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RESEARCH ARTICLE

PRINCIPAL COMPONENTS ANALYSIS OF THE STEM MORPHOLOGY AND ANATOMY OF WIND-EXPOSED *CAMELINA SATIVA*

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ABSTRACT

The purpose of this study was to determine to what level different degrees of wind exposure influence morphology and secondary growth in *Camelina sativa* L. crantz. The hypothesis was that wind-stressed *Camelina* develops greater lignified areas and invests carbon and energetic resources in sclerification. *Camelina* was harvested in the late flowering stage and analyzed in terms of stem anatomy and morphology. The results indicated that there was no significant difference in morphology or sclerification between groups. A Principal Components Analysis was performed on the data in order to elucidate *Camelina* morphology.

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INTRODUCTION

Camelina sativa is a promising new cultivar as an oil-bearing seed for industrial and nutritional applications. This is principally because *Camelina* oil has high concentrations of linolenic acid (Whitkop et al 2009). Nevertheless, because the species has only recently achieved commercial relevance, the factors which influence carbon allocation in *Camelina* in terms of secondary xylem thickness are not well understood. Moreover, it is unknown whether *Camelina* lignifies its shoot in order to stabilize reproductive parts. Further confounding the problem is the fact that the plant may respond differently when exposed to mechanical stresses such as wind stress. Extensive research is underway in various climates and field locations in the state of California; it is necessary to determine the agronomic conditions which are suitable to the plant, not the least of which is the degree of wind exposure. Carbon allocation clearly is an important part of the oilseed plant's development surrounding the time of reproduction and flower set. In order to further understand the plant's development, this study observes the height, stem diameter, and thickness of cross-sectional sclerified secondary growth in two varieties of three-month old *Camelina sativa*.

Although most people associate secondary growth with trees, secondary growth also occurs in other dicots such as cotton (Rost 1998). As noted by Dr. Barbara Gartner, options for decreasing stress are to lower the stress on the plant stem, or to add to the resistance to the stresses (1995). Clearly the plant can undertake only one of those options. Therefore the hypothesis is that wind-stressed *Camelina* will develop greater lignified areas and invest resources in sclerification rather than height to stabilize its reproductive shoots. The purpose of this study is to determine to what level different degrees of wind exposure influence stem morphology and secondary growth in *Camelina sativa* L. crantz.

MATERIALS AND METHODS

Plant material was collected from research plots at Cal Poly Pomona Chino Westwinds Ranch (California Institution for Men) during Winter 2013. Seed was provided by the Sustainable Oils Co.; the varieties evaluated herein were SO-30 and SO-40. The research plots were arranged in a randomized, blocked design. Thus, the field arrangement was optimal to take six samples from each variety. The samples from each variety were then selected arbitrarily from two distinct categories.

The first category was comprised of plots which were wind exposed. The second category was comprised of plots which were wind protected and designated as the control.

The *Camelina* was in the growth stage referred to as 7/ by the BBCH scale (Martinelli 2011). This is the late flowering stage, as indicated by the slash, where the flowers become white in prelude to silique formation. Each of the samples were evaluated for height, stem diameter, and thickness of the stem cross-sectional sclerified region. Height was measured using a construction tape measure without regard to lodging. Stem diameter was measured with a caliper. Finally, stem cross sections were prepared using Toluidine Blue and measured with a calibrated micrometer coupled with a compound light microscope. Morphology was explored statistically in terms of a non-inferential Principal Components Analysis in R as prescribed by Dr. David Moriarty (2012).

RESULTS

The values of the correlation matrix indicated that there was no strong correlation between any of the morphological parameters with respect to variety or wind exposure. The low results for the paired T-tests indicated that there was no statistically significant difference between any of the morphological variables with respect to variety or wind stress treatment. Therefore, the morphology was explored in terms of a non-inferential Principal Components Analysis.

Factor Loadings:

	Comp.1	Comp.2	Comp.3
avgdia	0.66748	-0.18127	0.72223
avght	0.64132	-0.35294	-0.68128
avgscl	0.37839	0.91792	-0.11933

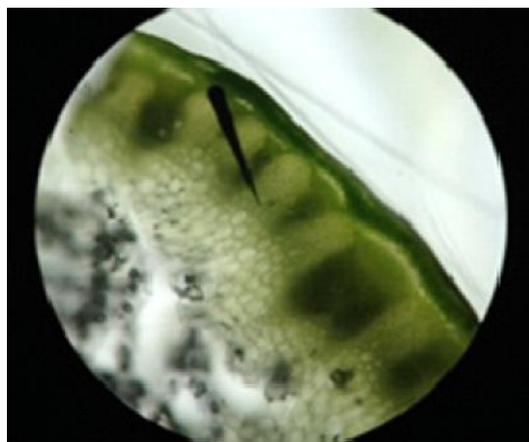


Figure 1. Cross-sectional view of untreated *Camelina sativa* stem with sclereids indicated

DISCUSSION

The Toluidine Blue stained slides show several qualitative characteristics. One can observe that *Camelina sativa* exhibits woody secondary growth collateral with secondary phloem in a circular pattern. There is also extensive lignification to the inside of the xylem, which is most notable in the SO-30 wind protected specimen above; its visibility is probably due to the level of light polarization, which differs slightly in this micrograph.

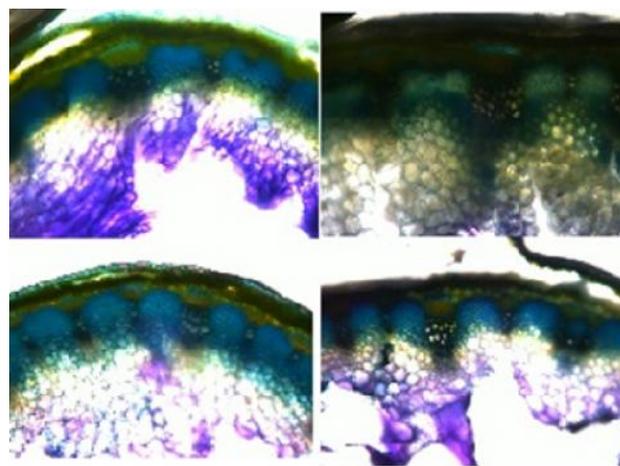


Figure 2. Toluidine Blue stained slides to indicate lignified regions. From left to right: SO-30 wind exposed, SO-30 wind protected, SO-40 wind exposed, SO-40 wind protected

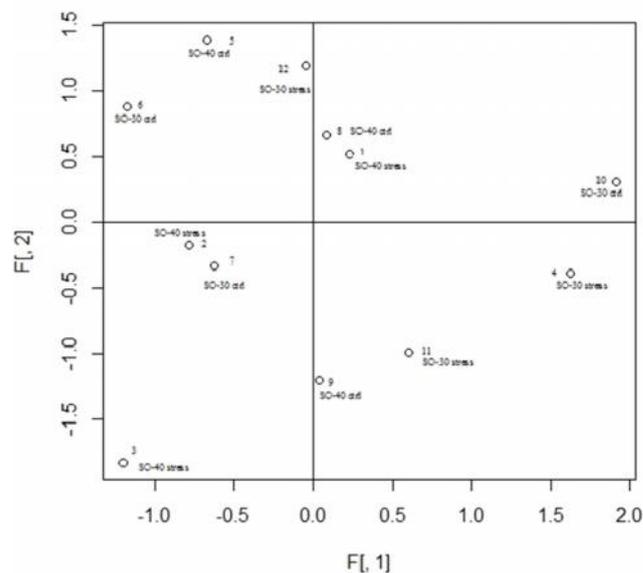


Figure 3. Plot of Principal Components 1 and 2. Note that SO-30 stress treatments tend to be high on PC1

It appears in regards to the cross-sectional slides that the exposed specimens may contain less aeranchyma and less spaces between the bright blue secondary xylem. However, this aspect of the stem anatomy was not quantified by the present study. The statistical output does not provide clear insight about the factors that influence secondary growth in *Camelina sativa*. The correlation matrix shows moderate associations, but nothing strong enough to be conclusive. Indeed, the paired T-tests resulted in no significant differences between the treatments or varieties in terms of height, diameter, or width of the sclerified regions within the stem. These results differ from Cordero's findings in *Cecropia* saplings, in which the wind stressed and wind exposed plants differed in stem diameter as well as photosynthetic parameters (1999). However, our study is somewhat concurrent with Retuerto's results in *Sinapsis alba*. Cordero reported that plants recovered better from wind stress in terms of compensatory growth than they did to density stress (2001). In regards to the Principal Components Analysis, it is clear from the factor loadings that plots that are high on PC1 are large with respect to both average diameter and average height.

Those same parameters also put a plot low on PC2. A large sclerified region is associated with a high score on PC2 or a moderately high score on PC1. Some interesting groupings arise in terms of plots which produced plants that had the largest overall size. It appears that the three largest specimens on the size component are all SO-30, although two of those were wind stressed and one was wind protected. Interestingly enough, the largest specimen with the highest sclerification was the SO-30 control. Another interesting group is in the quadrant representing plots producing plants with a large value for sclerification and a small score on the size component. The plot which fit most closely with this was plot 5, an SO-40 control plot. Nevertheless, the other two plots that are high on PC2 but low on PC1 are SO-30 plots, both interior and exterior. Although it is not statistically significant, one can see from the PCA that SO-30 followed a trend of growing taller and thicker, as well as having greater carbon allocation toward lignin even when the size component has a low score. However, no inferences can be made from the PCA morphological exploration.

Conclusion

The null hypothesis that *Camelina* would not develop greater lignified areas and achieve a lesser height was not rejected. There was no statistical difference in morphology or anatomy in wind-stressed versus controlled plots, nor between different cultivars as measured here. Further research is needed to determine the morphological consequences of mechanical stress on the secondary growth of Brassica species. While this field experiment offers a plethora of data, time series data would be useful in order to observe growth as it occurs in mustards such as *Camelina sativa* and *Sinapsis alba*.

One point that arises from looking at the slides prepared for this experiment is that although the sclerified bright blue regions are of similar thickness across all samples, on the exposed specimens it appears that there are less spaces laterally between these circular regions (dark blue). In addition, it appears that the exposed specimens exhibit less aerenchyma. However, those points must be explored quantitatively. Further research could also explore the density of the wood produced, because this aspect of carbon allocation was not detected in the present study.

This follows the suggestion of Givnish (1995) who stated that the energetic cost of growing to a certain height is lowest when a plant constructs stems of low-density wood. This makes sense since wood strength is proportional to its density (Ibid). In the case of wind stressed *Camelina*, one might still expect that higher density wood would be produced to stabilize the reproductive shoot.

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