Supplementary information

More than just lipid balls: quantitative analysis of plastoglobule attributes and their stress-related responses

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Fig. S1 TEM images showing some of the PGs traits analysed in the present study. **a** Small sized (ø<50 nm) PGs in phyllids of the moss *Syntrichia ruralis*. **b** Middle sized (100<ø<200 nm) PGs in leaves of the fern *Hymenoglossum cruentum*. **c** Large-sized (ø>200 nm) PGs in leaves of winter acclimated *Buxus sempervirens*. **d** PGs showing differential osmiophilicity in phyllids of the moss *Syntrichia ruralis* after rapid mild desiccation and subsequent rehydration. **e** Clusters of PGs (pearl necklace-like) in phyllids of the moss *Syntrichia ruralis* after rapid mild desiccation and subsequent rehydration. **f** Clusters of PGs (grape-like) in phyllids of the moss *Syntrichia ruralis* after rapid desiccation and subsequent rehydration. Bar size = 0.5 µm (**a** and **d**), 0.2 µm (**b**), 1 µm (**c**, **e**, **f**).

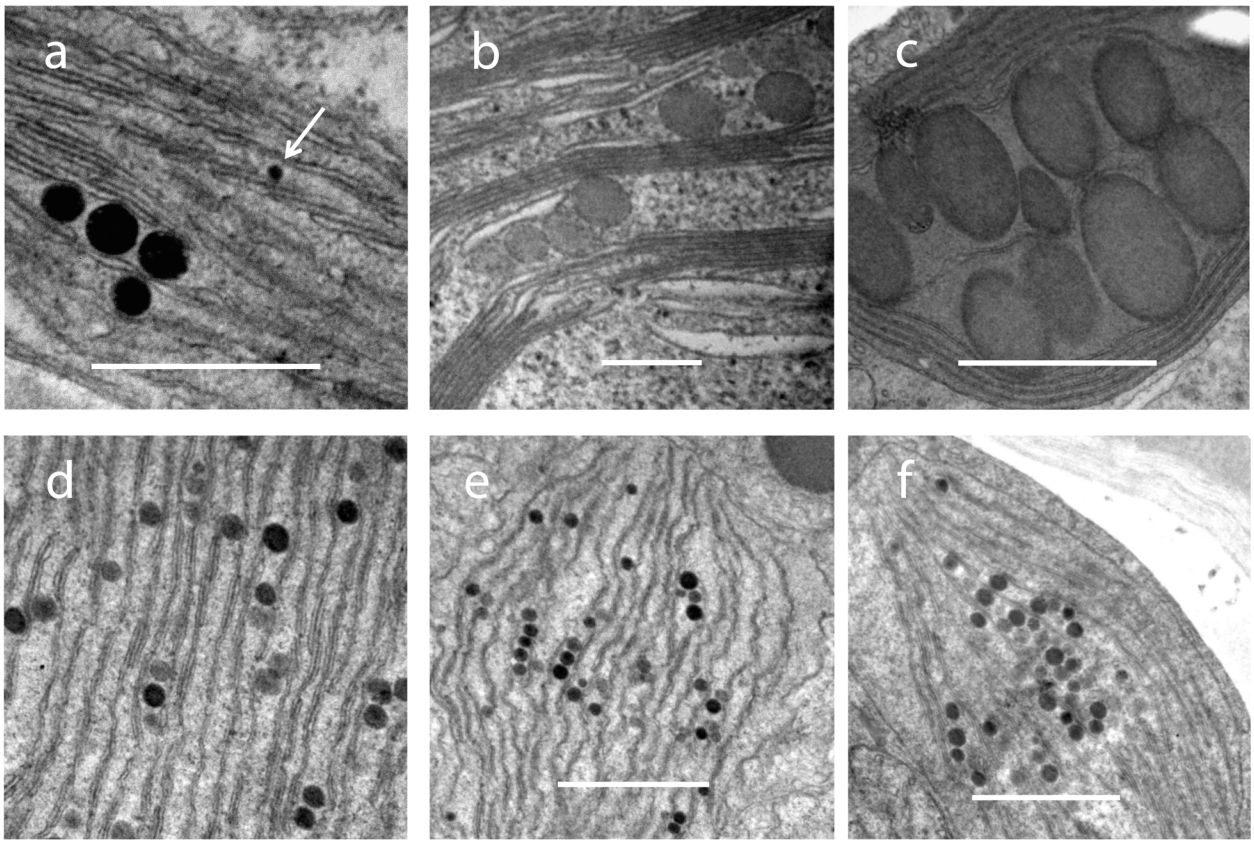


Fig. S2 Relation between numerical data provided by the authors of the article and data obtained by us through image analysis from figures of the same article. **a** Chloroplast cross section area (CA). **b** Total area of PG per chloroplast cross section. **c** Percentage of chloroplast cross section area occupied by the total PG area (%PG). **d** Number of PG per chloroplast cross section (NPG). **e** Diameter of PGs (ØPG). The continued line represents the linear trend line of the data and the dashed line the 1:1 relationship. Statistically significant differences are indicated by the asterisks (P<0.05).

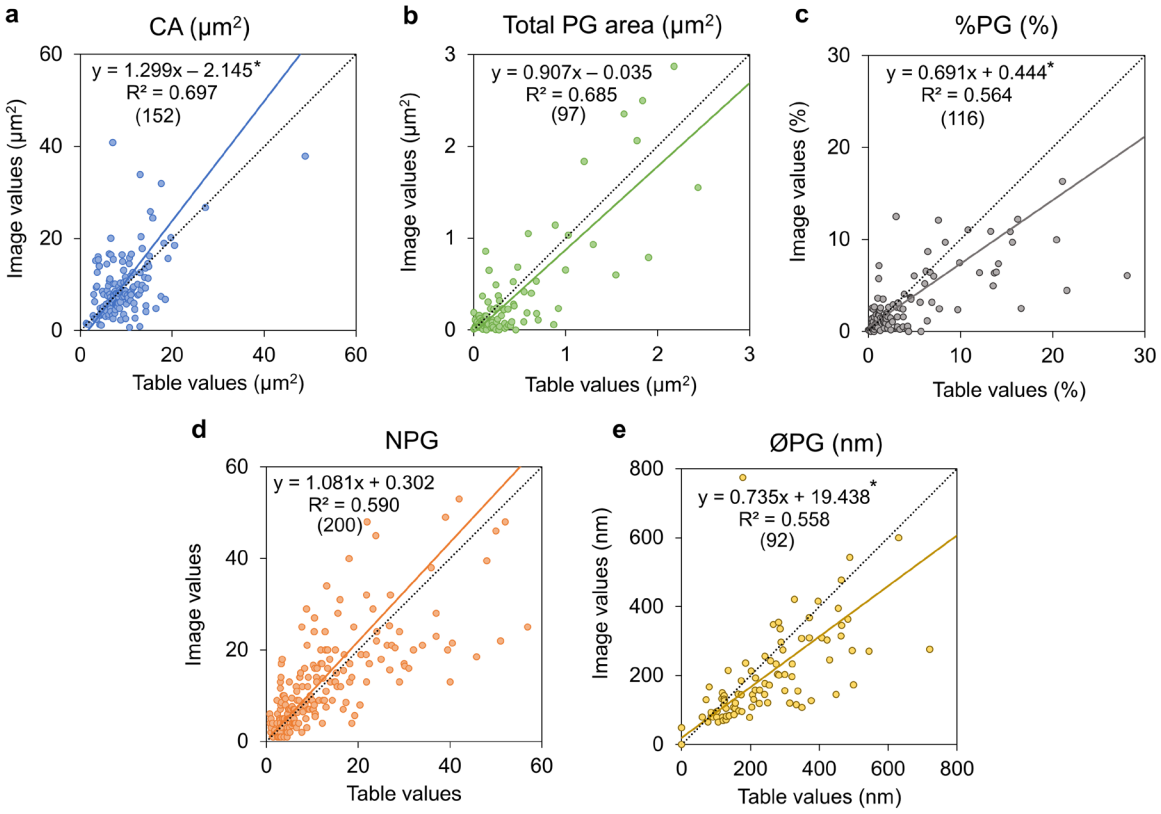
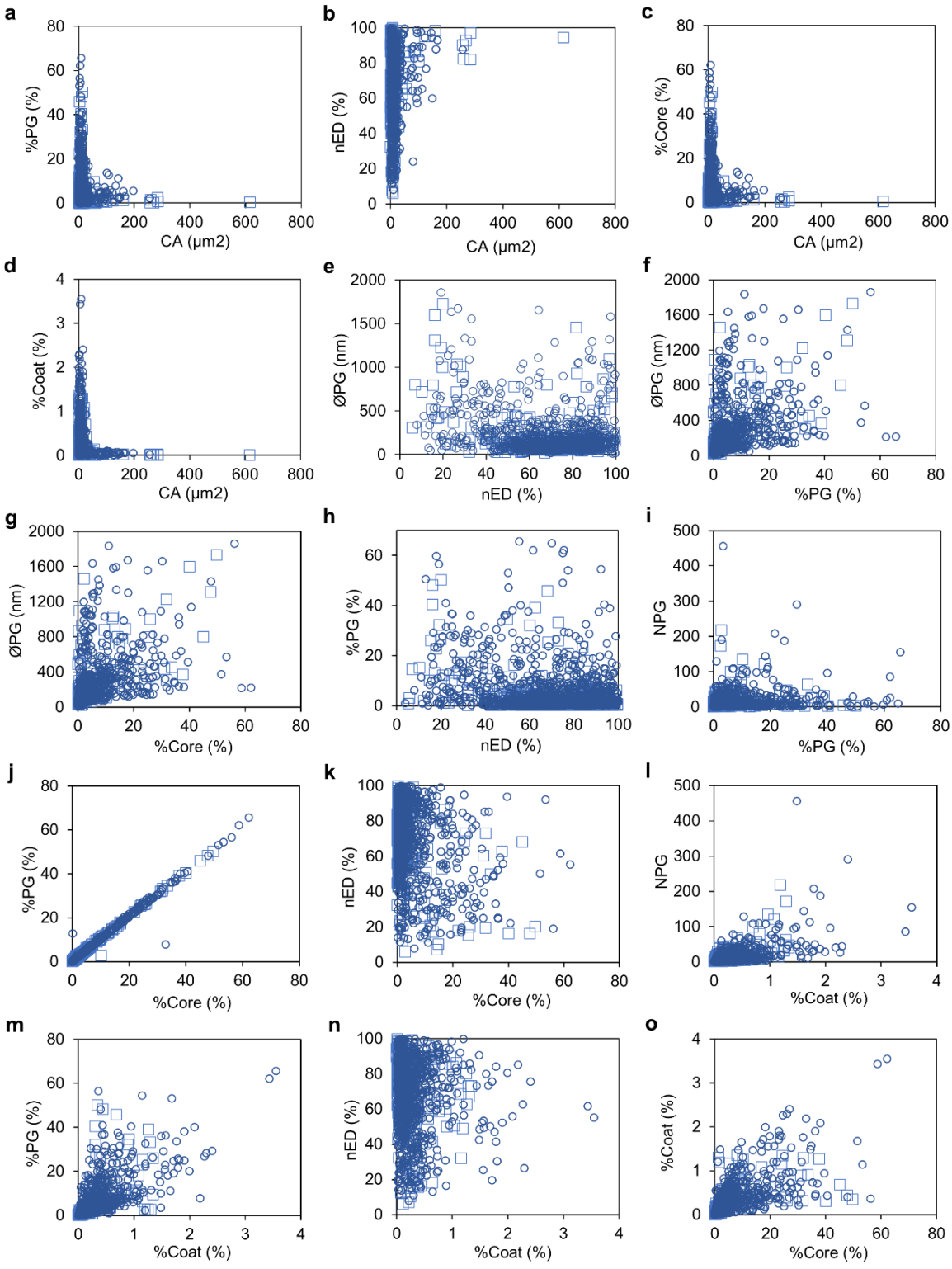


Fig. S3 Correlations between the parameters analysed in the study not shown in Fig. 3. **a** Percentage of chloroplast cross section area occupied by the total PG area (%PG) compared to chloroplast cross section area (CA). **b** Normalised electrodensity (nED) compared to CA. **c** Percentage of chloroplast cross section area occupied by the total core area (%Core) compared to CA. **d** Percentage of chloroplast cross section area occupied by the total coat area (%Coat) compared to CA. **e** Diameter of PG (ØPG) compared to nED. **f** ØPG compared to %PG. **g** ØPG compared to %Core. **h** %PG compared to nED. **i** Number of PGs per chloroplast cross section (NPG) compared to %PG. **j** %PGs compared to %Core. **k** nED compared to %Core. **l** NPG compared to %Coat. **m** % PGs compared to %Coat. **n** nED compared to %Coat. **o** %Coat compared to %Core. The circles represent the values of stress conditions and the squares the values of non-stress conditions.


a
%PG (%)
CA (µm2)
b
nED (%)
CA (µm2)
c
%Core (%)
CA (µm2)
d
%Coat (%)
CA (µm2)
e
ØPG (nm)
nED (%)
f
ØPG (nm)
%PG (%)
g
ØPG (nm)
%Core (%)
h
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l
NPG
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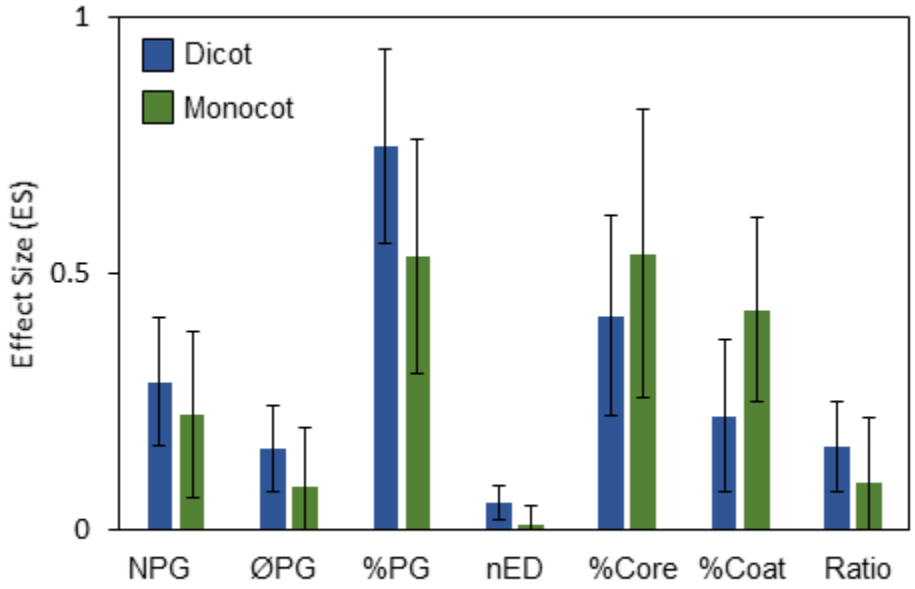
m
%PG (%)
%Coat (%)
n
nED (%)
%Coat (%)
o
%Coat (%)
%Core (%)

Fig. S4 Comparison of the response to toxicity between dicot and monocot species. nED, normalised electrodensity; NPG, number of PGs per chloroplast cross section; Ratio, %Core/%Coat ratio; ØPG, diameter of individual PG; %Coat, percentage of chloroplast cross section area occupied by the total coat area; %Core, percentage of chloroplast cross section area occupied by the total core area; %PG, percentage of chloroplast cross section area occupied by the total PG area.



Supplementary Table S1. Description of the different stress types present in this analysis.

Stress type	Description	Examples
Toxicity	Any damaging external substance applied to the plants.	*heavy metals*: lead, copper, silver, cadmium, chromium, titanium, boron, yttrium, nickel… *NaCl or sea water* (50mM, 100mM, 200mM…) *herbicides*: glyphosate, paraquat, paclobutrazol, atrazine, DTBP, fusillade, DCMU, isoxaflutole, diurion, difenzoquat… *other substances*: caffeine, phosphite, phosphate, industrial ash, mine soil, contaminated water, SO_2, NO_X, NaF, NH_4NO_3...
Senescence	The last stage of the development, when a coordinated degenerative process takes place.	Differences among a time period (days to weeks)
Pathogens	Any damaging pathogen or toxin released by the pathogens	*fungi*: *Botrytis cinerea*, *Capnodium, Acremonium zonatum, Cercospora beticola*… *virus*: plum pox virus (PPV)… *insects*: *Nothotrioza*, gall…
Deficiency	Deficiency of an essential element for the plant.	*nitrogen shortage:* 125mmolN/m^2 , 30% or 0% of the original dose, 40ppm or 120ppm… *magnesium shortage:* 30% or 0% of original dose… *phosphorous shortage:* 30% or 0% of original dose *boron shortage* *calcium shortage* *potassium shortage* …
Ozone	Application of elevated ozone levels on plants.	50ppb, 100ppb, 150ppb… 50-100 µl l^{-1} h^{-1} ...

Drought	Application of a shortage of water availability to plants.	*control*: well watered or -1.6MPa *drought:* PEG application, 7-10% of control watering, -4MPa, 50 or 70%RWC, total withdraw of water for an specific period of time (hours, days, weeks…)…
Temperature	Exposition to sub- or supra-optimal temperatures	*low temperature:* 4ºC for 2 hours, 5ºC, 10ºC, gradual decrease from 24/15ºC to 9/4ºC, gradual decrease from 23/15ºC to 8/1ºC, 2ºC during 10 days… *high temperature:* 40ºC for 40 min…
Light Intensity	Overall changes in light intensity, either high or low irradiance.	30%, 50% or 100% of max PFD 40, 50, 100, 200, 400 µmol m^{-2} s^{-1} 30, 60 or 90% of shadow …
Plant development	Studies where the plant was analysed during different developmental stages, in the absence of any additional stress factor.	Comparison of plant leaf expansion stages Comparison of young or old leaves Differences among ages (1 year vs. 5-6 years) …
Seasonal variation	Observation of seasonal-related changes in morphological traits	Spring, summer, autumn, winter
Desiccation/rehydration	Application of a shortage of water availability to plants that can tolerate a loss up to 95% of their water content and recover afterwards (resurrection plants).	*dehydration:*6 days, 8 days, 9 days, 13 days… *rehydration*: 1 hour, 2 hours, 4 hours, 8 hours, 12 hours and 24 hours…
Elevated CO_2	Application of elevated levels of CO_2 (e.g. above ambient concentration)	*control*: around 350- 400ppm *stress*: 600-750ppm
Others	Other types of stressors not included in the previous categories.	Age, high altitude, chlorosis, growth conditions, genotype, acid rain…