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Functional Traits in Palms

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ABSTRACT

Protocol to measure functional traits in palms that are strongly associated with growth and survival strategies, which correspond to structural traits, photosynthetic and biochemical traits, and dispersal traits.

GUIDELINES

Collected the data during wet season mainly to avoid stress by drought.

MATERIALS

- A perforating tool of known area.
- Pruning Tools.
- Metric tape.
- Refrigerator.
- Zip lock bag.
- Absorbent paper.

- Ohaus Scout Pro Portable balance or similar.
- Digital caliper (Mitutoyo absolute 500).
- Oven.
- Glass slide for microscopy.
- Microscope.
- Liquid transparent glue.
- Fluorometer, ex. PAM-2500 Portable Chlorophyll Fluorometer (Walz).
- 5 L Dimethyl sulfoxide (DMSO)
- Water–bath.
- Eppendorf tubes.
- Cuvette for spectrophotometer.
- Spectrophotometer Shimadzu UV-1800, Kyoto, Japan.
- Nikon Forestry Pro Laser Rangefinder/Height Meter.

1 Functional Traits in Palms

Structural traits

1.1 Sample leaves corresponding to non-senescent, completely expanded and without self-shadow being the third or the fourth leaf of the crown counting from the spear.

Then select ten to 20 leaflets from the second third section of the leaf to take lamina samples. Take lamina samples from the second third section of each leaflet with a perforating tool of 121 mm^2, avoiding the central vein.

1.2 Leaf Area (LA):
It is measured with a metric tape, taking the rachis length (b), without petiole, and the widest part of the leaf (a) tocalculate the approximated leaf area based on ellipse area: A=πa'b' (Eq. 1), were a' corresponds to a/2 and b' corresponds to b/2.

1.3 Specific Leaf Area (SLA), Leaf Dry Matter Content (LDMC) and Leaf Thickness (TH): Water saturate the lamina samples in a humid camera, made with a ziplock bag and a humid towel, for 12h, at 4^{o}C in complete darkness. You can store the ziplock bags in a black plastic bag and then put them on an Icopor box, or directly to the refrigerator.

Measure leaf fresh weight from the saturated lamina samples with an Ohaus Scout Pro Portable balance, or similar. To measure lamina thickness use a digital caliper.
After weighed, oven dry the lamina samples during 72h to obtain dry weight.
Specific leaf area and leaf dry matter content are calculated following Pérez-Harguindeguy et al. (2013).

1.4 Stomatal Density: Stomatal counting is performed preparing an epidermal impression of the leaf sample on a glass slide for microscopy using liquid transparent glue. After removing the leaf, the glass was placed under the microscope to count the number of stomata imprinted. Stomatal density is then calculated as the number of stomata per area.

Photosynthetic and biochemical traits

2 Physiological and biochemical measurements are potential indicators of palm responses to light. These are rapid light response curves (RLC), total chlorophyll content (ChlT mmol*g-1), and chlorophyll a/b ratio (Chlab).

2.1 Rapid light response curves are measured at field using a PAM-2500 Portable Chlorophyll Fluorometer (Walz), or similar. The RLC comes as a default routine of the PamWin-3 software of the PAM 2500 fluorometer (Walz, Effeltrich, Germany, 2008). Measurement is made on three leaflets collected, from 10 a.m. to 14 p.m. during sunny days. The leaflet is disposed under dark conditions for acclimation to obscurity for 1 minute approximately and then submitted to the increasing intensities of actinic light provided by the fluorometer. A mean value is calculated for each plant measured.

2.2 

Total chlorophyll and chlorophyll a/b ratio are measured at the laboratory. Chlorophyll is measured using dimethyl sulfoxide (DMSO) extraction method (Tait and Hik 2003). Lamina samples are immersed in one milliliter of DMSO and kept in the dark at field. Once at the laboratory, samples are placed in a water–bath maintained at 60^{O}C for one hour. After that, four to five repeated washings with DMSO are necessary to perform until total extraction.

Sometimes the chlorophyll extraction needs to be diluted several times, depending on color density. After dilution it is transferred to a cuvette for spectrophotometer readings at 480, 649.1 and 665.1 nm (for an UV spectrophotometer).

Finally, the chlorophyll concentration is calculated using the following equations for a UV: spectrophotometer (Wellburn, 1994):
Chla = 12.47A665.1 - 3.62A649.1 (Eq. 2)
Chlb = 25.06A649.1–6.5A665.1 (Eq. 3)
Chlx+c = (1000A480–1.29Chla–53.78Chlb)/220 (Eq. 4)

Once the formula is applied, final content is expressed in millimole (mmol) of chlorophyll per dry mass (g) and per area (mm2). Chlorophyll a/b ratio is calculated by dividing Chla by Chlb.

Dispersal Traits

3 Stem height (H m) is measured with a Nikon Forestry Pro Laser Rangefinder/Height Meter or with a metric tape depending on the species height. This trait is considered as both structural and dispersal trait.

Fruit size (FS, diameter in cm) and fruit number (FN, fruits per bunch) were derived from literature and botanical garden herbarium collections (COPPETEC-UFRJ 2019). Material selection was made based on species occurrences reported at the studied areas.

Fruit size is considered a surrogate for seed size, while some palms produce one seed per fruit and the fruit flesh is considerable thin around the seed.

Fruit number is derived as categorical variable, from one to four, being 1 for species with less than 20-30 fruits per bunch, 2 between 50-100, 3 between 100-500 and 4 for species with more than 500 fruits per bunch.