

Investigation of Metabolic Activity Versus Relative Water Content of Crop Leaves
Using Chlorophyll Content and Leaf-to-Soil Temperature Ratio

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Abstract

In this study, the relative water content (RWC) of the leaves of *Raphanus sativus* crops is measured, along with chlorophyll content index (CCI) and both leaf and soil temperatures. The relationships between CCI and leaf-to-soil temperature ratio are examined using linear regression. It was found that RWC has little impact on CCI, but that droughted plants have lower CCI on average than hydrated plants. The relationship between RWC and leaf-to-soil temperature ratio is stronger in droughted samples than in hydrated ones, suggesting that leaf temperature is more affected by water usage when under drought stress, and that susceptibility to heat stress is RWC-dependent.

1 Introduction

1.1 Background & Focus

Relative water content (RWC) is the measurement of crop hydration relative to saturated (aka turgid, hydrated) conditions, and is a standard indicator of plant water usage and drought stress (Arjenaki et al. 2012). RWC is calculated as follows:

$$\text{RWC} = \frac{m_{\text{fresh}} - m_{\text{dry}}}{m_{\text{turgid}} - m_{\text{dry}}} \times 100\%$$

Drought stress is a major concern for food producers, as it can significantly impact crop yield and food quality. Without sufficient hydration, plants are susceptible to overheating and reduced photosynthetic rate, leading to wilting and eventual crop loss.

This study focuses on the metabolic activity of *Raphanus sativus* (aka radish) crop leaves across differing levels of water usage, using RWC as an index. Plant leaves are selected because leaf tissue adapts most quickly to stress and is thus the most sensitive to dehydration. Furthermore, the leaves are the primary organs responsible for photosynthetic activity, and are as-such the site of a significant portion of a plant's metabolic activity.

1.2 Objectives & Hypotheses

The primary objective of this study is to investigate the existence of relationships between leaf RWC and both of: leaf chlorophyll content; and, the ratio between leaf temperature and soil temperature - these are chosen as reliable indicators of plant metabolic activity. It is firstly hypothesized that RWC will differ strongly between droughted and well-hydrated plants. It is also predicted that chlorophyll content will be lower in drought-stressed plants, and will correlate positively with RWC due to plant health factors. It is further hypothesized that the leaf-to-soil temperature ratio will be higher in droughted plants due to decreased transpiration, and will correlate negatively with RWC due to increased rates of evaporative cooling.

2 Methods

2.1 Sampling

A collection of crop plants have been cultivated under greenhouse conditions, with half of the plants receiving droughted treatment, and the other half as a control group. Drought treatment pots were watered one week prior to harvest to ensure that plants did not wilt or start to desiccate. Leaves from 4 plants (2 drought, 2 hydrated) were harvested by 15 sets of participants, producing 15 unique samples. Further ambient and soil measurements were also taken.

2.2 Data Collection

1. At-pot measurements are recorded, including: Soil moisture, electroconductivity, & temperature; light intensity; and relative humidity and leaf temperature.
2. Plants are harvested and each is labelled with the pot number. All soil is removed from the root system.
3. One complete leaf is sampled and the **fresh mass** (m_{fresh}) is taken. A photo is taken of the sample with its label and a ruler for scale.
4. Chlorophyll content readings are taken, and leaf samples are packaged inside a hydrated paper towel and, after resting for 24 hours, **turgid mass** (m_{turgid}) is taken.
5. Roots and shoots are separated and placed into a drying oven for one week, after which **dry mass** (m_{dry}) is taken.
6. Data from all samples are digitized and compiled into a single spreadsheet for analysis.

2.3 Analysis

Both chlorophyll content and leaf:soil temperature ratio are plotted against RWC for each sample group to determine if relationships exist. A linear regression is performed to determine the strength of each relationship.

3 Results

Table 1: Raw study data. Note: # indicates pot number, H indicates hydrated, D indicates droughted.

#	Trt.	Fresh (g)	Turgid (g)	Dry (g)	RWC	Soil °C	CCI	Leaf °C	L °C / S °C
1	H	0.93	1.21	0.02	76.5%	18.5	38.2	19.6	1.06
1	H	3.68	4.04	0.45	90.0%	18.3	32.5	19.8	1.08
1	H	5.70	7.85	1.02	68.5%	18.7	22.6	18.7	1.00
1	H	4.13	4.58	0.39	89.3%	18.3	2.50	18.6	1.02
2	H	4.91	5.46	0.27	89.4%	18.4	16.0	19.0	1.03
2	H	5.19	5.66	0.27	91.3%	18.7	14.3	18.7	1.00
2	H	5.55	6.32	0.34	87.1%	15.0	16.7	19.5	1.00
3	D	1.49	1.65	0.16	89.3%	19.4	18.3	20.0	1.03
3	D	1.65	1.97	0.22	81.7%	19.3	14.9	17.7	0.92
3	D	3.82	4.09	0.18	93.1%	19.0	16.8	21.5	1.13
4	D	12.07	13.77	1.19	86.5%	20.3	14.6	21.1	1.04
4	D	11.23	11.99	1.19	93.0%	19.8	11.5	20.3	1.03
4	D	9.60	10.25	0.66	93.2%	20.2	6.9	21.4	1.06
4	D	9.34	10.82	1.12	84.7%	19.9	30.4	20.5	1.03
4	D	7.03	7.65	0.58	91.2%	18.7	29.50	20.6	1.10

Table 2: Study data, averaged (mean) for each pot #.

#	Fresh (g)	Turgid (g)	Dry (g)	RWC	Soil °C	CCI	Leaf °C	L °C / S °C
1	3.61	4.42	0.47	81.1%	18.44	23.95	19.18	1.04
2	5.22	5.81	0.29	89.3%	17.39	15.66	19.08	1.11
3	2.32	2.57	0.19	88.0%	19.23	16.67	19.73	1.03
4	9.85	10.90	0.95	89.7%	19.78	18.58	20.78	1.05

Table 3: Study data, averaged (mean) for each treatment.

Trt.	Fresh (g)	Turgid (g)	Dry (g)	RWC	Soil °C	CCI	Leaf °C	L °C / S °C
H	4.30	5.02	0.39	84.6%	17.99	20.40	19.14	1.07
D	7.03	7.77	0.66	89.1%	19.58	17.86	20.39	1.04

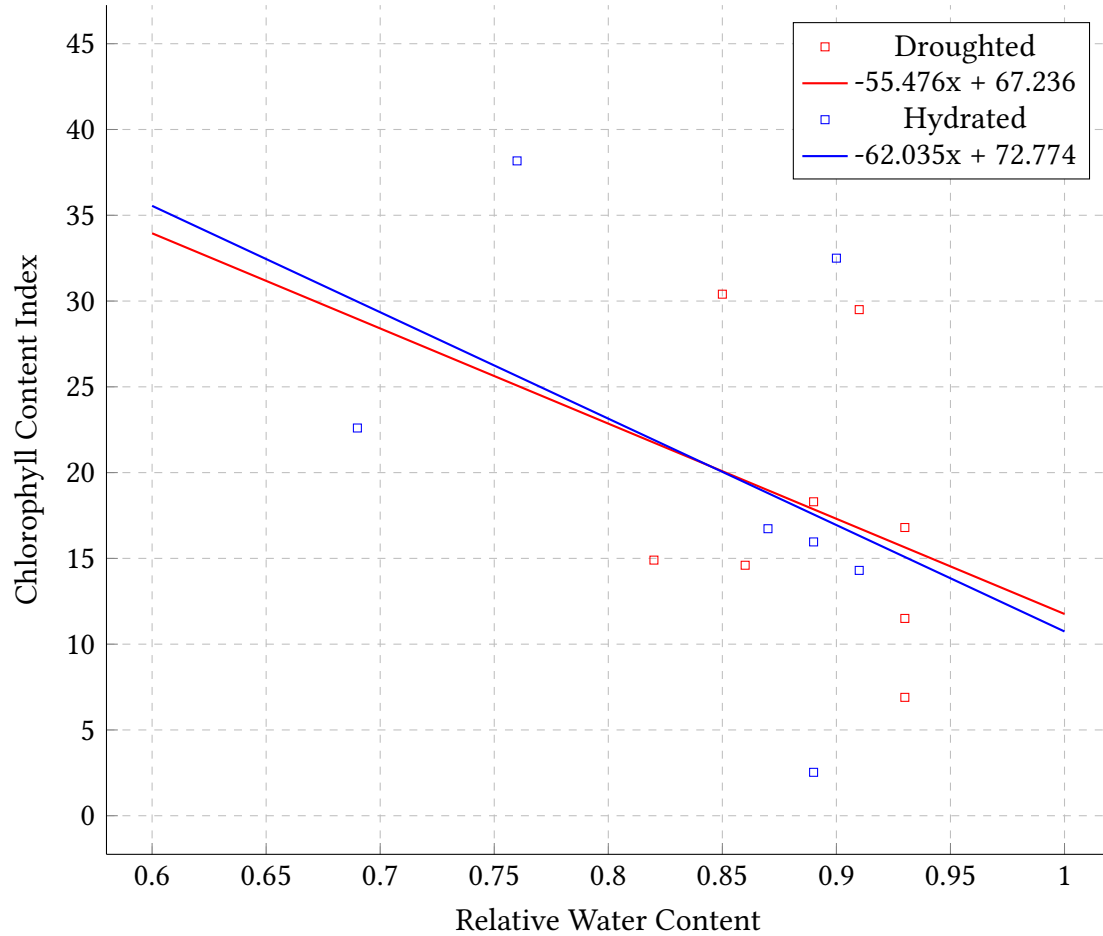


Figure 1: Chlorophyll Content Index (CCI) vs. Relative Water Content (RWC) in droughted (Pots #1 & #2) and hydrated (Pots #3 & #4) samples.

Droughted $R_D^2 = 0.0819$, Hydrated $R_H^2 = 0.1939$

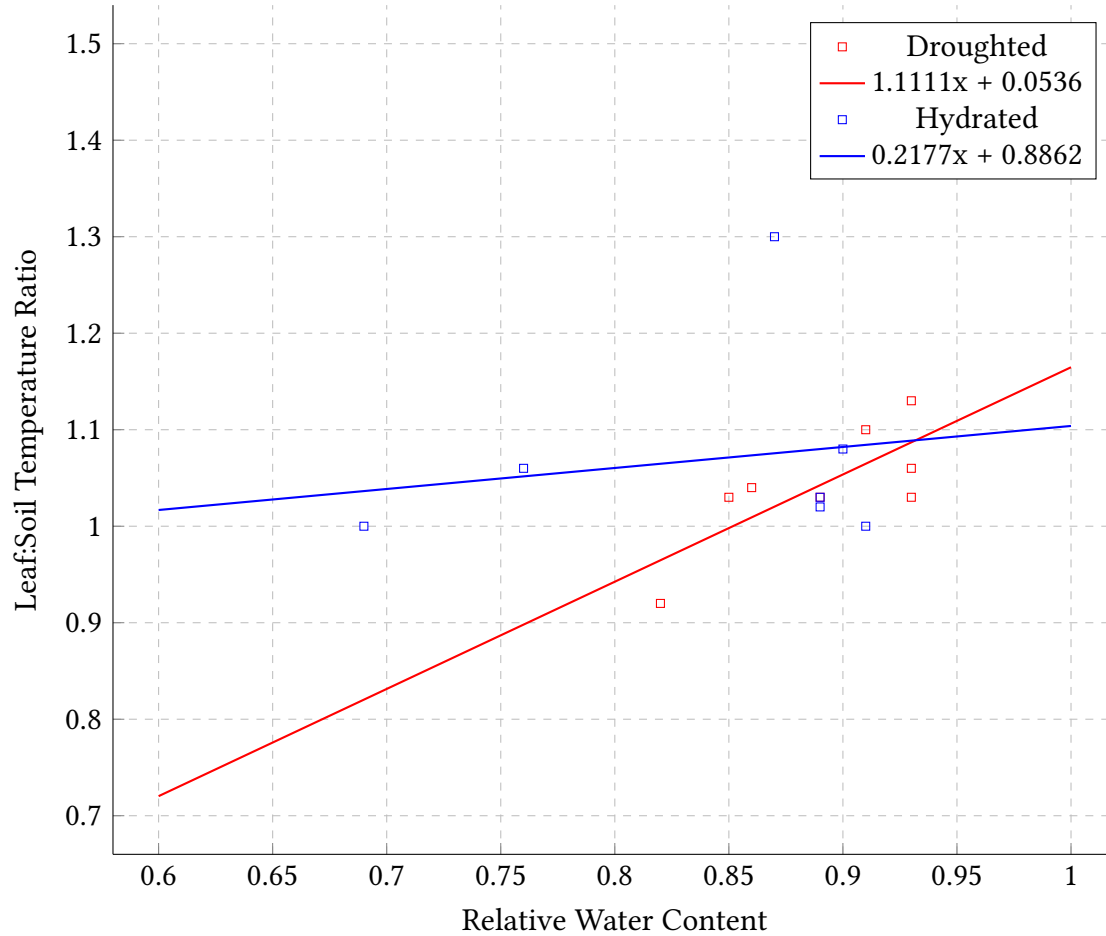


Figure 2: Leaf:Soil Temperature Ratio vs. Relative Water Content (RWC) in droughted (Pots #1 & #2) and hydrated (Pots #3 & #4) samples.

Droughted $R_D^2 = 0.5815$, Hydrated $R_H^2 = 0.0306$

4 Discussion

Relative Water Content

Drought treatment pots were watered one week prior to harvest to ensure that plants did not wilt or start to desiccate - this may have impacted the variance of RWC between droughted and hydrated samples: there was very little difference in mean RWC between the two groups (Droughted Mean RWC = 89.1%, Hydrated Mean RWC = 84.6%), and the RWC in droughted plants was in fact on-average higher than in hydrated plants. This suggests that the drought treatment may not have been severe enough to significantly impact RWC, and that other factors (such as differences in sampled leaf position and light exposure) may have had greater influence.

Chlorophyll Content Index

Chlorophyll content is a good indicator of photosynthetic activity, and as such was expected to be higher in well-hydrated plants. The relationship between RWC and CCI is weak, with R^2 values of 0.0819 and 0.1939 for droughted and hydrated samples, respectively.

There is, however, a significant difference between the two: droughted plants have a lower average CCI than hydrated plants (17.86 vs. 20.40), suggesting that drought stress has a negative impact on photosynthetic activity, which is supported by the literature (Yetik and Candoğan 2023). Furthermore, the strength of the relationship between RWC and CCI is more than double in hydrated samples, suggesting that the metabolic activity of hydrated plants is more affected by water usage than that of droughted plants.

Leaf:Soil Temperature Ratio

The leaf-to-soil temperature ratio was lower on average in droughted plants than in well-hydrated samples, suggesting that the metabolic activity of droughted plants is decreased - however, the difference is negligible (only 3%), and as such could likely be explained by differences in leaf position or light exposure. Furthermore, the relationship between RWC and leaf:soil temperature ratio is significantly stronger in drought-stressed samples than in hydrated ones ($R_D^2 = 0.5815 > R_H^2 = 0.0306$), suggesting that leaf temperature is far more affected by water usage when under

drought stress (more than 50% of variance). This may also indicate that droughted plants are more susceptible to heat stress.

More than one plant per pot may have also introduced crowding in the canopy, causing leaf:soil temperature to be consistently high and weakening the strength of correlation between RWC and leaf:soil temperature ratio. This may explain the positive y-intercepts in the regression equations, especially in hydrated samples where leaves are more likely to create a fuller canopy.

Conclusion

Contrary to findings in the literature, drought stress (even when combined with possible heat stress) did not impact dry biomass production Lama et al. 2023; rather, mean biomass of the drought-stressed group was higher than that of the hydrated group. The lack of significant difference in RWC between the two groups may also have contributed to this result.

Leaf relative water content might influence chlorophyll content more under hydrated conditions, but the relationship remains weak overall in this study, despite findings in the literature. By contrast, differences in chlorophyll content index on-average between the sample groups are significant - droughted plants display less metabolic activity, possibly due to heat stress, which is further indicated by the temperature-related findings and those in the literature (Fu et al. 2022). The increased variance of CCI with RWA in hydrated samples perhaps highlights the importance of irrigation and precision agriculture in maintaining water usage to optimize plant metabolic activity in non-water-stressed crops (Nishida et al. 2018). Finally, leaf relative water content significantly influences leaf-to-soil temperature ratio under drought conditions, but has little impact when plants are well-hydrated; in fact, droughted plants on-average are colder than hydrated plants, perhaps suggesting that they are less metabolically active.

All things considered, given the many limitations of this study - and concerns surrounding contradictory results - further research is needed to determine the impact of relative water content on plant metabolic activity.

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