Variation in temperature tolerance in grasses and palms and their relationship with leaf functional traits

Thesis submitted towards the partial fulfilment of BS-MS Dual degree programme

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Certificate

This is to certify that this dissertation entitled "Variation in temperature thermotolerance in grasses and palms and their relationship with leaf functional traits" towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Himanshu Lagachu at the Indian Institute of Science Education and Research (IISER), Pune under the supervision of Dr. Deepak Barua, associate professor, Biology department, IISER Pune, during the academic year 2019-2020.

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Declaration

I hereby declare that the matter embodied in the report entitled “Variation in temperature thermotolerance in grasses and palms and their relationship with leaf functional traits” are the results of the work carried out by me at the Department of Biology, Indian Institute of Science Education and Research (IISER), Pune under the supervision of Dr. Deepak Barua and the same has not been submitted elsewhere for any other degree.

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Contents

Abstract

There exists very little literature about the upper limits of temperature tolerance of grasses and palms. This information is essential for assessing their differential vulnerability to change in temperature. This study investigated the upper limits of temperature tolerance of grasses and palms and looked at their relation with leaf traits such as Leaf Mass per Area (LMA) and Leaf Dry Matter Content (LDMC). It was found that there was a significant difference between the upper limits of temperature tolerance of grasses and palms, with grasses having a much lower heat resistance than palms. With extreme temperature events and heatwaves predicted to increase in frequency in the future, grasses are at a high risk as their habitat temperatures are approaching their upper limit of temperature tolerance. This study found a positive correlation between temperature tolerance and LMA and LDMC. This differential vulnerability to change in temperature amongst high LMA and low LMA species could lead to directional changes in our vegetation towards high LMA species. Since high LMA species are slow resource acquiring species with low productivity, this could alter the sink strength of atmospheric carbon for our vegetation and further exacerbate climate change.

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Introduction

According to the ‘State of the Climate 2019’ published by the American Meteorological Society, 2019 was the 2nd warmest year recorded since records started in 1880. There was a +0.95°C departure from the average global land and ocean surface temperature. The five warmest years since records started in 1880–2019 have all occurred since 2015, while nine of the 10 warmest years have occurred since 2005. With global mean temperatures on the rise, heatwaves are projected to become more frequent. A rise of 1.5°C in the global mean temperature is projected to cause a 4-fold increase in the frequency of heatwaves from the current levels in India (Mukherjee et al., 2018). As a result many organisms are already being exposed to unprecedented extreme temperatures. And the frequency of these extreme events are projected to rise (Blunden et al., 2019). This exposure to extreme temperatures in plants could cause disruption of respiration and photosynthesis (Berry and Björkman, 1980; Huve et al., 2011; O’Sullivan et al., 2013), and permanent leaf tissue damage (Huve et al., 2011; O’Sullivan et al., 2013).

Different plants have different levels of tolerance to high temperature. This ability to tolerate high temperatures is called the ‘thermotolerance’ of that species. This study will be examining the thermotolerance of Palms (Arecaceae), Grasses and Bamboo (Poaceae) and Sedges (Cyperaceae). Poaceae or Gramineae is a family of monocotyledonous flowering plants commonly known as grasses. Bamboos belong to the sub-family Bambusoideae within the family of Poaceae. Plants commonly referred to as palms fall in the botanical family of Arecaceae in the monocot order Arecales.

Previous work done in our group has focused on thermotolerance in tropical woody species and tropical herbaceous species in Northern Western Ghats (Mohan, 2017; Poddar, 2018; Sastry and Barua, 2017; Sastry et al., 2017). Investigating thermotolerance in palms and grasses would help in gaining a more comprehensive understanding of plant thermotolerance in this region and help us compare thermotolerance variation between grasses, palms, herbs and woody tree species. Moreover grasses provide protection from soil erosion and are an essential part of the ecosystem and studying their thermotolerance limits could help us study and predict their differential vulnerability to change in temperatures and distribution in the future. Although some work has been done on heat shock proteins (Xu et. al., 2011) and root thermotolerance of grasses (Rachmilevitch et al., 2008; Xu et. al., 2015) there exist very little literature about the upper limits of temperature tolerance of the leaves of tropical grasses and palms.

Thermal Safety Margin is defined as the difference between the upper limits of temperature tolerance and maximum habitat temperature that a species faces (Deutsh et al., 2008; Doughty and Goulden, 2009; O'Sullivan et al., 2016). The maximum habitat temperature from the poles to the equator differs by 20°C, while the difference between the thermotolerance of the species found in these two habitats differed by approximately 8°C (O'Sullivan et al., 2016). Tropical species therefore have a narrower thermal niche breadth and in comparison with temperate species have lower thermal safety margins. Due to this, the upper limits of thermotolerance of temperate species is much higher than their current and projected future habitat temperatures but the same cannot be said about tropical species. In the case of increased frequency of high temperature events, tropical plants could prove to be vulnerable. Exposure to temperatures even near but below the T_{50} values could lead to irreversible damage to leaf tissue, ultimately affecting its performance in its habitat.

Previous studies have shown a positive relation between thermotolerance and leaf traits like Leaf Mass per Area and Leaf Dry Matter Content (Mohan, 2017; Poddar, 2018; Sastry and Barua, 2017) while other studies have shown no correlation (O'Sullivan et al., 2016). Leaf Mass per Area or LMA is positively correlated with stress tolerance and leaf life span, but negatively correlated with growth rate and photosynthetic rate (Wright et al., 2005). A change in forest cover from low LMA species to high LMA species could lead to a fall in primary productivity and the carbon uptake would be reduced. This means that species with a high LMA have a slower growth rate and are not as efficient a carbon sink as fast growing species with high photosynthetic rates.

To measure thermotolerance this study looked at the changes in dark-adapted chlorophyll *a* fluorescence, or Fv/Fm, with respect to temperature. This is one of the most common methods for measuring thermotolerance (Buchner et al., 2017; Krause, 2010), and is considered to be a good indicator of photosynthetic and organismal heat tolerance (Barua et al., 2003; Havaux et al., 1991). Light energy absorbed by chlorophylls associated with PSII can be used to drive photochemistry in which an electron (e−) is transferred from the reaction centre chlorophyll, P680, to the primary quinone acceptor of PSII, QA. Alternatively, absorbed light energy can be lost from PSII as chlorophyll fluorescence or heat. The processes of photochemistry, chlorophyll fluorescence, and heat loss are in direct competition for excitation energy (Baker, 2008). If the rate of one process increases the rates of the other two will decrease. So by measuring the change in the fluorescence with respect to temperature we get a measure of the efficiency of PSII photochemistry. In this study T_{50} was termed as the temperature when the Fv/Fm value fell by 50%, when compared to its value at room temperature. This T_{50} value was used as the upper limit of temperature tolerance.

This study will be investigating the upper limit of temperature tolerance of grasses and palms and try to assess their differential vulnerability. This study will look at T_{50} values and its relations with leaf traits namely Leaf Mass per Area (LMA) and Leaf Dry Matter Content (LDMC) and how they vary amongst different functional groups. This could help us understand how vulnerable these species are to heat stress, identify species that maybe particularly vulnerable and direct conservational efforts towards species more vulnerable to rising temperatures in its habitat.

Materials and Methods

Study sites and Sample Collection

Fifty-two species of plants were sampled for this experiment. The grass and palm species were chosen based on their availability and distribution in our sampling area, while the tree and herb species were chosen to represent the full spectrum of thermotolerance across their functional group. The sampling location were:

i) IISER Pune Campus, Pune, Maharashtra (18°32’41.1”N 73°48’25.4”E)

ii) J.E Farms, Pune, Maharashtra (18°37’46.5”N 73°43’07.5”E)

Pune is a city in the Indian State of Maharashtra and its climate is characterised by hot and dry summers from March to May and monsoons lasting from June to September/October. The average annual rainfall is 76.3 cm (India Meteorological Department) concentrated between July and October. The mean maximum temperature ranges from 37.4°C in April to 27.4°C in August. While the mean minimum temperature ranges from 12.2°C in January to 23°C in June (climate-data.org). Sampling for thermotolerance assays was carried out from mid July to end of September and additional data collection for leaf traits was done in early December. For all species fully mature leaves were selected which were exposed to direct sunlight.

For each species 4 to 6 replicate individuals were sampled (in cases where 6 replicates were not available, 4 or 5 replicates were sampled as per availability). For each replicate 12 to 15 leaves were sampled. In some cases where an individual didn't have 12 leaves, leaf samples from multiple individuals were collected, which were then considered to be one replicate.

The leaves were collected and packed in airtight ziplock bags which contained a wet tissue paper. The leaf samples were then transported to the lab where their fresh weights were measured, the leaves were scanned to measure leaf area and then thermotolerance assays were performed. After the thermotolerance assays, the leaves were dried in an oven at 70°C for 72 hours, and then weighed for dry weight.

Thermotolerance Assays

For the thermotolerance assays, a cork borer and a pair of sharp scissors were used to make leaf sections of approximately 0.8 cm in diameter. The leaf sections were carefully cut so as to avoid any major veins. 8 leaf disks per replicate were used in the thermotolerance assay. The leaf disks were then packed between two layers of muslin cloth and covered with an aluminium foil and then packed in airtight ziplock bags. The ziplock bags were then covered with a wire mesh to prevent it from floating and then placed in a water bath (Julabo, Model F25, Seelbach, Germany) where heat treatment was performed for 30 mins. In this manner 8 leaf disks were used per individual to perform 8 different heat assays (25°C, 40°C, 42.5°C, 45°C, 47.5°C, 50°C, 52.5°C and 55°C), with 25°C being the control. After 30 mins in the water bath, the leaf disks were taken out and placed in a petri dish with wet tissue paper. The petri dish was then covered and set aside for 24 hours for recovery.

After the completion of 24 hours the leaf disks were placed in a black opaque cloth for 30 mins for dark adaptation. After 30 mins dark adapted chlorophyll *a* fluorescence (*Fv/Fm*) was then measured with a PAM 2500 fluorometer (Walz, Effeltrich, Germany)

Measurement of Leaf Traits

Measurements of leaf traits was done by two methods:

i) Cutting leaf disks of approximately 0.8 cm in diameter and measuring leaf traits
ii) Measuring leaf traits of whole leaves (no cutting/punching)

For each species 6 replicate individuals were chosen and for each replicate 3 disks and 6 leaves were measured. Whole leaves of palms were not collected as the size of their leaves and sheath was too large and in most cases palms had 6-8 total leaves on the entire individual. The leaves were collected and put in an airtight ziplock bag with wet tissues for water saturation. The fresh-weights of the water saturated leaves/disks were measured as soon as it was removed from the ziplock bag. The leaves/disks were then scanned using a *CanoScan Lide 110 scanner* (Canon). The scanned images were used to calculate the area of the leaves/disks using the software ImageJ (version 1.51). After scanning the leaves/disks was completed, they were put inside brown paper bags and dried in an oven at 70°C for 48 hours minimum. After the drying process was completed, the dry-weights of the leaves/ disks were measured. Using the fresh-weight, dry-weight and leaf area data we calculated Leaf Area (LA), Leaf Mass per Area (LMA) and Leaf Dry Matter Content (LDMC). LMA is the ratio of the dry-weight to leaf area and LDMC is the ratio of the dry-weight to the fresh-weight.

Statistical Analysis

Temperature response curves (TRCs) of *Fv/Fm* were generated to estimate thermotolerance. A four-parameter logistic sigmoid curve was fitted to the chlorophyll *a* fluorescence (Fv/Fm) values across the range of temperatures examined using the R package ‘drc’.

$$f(x,(b,c,d,e)) = c + \frac{d-c}{1+exp[b(log(x)-log(e))]}$$

Here coefficient *b* denotes the steepness of the curve. *c* and *d* denotes the lower and upper asymptotes or limits of the response respectively and *e* denotes the halfway response between the upper and the lower limits (Ritz et al. 2015). When *Fv/Fm* drops to half of its control value, the corresponding temperature value was used as the T_{50}. Species level curves were then generated to determine the T_{50} of the particular species.

Kruskal–Wallis test by ranks (One-way ANOVA on ranks) was performed to see the effect of Plant Functional Types (PFT) and species on T_{50}. Similarly Kruskal–Wallis test was done for LMA, LDMC and LA. Mann–Whitney *U* test (Wilcoxon rank-sum test) was also performed to compare the functional groups for T_{50}, LMA, LDMC and LA. Spearman's rank correlation coefficient (Spearman’s ρ) was used to look at correlations between T_{50} and leaf traits (LMA, LDMC, LA). All statistical tests and correlations were done in R.

Results

Fv/Fm values in the temperature response curves dropped to zero between 47.5°C to 52.5°C for most grasses, while some bamboos dropped to zero or near zero between 50°C to 55°C, and palms showed the highest thermotolerance and only dropped to near zero at approximately 55°C. Representative temperature curves of different functional groups are shown in Figure 1.

Previous studies by this group has shown thermotolerance to vary between functional groups (Poddar, 2018) and our results confirm the same. Grasses had the lowest T_{50} amongst the functional groups. *Eleusine indica* (ELIN) had the lowest T_{50} amongst grasses at 44.39°C +/-0.16 and *Paspalum notatum* (PANO) had the highest amongst grasses at

48.49°C +/-0.30. Most grasses had a T_{50} value between 45°C to 47°C. Bamboos had a higher thermotolerance in comparison to grasses, *Sasa palmata* (SAPA) had the lowest T_{50} amongst bamboos at 47.74°C +/-0.21 while *Bambusa vulgaris* (BAVU) had a value of 51.61°C +/-0.19. Palms had the highest thermotolerance of the functional groups with its lowest *Dypsis lutescens* (DYLU) at 49.74°C +/-0.14 and its highest *Ravenala madagascariensis* (RAMA) at 53.37°C +/-0.64. Figure 5 shows the variation in thermotolerance between functional groups with each point representing a species. Kruskal–Wallis test by ranks on thermotolerance and leaf traits had a significant p-value for both PFT and species (table 1, table 2).

Spearman's correlation coefficient was used to look at the relationships between leaf traits (LMA, LDMC and LA) and T_{50}. LMA and LDMC had a significant positive correlation with T_{50} for both disks and whole leaves. LA for disks showed a significant positive correlation with T_{50} while LA for whole leaves did not show a significant correlation. The correlation coefficients are given in table 4.

On comparing mean maximum monthly habitat temperatures and T_{50}, it was observed that the T_{50} of all the species were above the mean maximum monthly temperatures. In the case of a future 6°C warming, the T_{50} values will still be above the mean maximum habitat temperatures. However it must be noted that these temperature values are just an average of the monthly highest temperatures. In this experiment, the leaves were subjected to just 30 minutes of heating, which could imply that even a short duration of exposure to temperatures higher than T_{50} could cause significant irreversible damage. On comparing maximum habitat temperatures recorded (not mean) and T_{50}, it was found that even a 3°C rise in the temperatures would mean that some of the grasses will be exposed to temperatures higher than their T_{50}. A 6°C rise would mean that all of the grasses would be exposed to temperatures higher than their T_{50} and even some bamboos. Thus putting them at a very high risk of habitat loss.

Discussion

Of the 52 species that this study investigated, T_{50} values ranged from the highest for *Ravenala madagascariensis* (RAMA) at 53.37°C +/-0.64 while the lowest was *Eleusine indica* (ELIN) at 44.39°C +/-0.16. T_{50} values differed greatly amongst functional groups with Palms having the highest thermotolerance, grasses the least with bamboos in-between. We observed a positive correlation between T_{50} and LMA and T_{50} and LDMC. An earlier study done by this group on tropical trees had similar results (Sastry and Barua, 2017; Mohan, 2017). We saw a weak positive correlation between T_{50} and LMA and T_{50} and LDMC for each functional group separately (grasses, palms and bamboos). This could be due to low sampling size. This could also be due the fact that palms have a high T_{50} and high LMA while grasses and bamboos have lower T_{50} and lower LMA and on plotting them together they even each other out to show a positive correlation between T_{50} and Leaf Traits.

The differential performance of the various functional groups could be an indicator of their performance under higher temperatures in the future. As the frequency of heatwaves and extreme temperature events rises (Mukherjee and Mishra, 2018; Blunden and Arndt, 2019) we could either see a shift in vegetation towards functional groups which have higher thermotolerance, towards species within the same functional group or towards individuals within the same species which are more heat resistant.

The highest temperatures recorded in Pune are around 42°C. In this study, multiple species showed drops in their *Fv/Fm* values at these temperatures when exposed for 30 minutes. So even if these temperatures are below the T_{50}s of all the observed species in this study, these could do irreversible damage to the leaves (Mohan, 2017). In the case of future warming by 3°C or in the extreme case of 6°C, the maximum habitat temperatures for even some palms (the most thermotolerant functional group in our study) will be higher than their T_{50}. This could cause irreversible damage to the leaves. Grasses with a lower T_{50} are at a higher risk and this should be investigated further as grasses are an important habitat for insects, small animals and birds and also to avoid environmental degradation like soil erosion.

Alternatively we might see a shift in the grass cover towards species with high thermotolerance and high LMA which can withstand higher temperatures in the future.

Studies have shown that high LMA species have a positive correlation with lower photosynthetic rate and slow leaf resource acquisition (Wright et al. 2005; Reich et al. 2014). If rising temperatures result in high LMA species outperforming low LMA species, a shift in vegetative cover towards species with high LMA, which are slow growing and less productive, could take place. Since vegetative cover act as carbon sinks, this could alter their sink strength for atmospheric carbon and lead to higher CO_2 concentration in the atmosphere and thus exacerbate further climate change.

Conclusion

There is a differential vulnerability to change in temperature amongst plant species within our study site. Species with high thermotolerance and high LMA could perform better than species with low thermotolerance and low LMA. This could result in compositional changes to our forest cover and our landscape. Average plant productivity and carbon sequestration could go down due to the selection pressure towards low productive (high LMA) species. This could reduce the carbon sink strength of our vegetative cover and have implications on climate change. Grasses are at a high risk as their habitat temperatures are approaching their upper limits of temperature tolerance and this could reduce grass cover in the future. However this needs to be investigated further and compositional changes need to be monitored closely, along with investigation of alternative heat tolerance strategies.

Figures and Tables

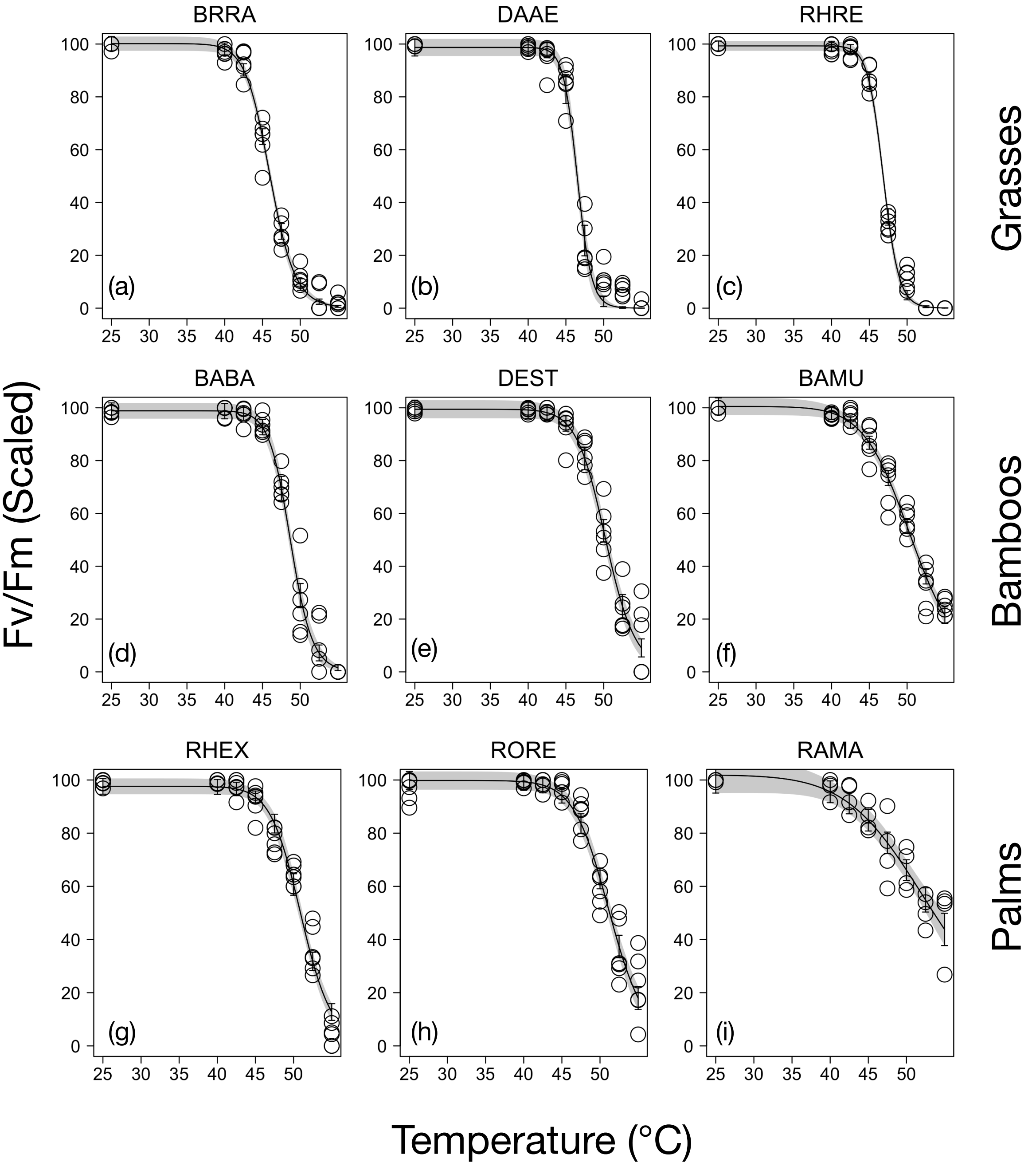

Figure 1: Representative species of functional groups i) Grass (low thermotolerance) ii) Bamboo (intermediate thermotolerance) iii) Palms (high thermotolerance)
a) TRC of Brachiaria ramosa, b) TRC of Dactyloctenium aegyptium, c) TRC of Rhynchelytrum repens, d) TRC of Bambusa bambos, e) TRC of Dendrocalamus strictus, f) TRC of Bambusa multiplex, g) TRC of Rhapis excelsa, h) TRC of Roystona regina, i) TRC of Ravenala madagascariensis.

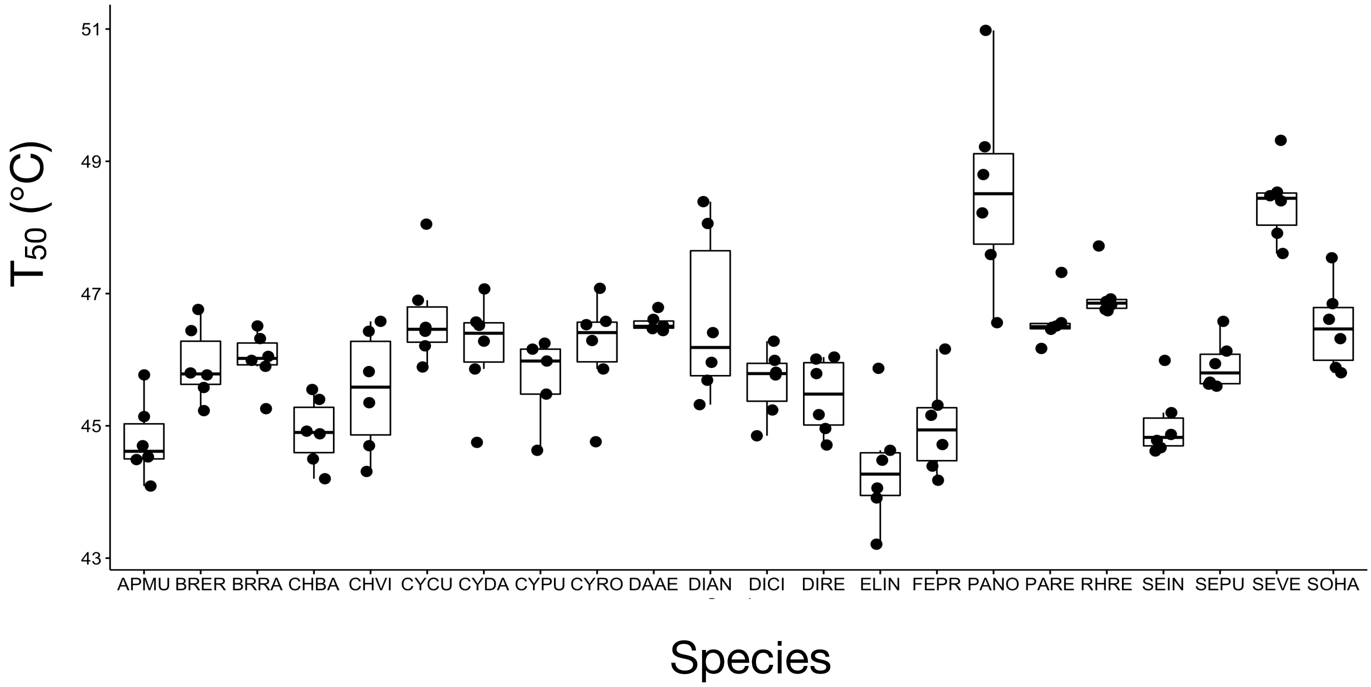

Figure 2: Variation in thermotolerance amongst Grasses. Each boxplot represents the distribution of T_{50} (°C) values for the respective species. Each point representing a replicate of that particular species.

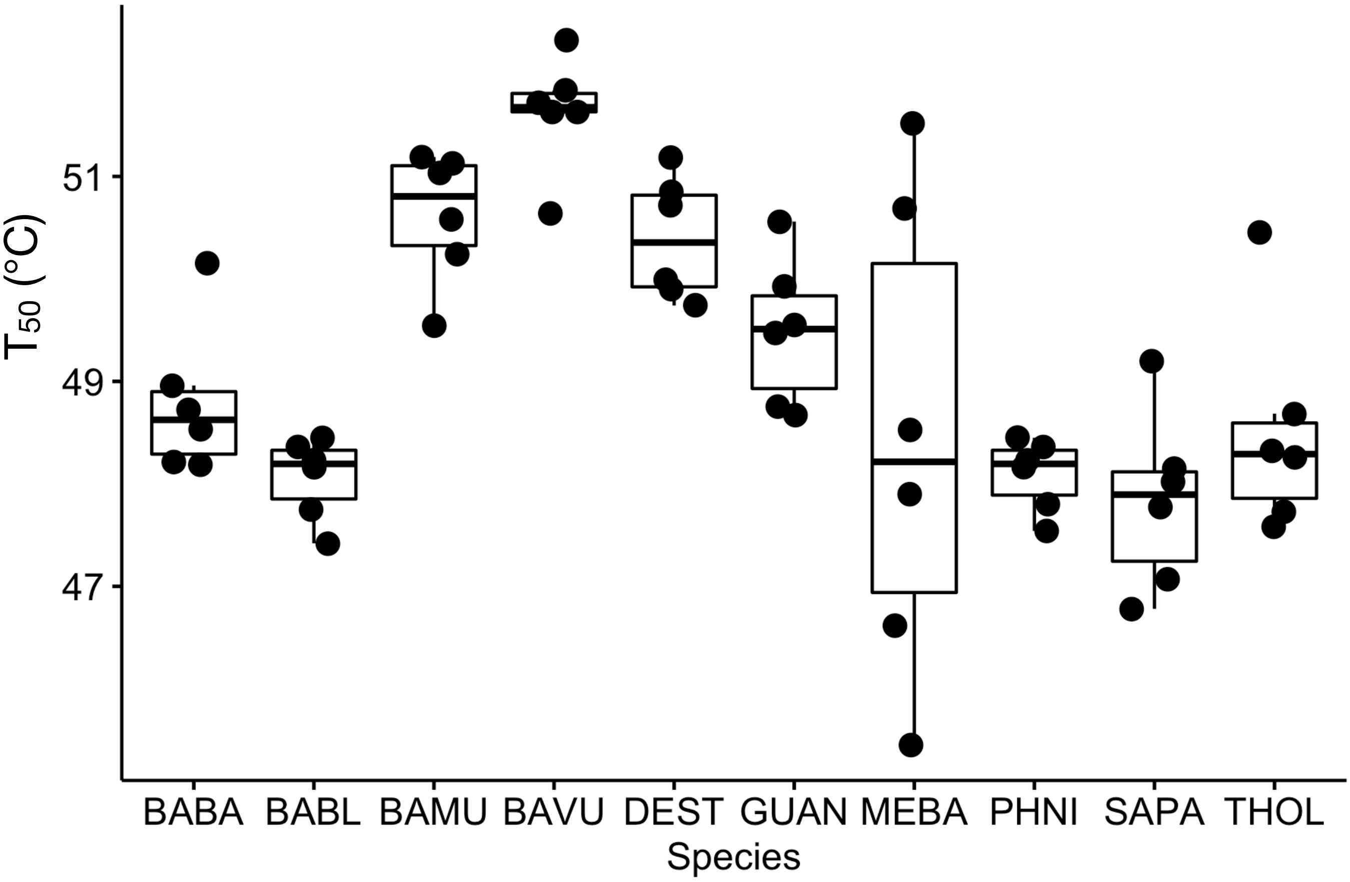

Figure 3: Variation in thermotolerance amongst Bamboos. Each boxplot represents the distribution of T_{50} (°C) values for the respective species. Each point representing a replicate of that particular species.

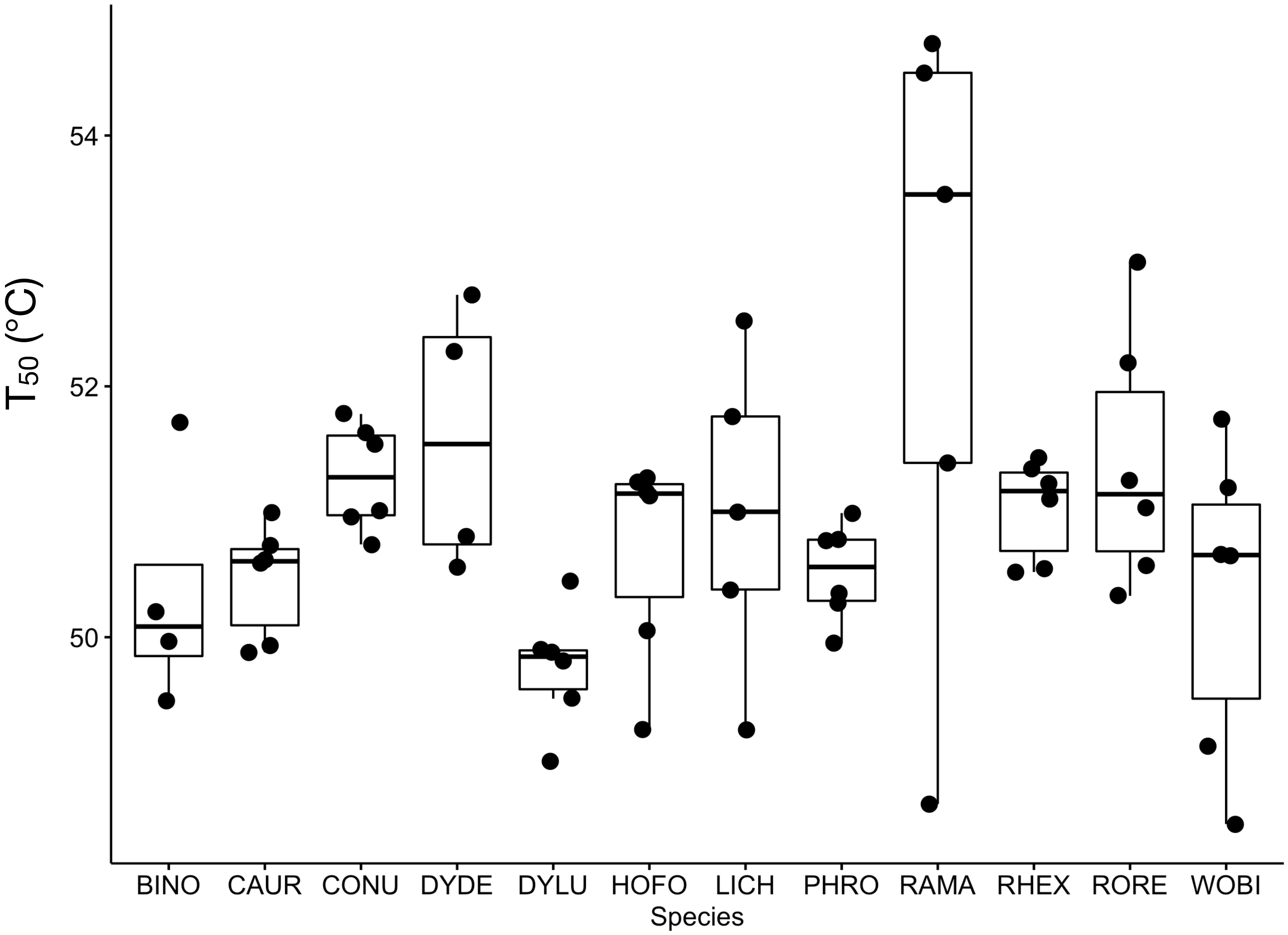

Figure 4: Variation in thermotolerance amongst Palms. Each boxplot represents the distribution of T_{50} (°C) values for the respective species. Each point representing a replicate of that particular species.

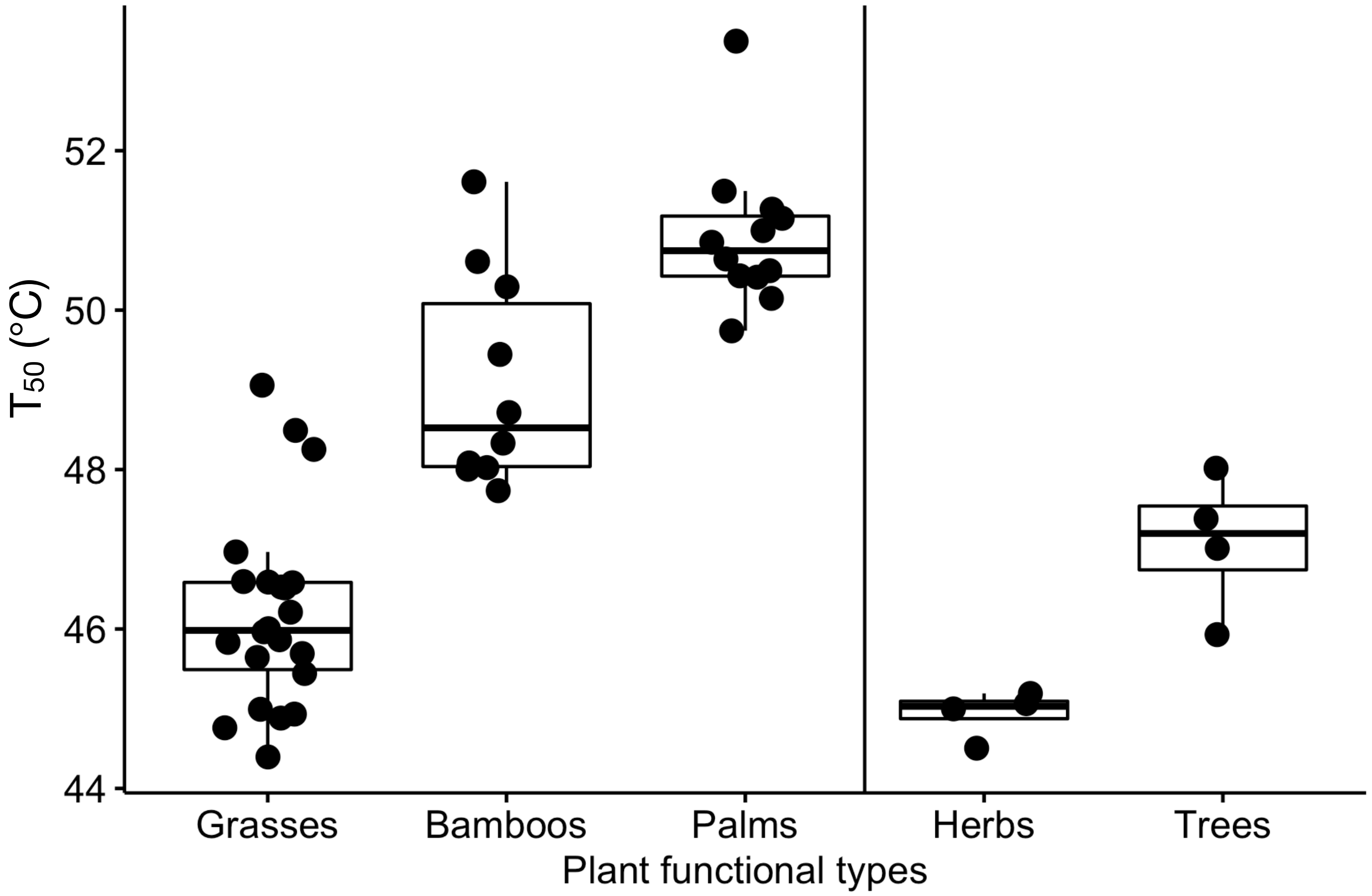

Figure 5: Variation in thermotolerance with plant functional type. Each boxplot represents the distribution of T_{50} (°C) values for the respective functional type. Each point representing the species mean T_{50} (°C).

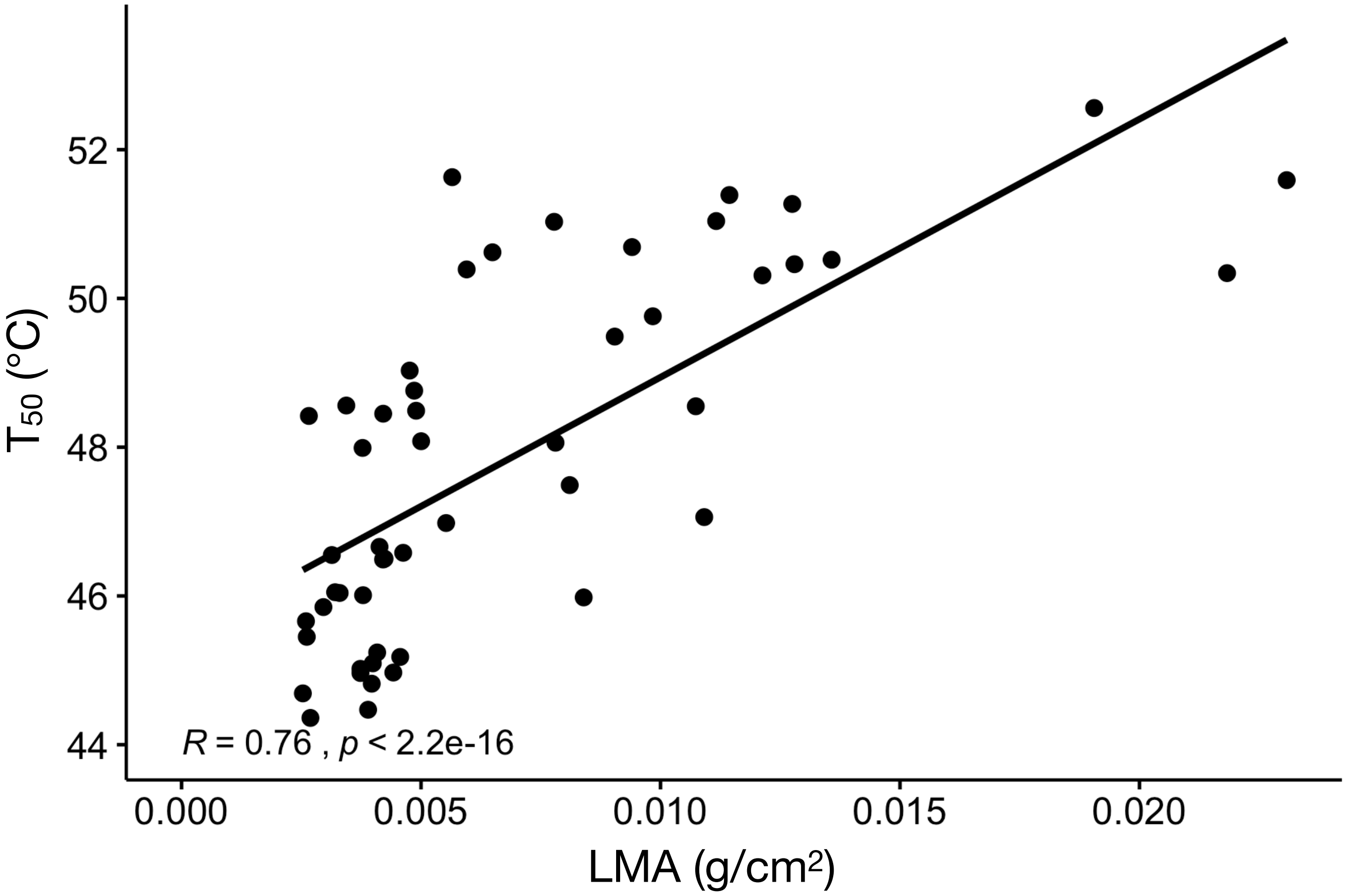

Figure 6: Relationship of T_{50} (°C) with LMA (g/cm^2) of leaf disk cuttings. Each data point representing the species mean T_{50} (°C) and LMA (g/cm^2) value

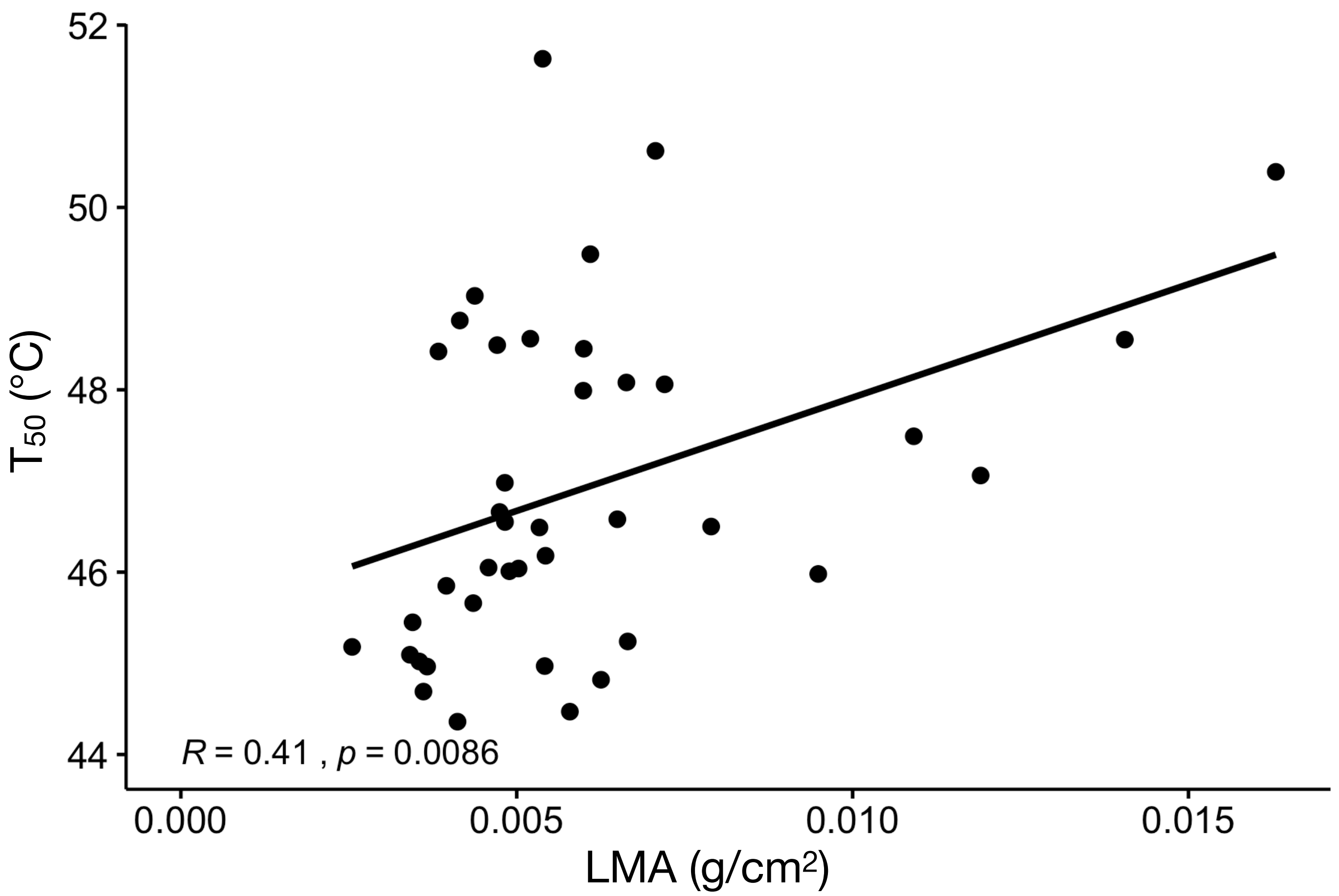

Figure 7: Relationship of T_{50} (°C) with LMA (g/cm^2) of whole leaves (no cuttings). Each data point representing the species mean T_{50} (°C) and LMA (g/cm^2) value.

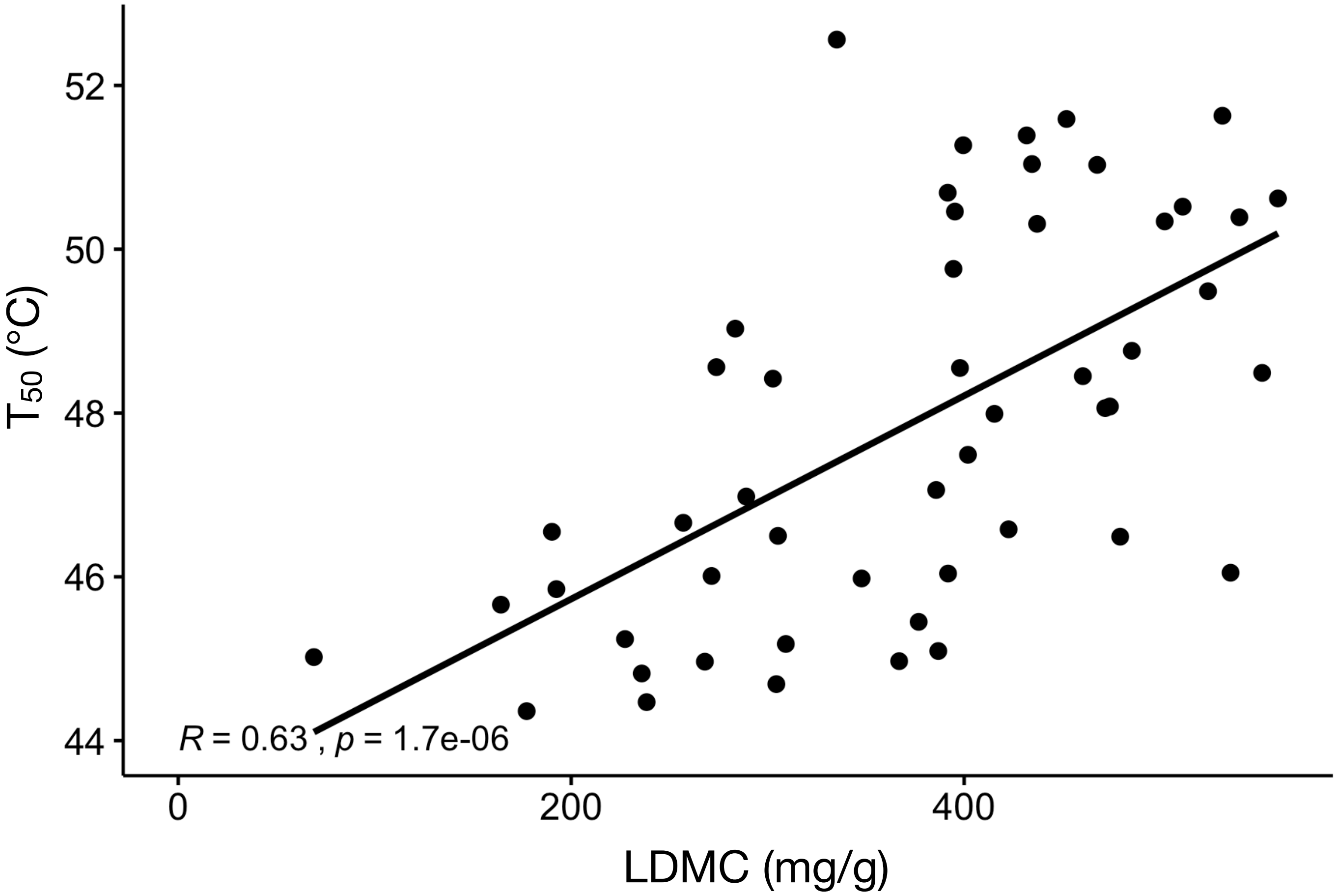

Figure 8: Relationship of T_{50} (°C) with LDMC (mg/g) of leaf disk cuttings. Each data point representing the species mean T_{50} (°C) and LDMC (mg/g) value

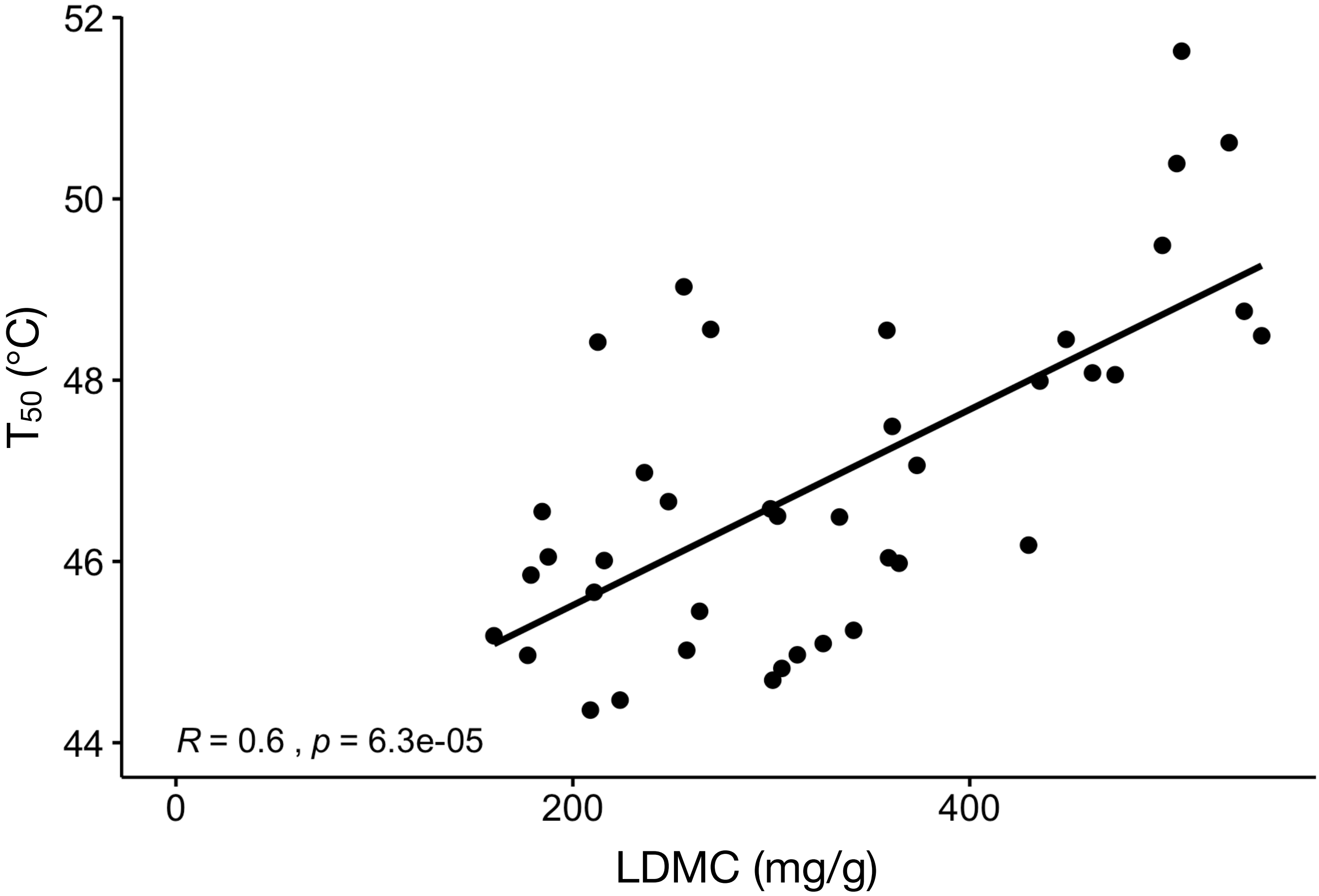

Figure 9: Relationship of T_{50} (°C) with LDMC (mg/g) of whole leaves (no cuttings). Each data point representing the species mean T_{50} (°C) and LDMC (mg/g) value.

Effect	DF	chi-squared	p-value
PFT	4	38.13	< 0.001
Species	51	271.29	< 0.001

Table 1: Variation in thermotolerance with plant functional type (PFT) and species. Results of a Kruskal–Wallis test by ranks (One-way ANOVA on ranks) examining the effect of species and PFT on T_{50}. P-values are significant ($\alpha < 0.05$).

Effect	df	chi-squared	p-value
a)Variation in Leaf Traits for LMA			
PFT	4	39.136	< 0.001
Species	50	259.29	< 0.001
b)Variation in Leaf Traits for LDMC			
PFT	4	29.555	< 0.001
Species	50	260.05	< 0.001

Table 2: Variation in leaf traits (a)LMA (b)LDMC for leaf disks with plant functional type (PFT) and species. Results of a Kruskal–Wallis test by ranks (One-way ANOVA on ranks) examining the effect of species and PFT on (a)LMA (b)LDMC for leaf disks. P-values are significant ($\alpha < 0.05$)

	df	chi-squared	p-value
a) Variation in Leaf Traits for LMA			
PFT	3	18.121	< 0.001
Species	39	193.09	< 0.001
b) Variation in Leaf Traits for LDMC			
PFT	3	27.459	< 0.001
Species	39	213.11	< 0.001

Table 3: Variation in leaf traits (a)LMA (b)LDMC for whole leaves with plant functional type (PFT) and species. Results of a Kruskal–Wallis test by ranks (One-way ANOVA on ranks) examining the effect of species and PFT on (a)LMA (b)LDMC for whole leaves. P-values are significant ($\alpha < 0.05$)

	p-value on comparison with T_{50}	r-value on comparison with T_{50}
a) Spearman correlation coefficient matrix of leaf disks traits and thermotolerance		
LMA	< 0.001	0.76
LDMC	< 0.001	0.63
a) Spearman correlation coefficient matrix of whole leaf traits and thermotolerance		
LMA	0.0086	0.41
LDMC	< 0.001	0.6

Table 4: Spearman correlation coefficient matrix of leaf traits and thermotolerance for (a)leaf disks and (b)whole leaves. P-values are significant ($\alpha < 0.05$)

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Appendix

Table S1: List of sampled species. Site abbreviations: I – IISER Pune, J – J.E Farms

	Species name	**Collection Site**	**Family**	**Acronym Used**	**Plant Type**
1	*Apluda mutica* L.	I	Poaceae	APMU	Grass
2	*Bambusa balcooa* Roxb.	J	Poaceae	BABL	Bamboo
3	*Bambusa bambos* (L.) Voss	I	Poaceae	BABA	Bamboo
4	*Bambusa multiplex* (Lour.) Raeusch. ex Schult.	I	Poaceae	BAMU	Bamboo
5	*Bambusa vulgaris* Schrad.	J	Poaceae	BAVU	Bamboo
6	*Bismarckia nobilis* Hildebr. & H.Wendl.	I	Arecaceae	BINO	Palm
7	*Brachiaria eruciformis* (Sm.) Griseb.	I	Poaceae	BRER	Grass
8	*Brachiaria ramosa* (L.) T.Q.Nguyen	I	Poaceae	BRRA	Grass
9	*Caryota urens* L.	I	Arecaceae	CAUR	Palm
10	*Chloris barbata* Sw.	I	Poaceae	CHBA	Grass
11	*Chloris virgata* Sw.	I	Poaceae	CHVI	Grass
12	*Chromolaena odorata* (L.) R.M.King & H.Rob.	I	Asteraceae	CHOD	Herb
13	*Cocos nucifera* L.	I	Arecaceae	CONU	Palm
14	*Cynodon dactylon* (L.) Pers.	I	Poaceae	CYDA	Grass
15	*Cyperus cuspidatus* Kunth	I	Poaceae	CYCU	Grass
16	*Cyperus pumilus* L.	I	Poaceae	CYPU	Grass
17	*Cyperus rotundus* L.	I	Poaceae	CYRO	Grass
18	*Dactyloctenium aegyptium* (L.) Willd.	I	Poaceae	DAAE	Grass
19	*Dalbergia sissoo* DC.	I	Fabaceae	DASI	Tree
20	*Dendrocalamus strictus* (Roxb.) Nees	I	Poaceae	DEST	Bamboo
21	*Desmodium triflorum* (L.) DC.	I	Fabaceae	DETR	Herb
22	*Dichanthium annulatum* (Forssk .) Stapf	I	Poaceae	DIAN	Grass
23	*Digitaria ciliaris* (Retz.) Koeler	I	Poaceae	DICI	Grass
24	*Dinebra retroflexa* (Vahl) Panz.	I	Poaceae	DIRE	Grass
25	*Dypsis decaryi* (Jum.) Beentje & J.Dransf.	I	Arecaceae	DYDE	Palm

	Species name	Collection Site	Family	Acronym Used	Plant Type
26	*Dypsis lutescens* (H.Wendl.) Beentje & J.Dransf	I	Arecaceae	DYLU	Palm
27	*Eleusine indica* (L.) Gaertn.	I	Poaceae	ELIN	Grass
28	*Ficus benghalensis* L.	I	Moraceae	FIBE	Tree
29	*Festuca pratensis*	I	Poaceae	FEPR	Grass
30	*Guadua angustifolia* Kunth	J	Poaceae	GUAN	Bamboo
31	*Howea forsteriana* (F.Muell.) Becc	I	Arecaceae	HOFO	Palm
32	*Ipomoea tricolor* Cav	I	Convolvulaceae	IPTR	Herb
33	*Lagerstroemia speciosa* (L.) Pers.	I	Lythraceae	LASP	Tree
34	*Livistona chinensis* (Jacq.) R.Br. ex Mart	I	Arecaceae	LICH	Palm
35	*Melocanna baccifera* (Roxb.) Kurz	J	Poaceae	MEBA	Bamboo
36	*Neolamarckia cadamba* (Roxb.) Bosser	I	Rubiaceae	NEKA	Tree
37	*Panicum repens* L.	I	Poaceae	PARE	Grass
38	*Paspalum notatum* Flüggé	I	Poaceae	PANO	Grass
39	*Phoenix roebelenii* O'Brien	I	Arecaceae	PHRO	Palm
40	*Phyllostachys nigra* (Lodd. ex Lindl.) Munro	J	Poaceae	PHNI	Bamboo
41	*Ravenala madagascariensis* So nn	I	Strelitziaceae	RAMA	Palm
42	*Rhapis excelsa* (Thunb.) Henry	I	Arecaceae	RHEX	Palm
43	*Rhynchelytrum repens* (Willd.) C.E.Hubb.	I	Poaceae	RHRE	Grass
44	*Roystonea regia* (Kunth) O.F.Cook	I	Arecaceae	RORE	Palm
45	*Sasa palmata* (Burb.) E.G.Camus	I	Poaceae	SAPA	Bamboo
46	*Senna obtusifolia* (L.) H.S.Irwin & Barneby	I	Fabaceae	SEOB	Herb
47	*Setaria intermedia* Roem. & Schult.	I	Poaceae	SEIN	Grass
48	*Setaria pumila* (Poir.) Roem. & Schult.	I	Poaceae	SEPU	Grass
49	*Setaria verticillata* (L.) P.Beauv.	I	Poaceae	SEVE	Grass
50	*Sorghum halepense* (L.) Pers.	I	Poaceae	SOHA	Grass

	Species name	**Collection Site**	**Family**	**Acronym Used**	**Plant Type**
51	*Thyrsostachys oliveri* Gamble	J	Poaceae	THOL	Bamboo
52	*Wodyetia bifurcata* A.K.Irvine	I	Arecaceae	WOBI	Palm

Table S2: Pairwise comparison matrix using Wilcoxon rank sum test for T_{50} and PFT

	Bamboo	Grass	Herb	Palms
Grass	0.0001	-	-	-
Herb	0.0040	0.0534	-	-
Palms	0.0057	3.6E-08	0.0027	-
Tree	0.0450	0.1120	0.0408	0.0027

Table S3: Pairwise comparison matrix using Wilcoxon rank sum test for LMA of leaf disks and PFT

	Bamboo	Grass	Herb	Palms
Grass	0.00022	-	-	-
Herb	0.03425	0.40917	-	-
Palms	6.2E-05	5.6E-08	0.00220	-
Tree	0.01332	0.00040	0.03571	0.06471

Table S4: Pairwise comparison matrix using Wilcoxon rank sum test for LDMC of leaf disks and PFT

	Bamboo	Grass	Herb	Palms
Grass	0.00012	-	-	-
Herb	0.00440	0.74783	-	-
Palms	0.00441	0.00082	0.00440	-
Tree	0.00440	0.08108	0.14286	0.14774