Progress Report: *Molecular Adaptations to Drought in Soybeans*

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Having determined that a number of chlorophyll-related metabolites (CRMs) exist in soybean leaf extracts for both a drought-susceptible cultivar (PANA) and a drought-tolerant cultivar, the first research objective of this proposal is to identify these CRMs. Research efforts this quarter have been dedicated toward preparation and isolation of these CRMs.

The CRMs contain the metal center Mg^{2+}, which is located in the porphyrin ring. Biologically, loss of Mg^{2+} from chlorophyll leads to production of pheophytin; hence, logically, if Mg^{2+} is removed from CRMs, they should produce pheophytin-related metabolites, which we will dub here PRMs. Because PRMs do not contain the metal center, and instead produce the conventional $(M+H)^+$ adducts produced by electrospray ionization, a method to remove Mg^{2+} from CRMs should help us facilitate their identities.

As such, we adapted a method for removal of Mg^{2+} from acetone extracts of spinach leaves [1] directly to leaves from both a drought-susceptible cultivar, PANA, and a drought-tolerant cultivar, PI 567731, of soybeans. A portion of the acetone extracts were then treated with acidic Dowex resin for 1 hr.(as opposed to 3 min used in the method described in ref. 1). Thin-layer chromatography (TLC) was conducted in closed-chambers using a mobile phase solvent o 70/30 hexanes/aceone as prescribed by Williamson [2]. The developed TLC plates of the extracts are shown in Figure 1. For the drought-susceptible cultivar PANA, TLC indicates that 1 hr. treatment of the acetone extract effectively converts all the chlorophyll a and b into pheophytin a and b (Figure 1, left), consistent with the results of ref. 1 using spinach leaves. For the drought-susceptible cultivar PI 567731, this is also observed, although a faint band of pheophytin a is detected prior to the treatment with Dowex resin (Figure 1, right). As noted in ref. 1, the demetallation process also enables detection of xanthophyll, which is obscured by the band due to chlorophyll b in the untreated acetone extract.

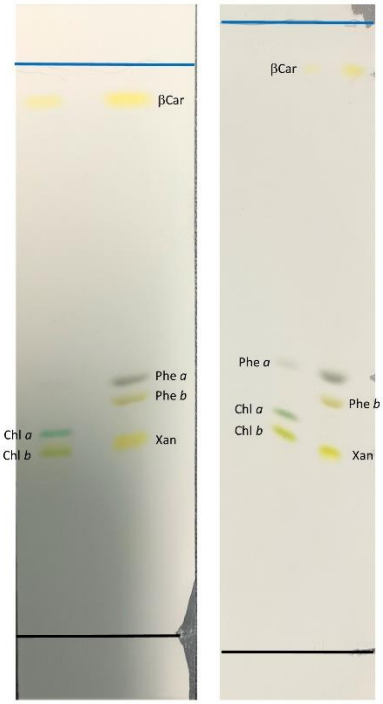

Figure 1. Thin-layer chromatogram plates of acetone extracts of (left) PANA and (right) PI 567731 leaves. In each plate, the untreated extract is the left-most column, and the extract treated with Dowex resin for demetallation is on the right. βCar = beta-carotene, Chl a = chlorophyll a, Chl b = chlorophyll b, Phe a = pheophytin a, Phe b = pheophytin b, and Xan = Xanthophyll.

Moving forward, this development will facilitate identification of the CRMs because demetallation of the CRM will lead to magnesium-free PRMs, which should be detectable by LC-MS.

References

1. Quatch, H. T.; Steeper, R. L.; Griffin, G. W. J. Chem. Ed. 2004, 81, 385-387.
2. Williamson, K. L. *Microscale and Macroscale Organic Experiments*, 34th Ed., Houghton-Mifflin: Boston, MA, 2003; pp. 162-166.