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An ASABE Meeting Presentation
DOI: https://doi.org/10.13031/aim.202200404
Paper Number: 2200404

Visual Measurements of Fluttering Leaf to Quantify Internal Water Stress

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Written for presentation at the
2022 ASABE Annual International Meeting
Sponsored by ASABE
Houston, Texas
July 17–20, 2022

ABSTRACT. Water stress directly affects crop growth and is used as a key indicator in evaluating the crop yield. There were several non-destructive approaches suggested to utilize the relationship between water stress and leaf vibration frequency, but the tendency of the frequency due to the dryness was controversial. In addition, we previously observed that the water stress induces either an increase or decrease in vibration frequency even with the same plant species, i.e., soybean plant. Here, we proposed a new perspective of leaf surface curvature effect to vibration frequency in order to understand this discrepancy. To characterize surface curvature changes during the plant drying process, leaves were monitored in both 2D and 3D tracking manner. As well, bending and flexural rigidity tests were conducted with soybean leaves. From video recordings, we found that the curvature of the leaves change from a flat surface to an upward curling topology as they grow. It was observed that the cupping shapes appeared randomly. The topological changes influenced the overall stiffness of the leaf and further contributed to increasing the frequency. Thus, the frequency of fluttering leaves can show contrasting results caused by the shape deformation. These results indicate that the morphological feature of leaves is key to the vibration frequency.

Keywords. *Curvature, leaf fluttering, water stress*

Introduction

Optimization of crop yields is a crucial challenge to be solved amid the prediction of a global food crisis caused by climate change. Among several indicators of crop yield, water stress is undoubtedly the dominant factor to regulate the production (Osakabe et al., 2014). Severe water stress negatively impacts the plant growth physiologically, and decreases plant production (Rashidi et al., 2007; Farooq et al., 2009; Chaves et al., 1991). Therefore, early detection of water stress level in plants is important to mitigate water stress and maximize crop yields.

These efforts include studies attempting to estimate a plant's level of drought through measuring the frequency of leaf vibrations in a non-destructive way. The frequency-based approach is based on beam theory. According to beam theory, the frequency of a beam shape body is proportional to the square root of that object's flexural rigidity (Timoshenko, 1983). Plant water stress affects the turgor pressure of leaf cells, which consequently regulates leaf stiffness. When water is sufficient, the plant cell maintains high turgor pressure and high rigidity (Faisal et al., 2010; Nilsson et al., 1958). In contrast, as moisture is decreased, the leaves become more flexible and less stiff, thus lowering the fluttering frequency. Numerous studies have shown that the leaf vibration frequency decreases in a dry environment (Sano et al., 2013; Sano et al., 2015; de Langre et al., 2019). However, this hypothesis is controversial due to conflicting results showing that leaf vibration frequency increases in a dry environment (Caicedo-Lopez et al., 2020; Jung et al., 2021) The mechanism needs further discussion, so an intuitive explanation using general beam theory is insufficient for explaining an increase in vibration frequency.

Here, we propose visual measurements to evaluate the causes of the two opposing trends, particularly the frequency-increasing case. As an initial result of frequency experiments with soybean plants, both an increasing trend and a decreasing trend are observed. We argue that morphological factors affect the changes of the frequency in addition to the environment. In order to capture the morphology contributing to this antithetical tendency, Raspberry pi camera, GoPro, and high-speed camera are utilized as well as normal DSLR cameras. Complementary to the general two-dimensional imaging method, a three-dimensional tracking method using two cameras, is introduced to measure both the fluttering frequency and the morphological surface change. Through these recording tools, changes in leaf shape during the drying process are monitored. Leaves show phenomena of sagging down or curling up from edges, where curling up is the main cause of rigidity changes, which greatly contributes to increasing the vibration frequency of the leaves. Such curling correlates to the vibration frequency increasing and enables physical understanding of the two seemingly incompatible results.

Materials and Methods

Growing plants

Soybean (Glycine max) plants are used as the test plant and raised in the indoor growth chamber. The soybean plants at V(n) stage, which have nth trifoliolate, are selected to test. For the test, we divide soybean plants into two groups, control and dry. Control plants have been supplied with sufficient water but for the dry group or water stress group, we stop the water supply and let them dry (Figure 1(a)).

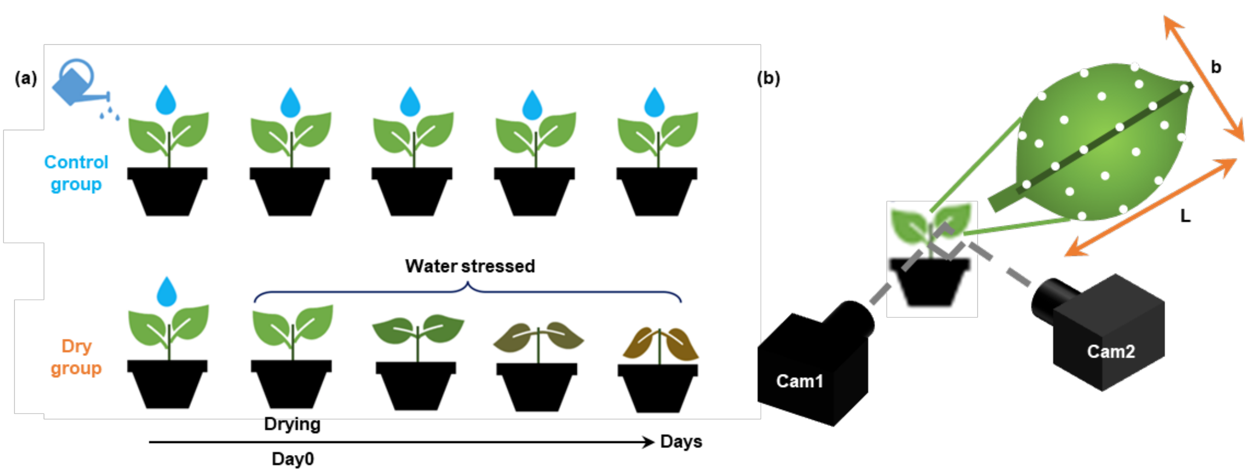

Figure 1. **(a) Setting up the experimental group and the control group. The experimental group is the dry group, and water supply is stopped and water stress is applied. The control group keeps uptaking water. (b) Experimental setup to analyze a surface in 3D using two cameras. Here, the two cameras are mounted at the same height, maintained at a 90 degree angle, and focused on the leaf. The leaves are painted with white dots for 3D tracking.**

Dynamic test

Fluttering events are generated by tapping the tip of the leaf. All the dynamic tests are conducted triplicate and captured with a high-speed camera, FASTCAM Nova S9 (Photron, Japan) at 250 fps. In these videos, all tip positions are tracked by Tracker software. These tracking data are plugged into the MATLAB Fast Fourier Transform (FFT) function so that vibration frequencies are computed.

Imaging

The changes while plants dry are monitored with full shots of the entire plant and close-up shots of individual leaves in time-lapse. The full shots are taken with a Raspberry Pi camera rev1.3 connected to a Raspberry Pi. Individual leaves are captured using a GoPro Hero 10 camera (GoPro, San Mateo, CA, USA), and leaves are placed on a Petri dish to keep their position. At this time, the leaves are intact and connected to the main stem with only the petiole part weakly fixed with paper.

Curvature test

To determine the natural vibration frequencies of a sheet for a particular curvature, 8 different radii (r = [0, 50, 100, 150, 200, 250, 300, 350, 400] mm) of curved 3D-printed sheet clamps are used. In this setup, three different types of sheets are used: a 0.005 and a 0.010-inch thick polycarbonate sheet and a 0.003-inch thick copper sheet. The sheets are cut to 4x8 cm, and they are measured for mass and thickness. Each sheet is clamped along one edge so that the transversal curvature is zero. A 4x6 cm section of each sheet is made into a cantilever, where 2 cm of the longest edge of the sheet is clamped.

A light force is applied to the tip of a clamped sheet and released to induce vibration. A high-speed camera, FASTCAM Nova S9 (Photron, Japan) is used to capture the deflection of the tip of each sheet. Footage is captured at 500 fps using a 105 mm lens. Data for tip deflection is captured by tracking a marker point at the tip of each sheet. Frequency data is analyzed using a FFT function in MATLAB. Three trials are conducted for each sheet type and degree of curvature. The natural frequency is averaged for each material and curvature.

Measurement of a leaf curvature

For the measurement of the change in surface curvature, 3D tracking is performed using two Nikon D7100 DSLR cameras (Nikon, Japan) (Figure 1(b)). The two DSLRs are placed at a 90 degree angle. Checkerboard images are taken for calibration. Meanwhile, leaves are painted with white dye in a grid pattern arrangement to recognize the shape and can then be made into a mesh grid. The pictures are taken when the dots painted on the leaves are visible from both cameras. For the post processing, the positions of the dots on the leaves of the images taken by the two cameras are obtained through MATLAB. Next, 2D positions are converted to points in 3D through stereo camera calibration and triangulate function. The 3D positions are surface-fitted and from the surface equation, the mean curvature is calculated.

Results and Discussion

Dynamic test and shape changes

From a dynamic fluttering test, the effect of water stress on a leaf's frequency is tested. Figure 2 shows a graph of tip displacement versus times on days 0 and 4 for the control group and water-stressed plants respectively. For the control group with water supply, the period of oscillation remains similar over time. On the other hand, in the water stress group, the period of oscillation is increased or decreased. This means that even within the same species, the effects of water stress can present in two different directions. To differentiate them, we label the group with increased frequency as f-increase and the group with decreased frequency as f-decrease.

To elucidate the cause of the two different trends in frequency, the physical shape of the plants is monitored. The drying of plants is recorded through time-lapse in between 4 to 7 days, which shows that the individual leaves change their shape over time. Shown in figure 3(a), some leaves sag down, and in figure 3(b), some leaves curl up. When the leaves sag, the turgor pressure of the stems and leaves is reduced and eventually the leaves become pliable. To quantify the relation between turgor pressure and leaf vibration, we use the frequency equation of a beam-like equation. (Timoshenko, 1983).

$$f = \frac{3.5161}{2\pi}\sqrt{\frac{EI}{mL^3}} \tag{1}$$

where
f= natural frequency of flat sheet
EI=flexural rigidity [Pa m-^{4}]
E = Young's Modulus [Pa]
$I = bt^3/12$ [m-^{4}]
b = width [m]
t = thickness [m]
m = mass [kg]
L = length [m]

According to this equation, frequency is proportional to the square root of flexural rigidity. This can be interpreted as lowering the flexural rigidity by having less turgor pressure, which leads to the reduction of fluttering frequency of some leaves. Meanwhile, curling leaves upward is expected to increase the overall strength of the leaves and raise the frequency. This is a result inferred through a study (Pini et al, 2016) that revealed the relationship between curvature and spring constant, k_0.

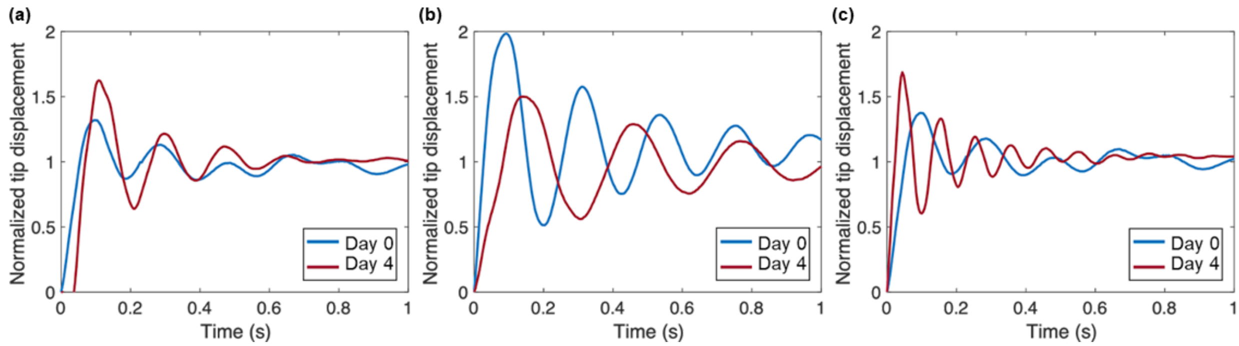

Figure 2. Individual plots of normalized tip displacement versus time. (a) controlled group, (b) f-decreasing group, and (c) f-increasing group

Figure 3. Qualitative image sequences showing the drying process of plants. (a) Changes in overall appearance of the soybean plant as it dries. The plant has an overall droopy appearance. (b) Observation of individual leaves in an environment with high water stress after stopping the water supply. The leaves gradually curl up.

Curvature effect test

Through previous leaf observation, it is verified that the tip of the leaf curls up when the leaf dries. In addition, mechanical tests are performed using plastic and copper sheets to investigate the effect of curvature on vibration frequencies of thin sheets. Eight different levels of curvature are artificially applied to the sheets, and changes in frequency are analyzed with respect to the theoretical model shown in equation (2). For equation (2), it is modified from the study on the relation between curvature and spring constant of thin sheets (Pini et al., 2016), to apply it to our experiments.

$$f = \frac{1}{2\pi}\sqrt{\frac{k_0}{m}\left(\frac{L^2}{60}\frac{\beta^4}{\eta^2}\nu^2\kappa^2 e^{-3.095\beta} + 1\right)} \tag{2}$$

where
$k_0 = m(2\pi f_0)^2$
$\beta = b/L$
$\eta = t/L$
ν = Poisson's ratio
κ = dynamic curvature at the transversal axis of the plane [1/m].

Experimental data for the curvature-dependent frequencies of each sheet is plotted (Figure 4). The base frequency (f_0), defined as the frequency when curvature is zero, is determined by equation (1). The base frequency of each sheet is dependent on the mechanical and geometric properties of each sheet, determined by each sheet's material, thickness, and length. As the transversal curvature of the sheet increases, the vibration frequency rises. This relationship between curvature and frequency was validated through experimental data.

The experimental values are compared to theoretical frequencies characterized by equations (1) and (2). Both the experimental data and the theoretical model predict an increase in frequency based on transversal curvature. The experimental data appears to be a good fit for the theoretical function, given by the diagonal line in figure 4(b). Overall, these results propose that the change in curvature is a direct factor in regulating the vibration frequency of flat, cantilever sheets.

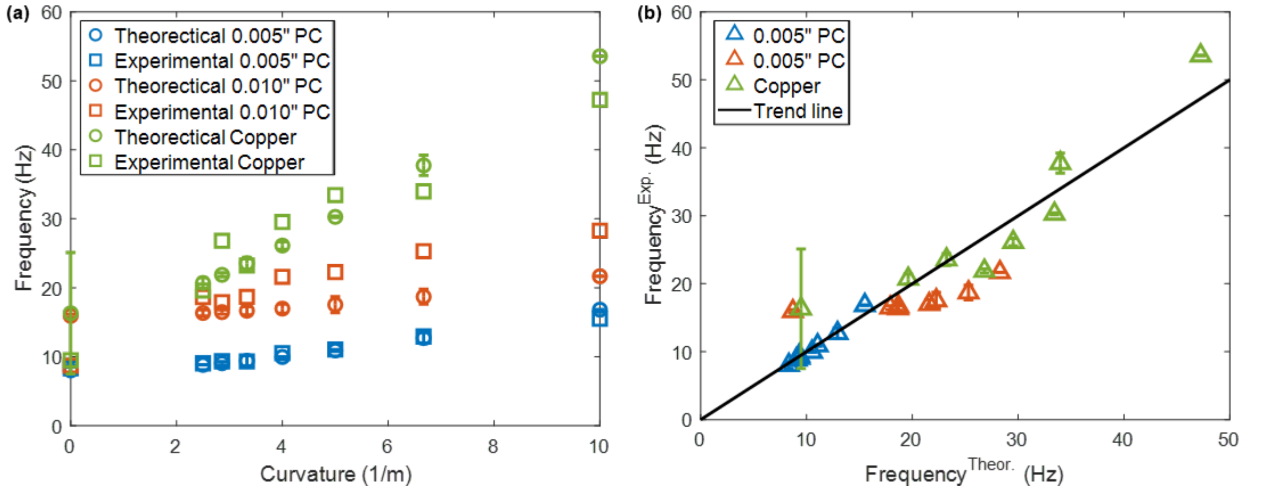

Figure 4. (a) Theoretical curvature-dependent frequency compared to experimental data, (b) fit of experimental data to theoretical function.

Measurement of a leaf curvature

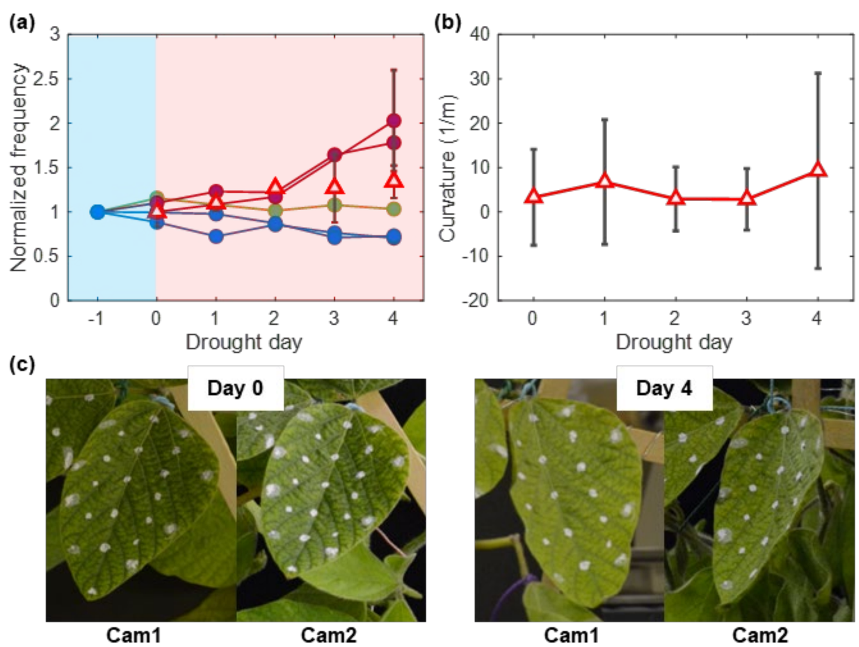

Figure 5. (a) The change in leaf frequency after shutting off the water supply. For the green line, unlike the other lines, it represents the control group, and the water supply is continuously maintained. Reds mean f-increased and blues mean f-decreased case. Only for the red triangle symbol, surface curvature is also measured. (b) Curvature measurements of f-increased leaf with triangle symbol. (c) The shape of the leaves by two cameras on day 0 and day 4.

Previous mechanical tests using plastic sheets confirmed that increasing the surface curvature eventually increased the frequency by reinforcing the stiffness of the leaf. Moreover, we apply this result to actual leaves to validate that the curvature effects can be utilized. Prior to this, we certify whether f-increase and f-decrease can be reproducible. Figure 5(a) represents the change in frequency per day. This figure shows the normalized frequency changes from one day before the water supply was stopped (drought day=0) to the fourth day after stopping the water supply. In the case of the control group, there is a slight difference depending on the date, but overall the normalized frequency stays at 1. Whereas the water stress groups are distinguished clearly to the two groups as shown in figure 2. In other words, our experimental data is reproducible.

Next, 3D tracking of the leaf shape, whose frequency increases among the dry group, is performed. The leaf showing the triangular symbol in figure 5 is a tracked leaf. Figure 5(b) illustrates the surface curvature alteration over time. The curvature was 3.3 m^{-1} on day 0 and 9.3 m^{-1} on day 4, an increase of about 2.8 times. However, on the first day, the value of the curvature jumped to 6.7 m^{-1}, and the error bars are large overall. Therefore, although it tends to grow, the measurement reliability is low due to a large error. Large error bars can come from errors in calibration, fitting, quality of the image, accuracy of tracking, etc. On the other hand, on day 4 in figure 5(c), the edges are rolled up. Compared with day 0 having all the white dots visible, the dots on the edges are clearly hidden on day 4, meaning that the leaf boundaries on day 4 are curled up. From these results, 3D tracking using two cameras still needs to be improved in various aspects. It is also necessary to discuss how to track points that become covered when the leaves roll up. Although there are errors in the measurement of surface curvature, it is meaningful that curvature measurement is possible and the trend is consistent.

Conclusions

With various visual measurements, how plant leaves respond to water stress is evaluated from various physical features. As shown in the literature, as the water stress progresses, the frequency of the leaves increases or decreases. If a frequency drops, it is presumably due to the decrease in flexural rigidity as its turgor pressure decreases. While the frequency increases, the rising of the curvature is considered to be the main cause. These results suggest that a more complex and accurate analysis is needed, given that plants simply do not show frequency changes in one direction, but in both directions.

Acknowledgements

This work was supported by the National Science Foundation (NSF) under Grant No. ISO-2120739.

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