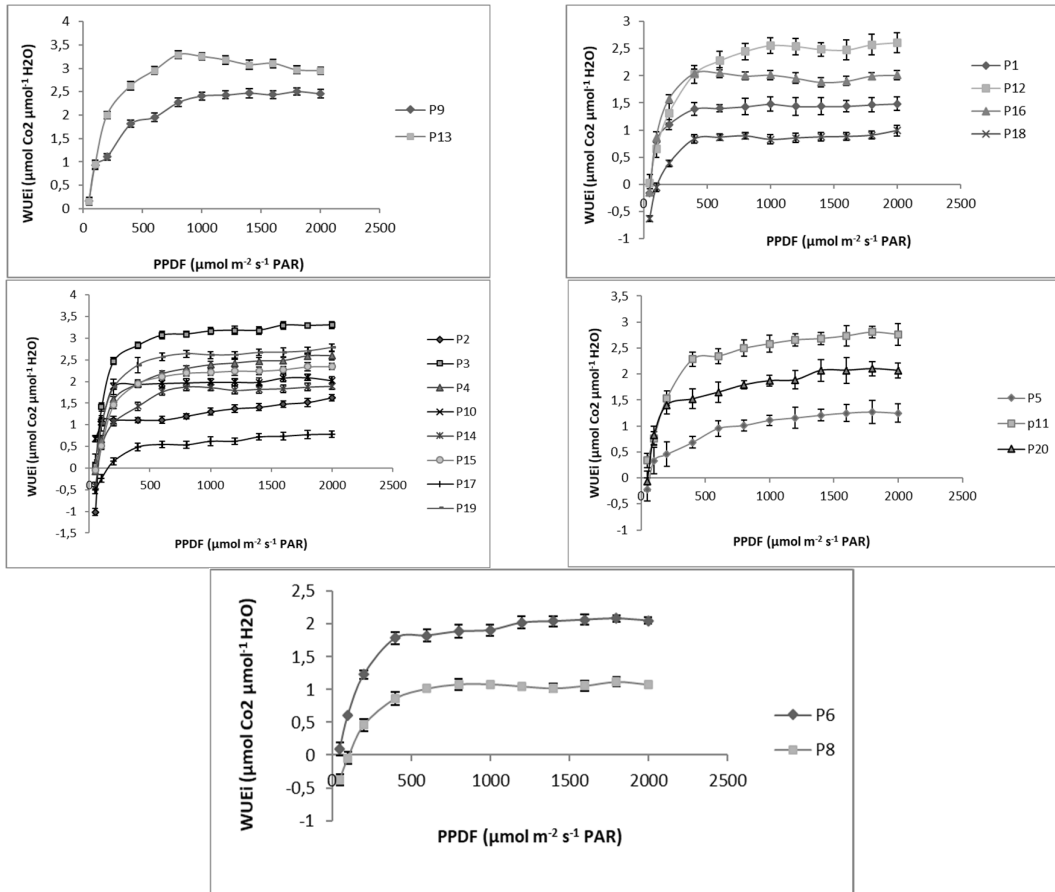
Values are means  $\pm$  SE of at least three independent measurements.



**Figure 3.** Response of transpiration rate ( $E$ ) as a function of PPFD in an atmosphere of 400 ppm  $\text{CO}_2$  and 25 °C  
 Values are means  $\pm$  SE of at least three independent measurements.

Instantaneous water use efficiency ( $\text{WUE}_i$ ) was estimated by relating the net assimilation of photosynthesis ( $A_n$ ) to transpiration. A remarkable increase in  $\text{WUE}_i$  with increasing irradiance was observed.  $\text{WUE}_i$  is completely saturated at light intensities  $<600 \mu\text{mol m}^{-2} \text{s}^{-1}$  for almost all provenances (Figure 4). Provenances P3, P11, P19, P13 and P12 are the most efficient in using water under saturated light.





**Figure 4.** Response of gas exchange to light: instantaneous water use efficiency (WUE<sub>i</sub>) as a function of PPDF in an atmosphere of 400 ppm CO<sub>2</sub> and 25 °C  
 Values are means ± SE of at least three independent measurements.

#### *Photosynthetic pigments*

The analysis of variance of the chlorophyll pigments showed a highly significant difference between the 19 sources ( $P < 0.0001$ ). The needles from provenances P12, P8 and P19 have the highest content, while provenance P19 has the lowest total chlorophyll concentration (chl a+chl b). The carotenoid content was highest in P4, P5 and P13 and lowest in P17, P8 and P19 (Table 3).

**Table 3.** Leaf pigments content: Chl a+b and total carotenoid concentrations per unit leaf area and chlorophyll a/b and carotenoids/total chlorophyll (Car/Chl) ratios

|     | Mg Chl a<br>/cm <sup>2</sup> | Mg Chl b<br>/cm <sup>2</sup> | Mg Chl a-b<br>/cm <sup>2</sup> | Mg<br>carotenoid/cm <sup>2</sup> | Chl a/<br>Chl b    | Car. /<br>Chl a-b  | LMA<br>(g/cm <sup>2</sup> ) |
|-----|------------------------------|------------------------------|--------------------------------|----------------------------------|--------------------|--------------------|-----------------------------|
| P1  | 1,41b                        | 0,452gf                      | 1,8617dc                       | 0,284cd                          | 3,113 <sup>c</sup> | 0,153 <sup>c</sup> | 1,84b                       |
| P2  | 0,41ih                       | 1,33cd                       | 1,679cd                        | 0,137ef                          | 0,308q             | 0,079l             | 0,83d                       |
| P3  | 0,812a                       | 0,867e                       | 1,6793cd                       | 0,0596gh                         | 0,663k             | 0,036o             | 1,05dc                      |
| P4  | 0,923efd                     | 1,562ab                      | 24855ab                        | 0,509a                           | 0,591n             | 0,205c             | 1,92b                       |
| P5  | 1,182bcd                     | 0,319gh                      | 1,5017de                       | 0,343bc                          | 3,698c             | 0,229b             | 0,97dc                      |
| P6  | 1,166bcd                     | 0,769e                       | 1,936dc                        | 0,249d                           | 1,515h             | 0,129h             | 2,48a                       |
| P8  | 0,745efg                     | 1,235d                       | 1,9807dc                       | 0,0286h                          | 0,603m             | 0,014q             | 1,06dc                      |
| P9  | 0,368ih                      | 0,61f                        | 0,9783f                        | 0,146ef                          | 0,603m             | 0,15f              | 0,99dc                      |
| P10 | 1,28bc                       | 0,599f                       | 1,8793dc                       | 0,315c                           | 2,137g             | 0,168d             | 1,4c                        |
| P11 | 0,5997fgh                    | 0,93e                        | 1,53cde                        | 0,185ef                          | 0,644l             | 0,121i             | 1,10dc                      |
| P12 | 0,6247efgh                   | 1,473bc                      | 2,0977bc                       | 0,102fgh                         | 0,424o             | 0,049n             | 1,08dc                      |
| P13 | 1,22bcd                      | 0,375gh                      | 1,5953cde                      | 0,377b                           | 3,251d             | 0,236a             | 2,16ab                      |
| P14 | 0,5697gh                     | 0,599f                       | 0,7933f                        | 0,11fg                           | 2,545f             | 0,139g             | 1,08dc                      |
| P15 | 0,8efg                       | 1,206d                       | 2,0057                         | 0,576gh                          | 0,663k             | 0,029p             | 1,10dc                      |
| P16 | 0,1837i                      | 1,67ab                       | 1,8537dc                       | 0,104fgh                         | 0,11l              | 0,056m             | 1,12dc                      |
| P17 | 1,8147a                      | 0,279gh                      | 2,094bc                        | 0,0603gh                         | 6,493a             | 0,29p              | 1,24dc                      |
| P18 | 0,4887gh                     | 1,163d                       | 1,652cd                        | 0,188 <sup>c</sup>               | 0,42p              | 0,114j             | 0,98dc                      |
| P19 | 1,124bcd                     | 1,43bc                       | 2,554ab                        | 0,033gh                          | 0,786j             | 0,013r             | 1,40dc                      |
| P20 | 0,9507ecd                    | 0,205h                       | 1,155ef                        | 0,105fgh                         | 4,637b             | 0,091k             | 1,30dc                      |

The values shown represent the mean of at least three replicates with standard errors.

## Discussion

The efficiency of carbon assimilation capacity as a function of photosynthetic photon flux density (PPFD) of black pine has been assessed. Several authors have emphasized the importance of photosynthetic performance as an element of selection (Valladeras *et al.*, 2010). Bassow and Bazzaz (1997) mentioned that within the same forest, photosynthesis can vary greatly among species and within an individual tree. In our study, the variability of photosynthetic efficiency within *Pinus nigra* species planted in the same pedoclimatic conditions was assessed. High intraspecific variability in terms of photosynthesis capacity was found ( $p < 0.05$ ). These results demonstrated that the ecological origin of provenances determined their photosynthetic performance.

This variation was observed in individuals under the same environmental conditions, indicating a high degree of intrapopulation genotypic variability in black pine, as has been reported for other species. Indeed, Blaguer *et al.* (2001), from the analysis of the response in light of different populations of *Q. coccifera* from different origins, concluded the existence of an eco-typical difference and not phenotypic plasticity. In addition, several studies showed that the light curves differ between species and within the same species (Lambers *et al.*, 2006; Aber *et al.*, 2001; Lachapelle 2011; Huang, 2021). Using the photosynthesis-light curve, the parameters Amax, Rd, LCP and  $\phi$  were estimated. Several studies have compared the photosynthetic performance of different species on the basis of these criteria (Leverenz *et al.*, 1996; Sullivan *et al.*, 1997; Evans *et al.*, 2001; Tang *et al.*, 2003; Hiroaki *et al.*, 2012; Guzmán *et al.*, 2013; Rzigui *et al.*, 2015 Guzmán *et al.*, 2016; Yang *et al.*, 2020). Leverenz (1995) showed that the variations in Amax between species were small compared to variations within a species. In this study, the maximum rate of photosynthesis ( $>1500 \mu\text{mol de photons. m}^{-2}. \text{s}^{-1}$ ) were reached by the four provenances Marghèse (P20) (*laricio* ssp), Les Barres (P19) (*laricio* ssp), Puget-Thénier (P6) (*nigra* ssp) and Saint-Guilhem (P12) (*salzmanni* ssp). The increase in photosynthesis as a function of light

intensities is limited by the carboxylation capacity of Rubisco or by the metabolism of trisic phosphates (Sharkey, 1987; Harley *et al.*, 1992; Yang *et al.*, 2015). Chazdon and Field (1987) showed, among Piper species, that Amax was strongly dependent on leaf nitrogen and on all descriptors of the light environment in the leaf microsite. In our study, the maximum values of photosynthesis (Amax) are positively correlated with the total chlorophyll contents of the species *Pinus nigra* ( $r^2=0.768$ ). Leverenz (1987) showed that the photosynthetic rates of the needles of five conifers (*Picea abies* (L.), *Picea glauca*, Voss, *Picea mariana* Bsp, *Picea sitchensis* Carr., *Pinus sylvestris* L.) were strongly correlated with the amount of chlorophyll. Amax and LMA are positively correlated for the species *P. nigra* ( $r^2=0.617$ ). In the same context, Wright *et al.* (2004) showed that the maximum net photosynthesis rate (Amax), specific leaf area (LMA) covariate among themselves, for all 2548 species sampled across the planet. This relationship could derive from a greater contribution of mesophyllian tissues containing chloroplasts reported to the biomass (Nouvellon *et al.*, 2010; Wayka *et al.*, 2012). Apparent quantum yield, the equivalent of moles of absorbed photons that are converted into moles of fixed CO<sub>2</sub>, was higher in provenances; Crimée, Les Barres, Brouzet-lès-Alès, Trenta, Barres suggesting that the efficiency of the Calvin cycle, in terms of the use of ATP and NADPH, is higher in the needles of these provenances compared to other ones (Jones, 1998; Bauerle *et al.*, 2020). Thus, this parameter of leaf photosynthesis has been shown efficiency in needles of these provenances in terms of carbon uptake.

The ability of a species to acclimate and adapt to environmental conditions is associated directly/indirectly with its ability to modulate photosynthesis and water vapor exchange (Ayuko *et al.*, 2008; Dieleman and Meinen, 2008; Kruse *et al.*, 2008). The WUEi is completely saturated at light intensities close to  $<600 \mu\text{mol m}^{-2} \text{s}^{-1}$  almost for all the origins of *Pinus nigra*. When the light is weak, gas exchange and therefore the WUEi depend on photosynthetic reactions limited to light, while, under high light intensities, The WUEi is mainly controlled by stomatal conductance (Lawson, 2009; Hummel *et al.*, 2010; Eisenach *et al.*, 2012; Lawson and Blatt, 2014, Fkiri *et al.*, 2020). Cornic *et al.* (1989) noted that heterogeneous stomatal closure caused a decrease in net photosynthesis at high PPFD and a decrease in the apparent quantum yield. Thus, high stomatal conductance and WUEi under limited photosynthetic conditions could be advantageous for provenance/species performance.

## Conclusions

In this study pattern of photosynthetic response to variations in photosynthetic photon flux densities was investigated. Results obtained showed that the photosynthetic performance is eco-typical controlled. Indeed, the nineteen provenances of *P. nigra* originate from various origins around the Mediterranean area planted under the same environmental conditions demonstrated a significant difference in terms of photosynthetic capacity. Therefore, these parameters taken from the light curve could be used in the selection of performance provenances. Thus, Provenances that show better photosynthetic performance are recommended for reforestation or afforestation in like conditions because they sequester much more carbon than others and therefore, they could be a suitable carbon reservoir.

## Authors' Contributions

Methodology FS, RT and NZ; Supervision M.L.K, KA and NZ; Validation NZ; Writing - original draft: FS; Writing - review and editing RT, GF, HG and SY.

All authors read and approved the final manuscript.

## Ethical approval (for researches involving animals or humans)

Not applicable.

## Acknowledgements

This work was supported and funded by National Institute of Rural Engineering, Water and Forest.

## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

## References

- Balaguer L, Martinez-Ferri E, Valladares F, Perez-Corona ME, Baquedano FJ, Castillo FJ, Manrique E (2001). Population divergence in the plasticity of the response of *Quercus coccifera* to the light environment. *Functional Ecology* 15(1):124-135. <https://doi.org/10.1046/j.1365-2435.2001.00505.x>
- Bassow S, Bazzaz F (1997). Intra- and inter-specific variation in canopy photosynthesis in a mixed deciduous forest. *Oecologia* 109:507-515. <https://doi.org/10.1007/s004420050111>
- Bauerle WL, McCullough C, Iversen M, Hazlett M (2020). Leaf age and position effects on quantum yield and photosynthetic capacity in hemp crowns. *Plants* 9(2):271. <https://doi.org/10.3390/plants9020271>
- Chazdon RL, Field CB (1987). Determinants of photosynthetic capacity in six rainforest piper species. *Oecologia* 73:222-230. <https://doi.org/10.1007/BF00377511>
- Chen Y, Xu DQ (2006). Two patterns of leaf photosynthetic response to irradiance transition from saturating to limiting one in some plant species. *New Phytologist* 169:789-798. <https://doi.org/10.1111/j.1469-8137.2005.01624.x>
- Cornic G, Le Gouallec JL, Briantais JM, Hodges M (1989). Effect of dehydration and high light on photosynthesis of two C3 plants {*Phaseolus vulgaris* L. and *Elatostema repens* (Lour.) Hall f.}. *Planta* 111:84-90. <https://doi.org/10.1007/BF00392157>
- Diamond SE, Martin RA (2021). Physiological adaptation to cities as a proxy to forecast global-scale responses to climate change. *Journal of Experimental Biology*. <https://doi.org/10.1242/jeb.229336>
- Fkiri S, Rzigui T, Elkhorchani A, Hassine AB, Khaldi A, Khouja ML, Nasr Z (2020). Tree growth and leaf gas exchange variability of three Mediterranean *Pinus* spp. growing in a common garden in Northeastern Tunisia. *Euro-Mediterranean Journal for Environmental Integration* 5(1):1-9. <https://doi.org/10.1007/s41207-019-0136-7>
- Fox RJ, Donelson JM, Schunter C, Ravasi T, Gaitán-Espitia JD (2019). Beyond buying time: the role of plasticity in phenotypic adaptation to rapid environmental change. *Philosophical Transactions of the Royal Society B*: 374(1768)20180174. <https://doi.org/10.1098/rstb.2018.0174>
- Fu WG, Li PP, Bian XM, Wu YY, Cao QY (2006). Diurnal photosynthetic changes of *Phragmites communis* in the wetland lying in Beigushan mountain of Zhenjiang prefecture. *Acta Botanica Boreali-Occidentalia Sinica* 26:0496-0501. <https://doi.org/10.1007/s11099-007-0110-5>
- Gao J, Meng P, Wu B, Zhang JS, Chu JM (2006). Photosynthesis and transpiration of *Salvia miltiorrhiza* in tree-herb system of *Prunus dulcis* and *Salvia miltiorrhiza*. *Journal of Beijing Forest University* 28:64-67.
- Giovannelli G, Roig A, Spanu I, Vendramin GG, Fady B (2017). A new set of nuclear microsatellites for an ecologically and economically important conifer: the European black pine (*Pinus nigra* Arn.). *Plant Molecular Biology Reporter* 35:379-388. <https://doi.org/10.1007/s11105-017-1029-z>
- Huang M Y, Wong SL, Weng JH (2021). Rapid light-response curve of chlorophyll fluorescence in terrestrial plants: Relationship to CO<sub>2</sub> Exchange among five woody and four fern species adapted to different light and water regimes. *Plants* 10(3):445. <https://doi.org/10.3390/plants10030445>

- Köse N, Akkemik Ü, Dalfes N, Özeren MS, Tolunay D (2012). Tree-ring growth of *Pinus nigra* Arn. subsp. *pallasiana* under different climate conditions throughout western Anatolia. *Dendrochronologia*: 30: 295–301. <https://doi.org/10.1016/j.dendro.2012.04.003>.
- Kremer A, Kleinschmit J, Cottrell J, Cundall EP, Deans JD, Ducouso A, ... Funk C (2018). Proteomic analysis of the phycobiliprotein antenna of the cryptophyte alga *Guillardia theta* cultured under different light intensities. *Photosynthesis Research* 135:149-163. <https://doi.org/10.1007/s11120-017-0400-0>
- Kreyling J, Wiesenberg LB, Thiel G, Wohlfart D, Huber C, Walter G, ... Beierkuhnlein M (2012). Cold hardiness of *Pinus nigra* Arnold as influenced by geographic origin, warming, and extreme summer drought. *Environmental and Experimental Botany* 78: 99-108. <https://doi.org/10.1016/j.envexpbot.2011.12.026>.
- Kyei-Boahen S, Lada R, Astatkie T, Gordon R, Caldwell C (2003). Photosynthetic response of carrots to varying irradiances. *Photosynthetica* 41:301-305. <https://doi.org/10.1023/B:PHOT.0000011967.74465.cc>.
- Lefèvre F, Fady B, Jean F, Davi H, Pichot C, Oddou-Muratorio S (2015). Les processus biologiques de réponse des arbres et forêts au changement climatique : adaptation et plasticité phénotypique. *Innovations Agronomiques* 47:63-79.
- Levanic T, Popa I, Poljansek S, Nechita C (2013). A 323-year long reconstruction of drought for SW Romania based on black pine (*Pinus nigra*) tree-ring widths. *International Journal of Biometeorology* 57:703-714. <https://doi.org/10.1007/s00484-012-0596-9>
- Liu YF, Xiao LT, Tong JH, Li XB, (2005). Primary application on the non-rectangular hyperbola model for photosynthetic light-response curve. *Chinese Agriculture Sciences Bulletin* 121:76-79.
- Lowe AJ, Munro RC, Petit RJ, Stephan BR (2002). Is there a correlation between chloroplastic and nuclear divergence, or what are the roles of history and selection on genetic diversity in European oaks?. *Forest Ecology and Management* 156:75-87. [https://doi.org/10.1016/S0378-1127\(01\)00635-1](https://doi.org/10.1016/S0378-1127(01)00635-1)
- Marschall M, Proctor CF, (2004). Are bryophytes shade plants? Photosynthetic light responses and proportions of chlorophyll a, chlorophyll b and total carotenoids. *Annals of Botany* 94:593-603. <https://doi.org/10.1093/aob/mch178>
- McKown AD, Klápště J, Guy RD, Gerald A, Porth I, Hannemann J, ... Douglas CJ (2014). Genome-wide association implicates numerous genes underlying ecological trait variation in natural populations of *Populus trichocarpa*. *New Phytology* 203:535-553. <https://doi.org/10.1111/nph.12815>
- Nouvellon Y, Laclau JP, Epron D, Kinana A, Mabiala A, Rouspard O, Saint-André L (2010). Within-stand and seasonal variations of specific leaf area in a clonal eucalyptus plantation in the Republic of Congo. *Forest of Ecology and Management* 259(9):1796-1807. <https://doi.org/10.1016/j.foreco.2009.05.023>
- Ögren E (1993) Convexity of the photosynthetic light-response curve in relation to intensity and direction of light during growth. *Plant Physiology* 101:1013-1019. <https://doi.org/10.1104/pp.101.3.101>
- Schaberg P, DeHayes D, Hawley G, Nijensohn S (2008). Anthropogenic alterations of genetic diversity within tree populations: implications for forest ecosystem resilience. *Forest Ecology Management* 256:855-862. <https://doi.org/10.1016/j.foreco.2008.06.038>
- Scho M, Kohnle U, Albrecht A, Lenk E (2010). Growth analyses of four provenances of European Black Pine (*Pinus nigra*) growing on dry sites in southwest Germany (Baden-Wuerttemberg). *Allgemeine Forst und Jagdzeitung* 181:104-116.
- Shishkova V, Panayotov M (2013). Climate growth relationship of *Pinus nigra* tree-ring width chronology from the Rhodope Mountains, Bulgaria. *Bulgarian Journal of Agricultural Sciences* 19:9-90.
- Sow MD, Allona I, Ambrose C, Conde D, Fichot R, Gribkova S, ..., Maury S (2018). Epigenetics in forest trees: state of the art and potential implications for breeding and management in a context of climate change. *Advances in Botanical Research*: 88:387-453. <https://doi.org/10.1016/bs.abr.2018.09.003>
- Strumia G, Wimmer R, Grabner M (1997). Dendroclimatic sensitivity of *Pinus nigra* Arnold in Austria. *Dendrochronologia* 15:129-137. <https://doi.org/10.3959/2011-7.1>
- Thiel D, Laura Nagy L, Beierkuhnlein C, Huber G, Jentsch A, Konnert M, Kreyling J (2012). Uniform drought and warming responses in *Pinus nigra* provenances despite specific overall performances. *Forest of Ecology and Management* 270:200-208. <https://doi.org/10.1016/j.foreco.2012.01.034>
- Vogelmann TC (1989) Penetration of light into plants. *Photochemistry and Photobiology* 50:895-902. <https://doi.org/10.1111/j.1751-1097.1989.tb02919.x>

- Wang HT, Meng YY, Cao XP, Ai JN, Zhou JN, Xue S, Wang W (2015). Coordinated response of photosynthesis, carbon assimilation, and triacylglycerol accumulation to nitrogen starvation in the marine microalgae *Isochrysis zhangjiangensis* (Haptophyta). *Bioresources Technology* 177:282-288. <https://doi.org/10.1016/j.biortech.2014.11.028>
- Yang JT, Preiser AL, Li Z, Weise SE, Sharkey TD (2016). Triose phosphate use limitation of photosynthesis: short-term and long-term effects. *Planta* 243:687-698. <https://doi.org/10.1007/s00425-015-2436-8>
- Ye ZP (2007) A new model for relationship between irradiance and the rate of photosynthesis in *Oryza sativa*. *Photosynthetica* 45:637-640. <https://doi.org/10.1007/s11099-007-0110-5>