







# Redundancy and independence of chlorophyll fluorescence (OJIP transient) parameters in plant ecological studies

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#### Introduction

Chlorophyll fluorescence (ChIF) analysis, especially the prompt fluorescence (PT), offers a large array of parameters useful to study the responses of plants under environmental stress. Stress factors induce specific physiological responses, related to the efficiency of the photosynthetic apparatus, that can be assessed with a proper selection of parameters. The processes connected to solar radiation (energy) absorption, trapping, transport and dissipation are often correlated each other's, and consequently also the parameters adopted to analyze this photochemical processes.

In ecological field studies, where many samples are often used, there is the need to screen the analyzed populations with few, possibly independent, parameters.

In this study we re-analyze large datasets to individuate the main relationships between PT parameters (i.e. JIP test parameters, Strasser et al. 2010) to select the more appropriate ones for a quick classification of plants in relation to their photosynthetic characteristics,.

#### Table 1

A\_FunDivEUROPE – 2012-13 - Field conditions - forest (Pollastrini et al., 2016)

Nr. of observations: 18188. Variables: site (Finland, Italy, Poland, Romania, Spain); tree species or functional group (conifers, broadleaves); tree richness (1 to 5 speciles); Leaf position (top, bottom).

**B\_Tuscany (IT) – 1998** - Field conditions - *Quercus ilex* forest (Bussotti, 2004) Nr of obs.: 577. Variables: site (Colognole; Cala Violina); month of the year; time of the day (pre-dawn, midday).

C\_Gembloux (BE) – Field conditions – Grassland (Digrado et al., 2017, 2018).

Nr of obs.: 14745 Variables: plant species; month of the year; hour of the day.

**D\_Curno (IT) beech** – 2008 - Experimental plantation (Desotgiu et al., 2012).

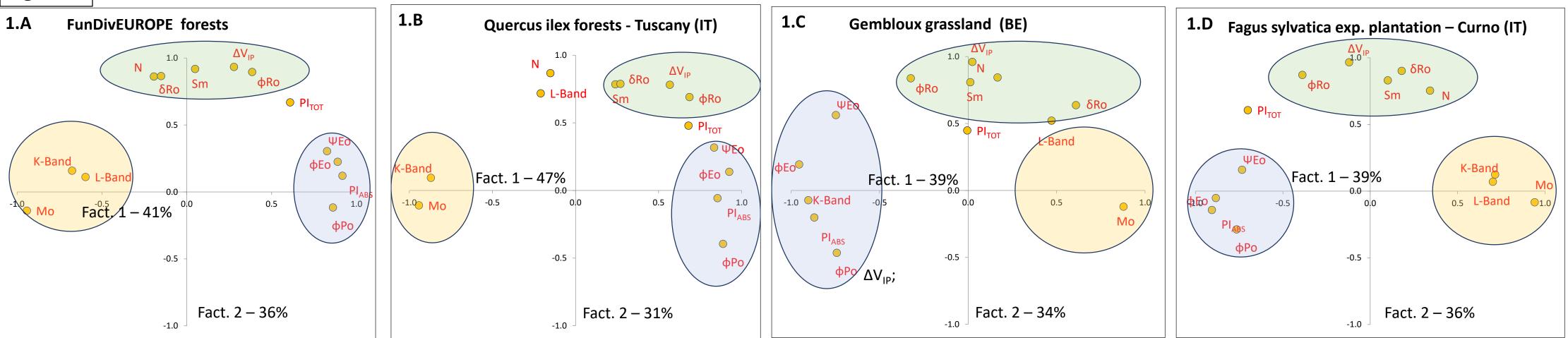
Nr of obs.: 1737 Variables: month of the year; hour of the day; leaf position (sun, shade).

#### Methods

Here, we present 5 cases of study, varied out over time by the authors, accounting in total more than 35,000 ChIF measurements. These cases of study are listed in Table 1. All results are already published. The datasets were checked for incongruences and anomalous values of the assessed parameters. Principal Component Analysis was carried out to individuate homogeneous groups of parameters.

Fig. 1

**E\_Curno (IT) poplar - 2008** - Open-top chamber experiment (Desotgiu et al., 2012, 2013). Nr of obs.: 1709. Variables: month of the year; hour of the day; leaf position; ozone treatment; water treatment.



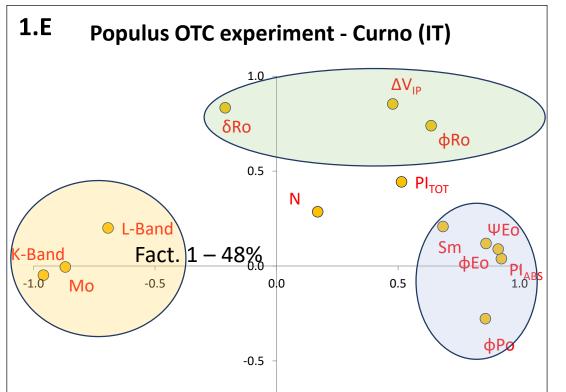


FIG. 1 - Results of the PCA analysis for each separate dataset. Description of ChIF parameters applied.  $\varphi Po$  (=Fv/Fm): Trapping probability, i.e.maximum quantum yield of primary photochemistry in dark adapted samples;  $\Psi_{Eo}$ : probability that a photon trapped by the PSII reaction centre enters in the electron transport chain;  $\varphi Eo$ : maximum quantum yield for electron transport;  $\varphi Ro$ : maximum quantum yield for the reduction of final acceptors of electrons beyond PSI;  $\delta_{Ro}$ : probability that a nelectron is transported from reduced PQ to the electron acceptor side of PSI;  $\Delta V_{IP}$ : amplitude of the IP phase; Mo = initial slope of the normalized transient; Sm: total content of electron carriers for reaction centre; N: turnover number; K-Band: relative variable fluorescence at 300 µs; L-Band: relative variable fluorescence at 100 µs;  $PI_{ABS}$ : Performance Index on absorption basis;  $PI_{TOT}$ : Performance Index total.

### Results (Fig. 1)

<sup>s</sup> The PCA analyses evidenced three distinct clusters of parameters in each examined dataset. The parameters connected to the trapping of sun energy (Fv/Fm) and to the first photochemical events of electron transport (Ψeo, φEo, Pl<sub>ABS</sub>) form a cluster on the Factor 1 (Clusters 1, light blue), together with (but in a opposite position) the parameters connected to energy dissipation (Mo, K-Band, L-band. Clusters 2, yellow area). On the Factor 2 there are the parameters connected to the reduction of the final acceptors electron beyond the PSI (ΔV<sub>IP</sub>, φRo, δRo, Clusters 3, green areas) together with Sm and N. PI<sub>TOT</sub> is in intermediate position between Cluster 1 and Cluster 3. The overall photosynthetic efficiency can be synthetized with a parameter of the Cluster 1 (Fv/Fm) and a parameter of the Cluster 3 (ΔV<sub>IP</sub>).

Fact. 2 – 23%

Fig. 2 0.90 0.9 1.00 0.90 Fv/Fm Fv/Fm Fv/Fm Fv/Fm **2.C 2.**A **2.**B **2.D** 0.8 0.85 0.90 0.85 0.7 0.80 CV 0.6 0.80 0.80 0.75 0.5 0.70 0.70 0.75 0.4 0.65 0.3 0.70 0.60 0.60 Gembloux grassland (BE) 0.2 **Quercus ilex forest – Tuscany (IT)** FunDivEUROPE (Spain) 0.50 **FunDivEUROPE** (all sites) 0.65 Fv/Fm<0.5 0.55 0.1  $\Delta V_{IP}$  $\Delta V_{IP}$  $\Delta V_{IP}$  $\Delta V_{IP}$ 0.50 1.00 0.00 0.20 0.40 0.30 0.15 0.20 0.00 0.20 0.25 0.35 • CL PD CL MD CV MD • PN • PS • QF • CV PD 

**FIG. 2** – **Examples of correlation betwee Fv/Fm and**  $\Delta V_{IP}$  **in the different (total or partial) datasets. 2.B**. PN: Pinus nigra; PS: Pinus sylvestris; QI: Quercus ilex; QF: Quercus faginea. **2.C.** Site: Colognole (CL); Cala Violina (CV); Time of the day: PD (predawn); MD (midday). **2.E**.. Time of the day: PD (predawn); MD (midday); leaf position: Light-sun exposed leaves (L); Shade leaves (Sh). All the correlations shown in Fig. 2 are significant with P<0.05.

#### Results (Fig. 2)

In most cases the correlation between Fv/Fm and  $\Delta V_{IP}$  is positive (2.A; 2B), thus indicating a co-ordination between the events described by these parameters. Under stress condition, this positive correlation is due to a depression, at the same time, of both parameters (2C, circled: winter stress at CL; 2D, circled: extreme heat and drought stress).

Negative correlations derive from the trade-off between the trapping capicity (Fv/Fm) and the reduction of final acceptors ( $\Delta V_{IP}$ ) (2C, CV; 2.D; 2.E), as down-regulation mechanism in high light conditions.

#### Conclusions

<sup>0.45</sup> Fv/Fm and  $\Delta V_{IP}$  confirmed to be independent and complementary parameters able to characterize, in combination, the photosynthetic

