

# Characterizing proteases in sorghum drought response using activity- based protein profiling (ABPP)

September 2022

Vivian S. Lin  
Natalie C. Sadler  
Pubudu Handakumbura  
Kylee Tate  
Gerard X. Lomas  
Jennifer Ramirez

## DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor Battelle Memorial Institute, nor any of their employees, **makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights.** Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or Battelle Memorial Institute. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

PACIFIC NORTHWEST NATIONAL LABORATORY  
*operated by*  
BATTELLE  
*for the*  
UNITED STATES DEPARTMENT OF ENERGY  
*under Contract DE-AC05-76RL01830*

Printed in the United States of America

Available to DOE and DOE contractors from  
the Office of Scientific and Technical  
Information,  
P.O. Box 62, Oak Ridge, TN 37831-0062  
[www.osti.gov](http://www.osti.gov)  
ph: (865) 576-8401  
fox: (865) 576-5728  
email: [reports@osti.gov](mailto:reports@osti.gov)

Available to the public from the National Technical Information Service  
5301 Shawnee Rd., Alexandria, VA 22312  
ph: (800) 553-NTIS (6847)  
or (703) 605-6000  
email: [info@ntis.gov](mailto:info@ntis.gov)  
Online ordering: <http://www.ntis.gov>

# **Characterizing proteases in sorghum drought response using activity-based protein profiling (ABPP)**

September 2022

Vivian S. Lin  
Natalie C. Sadler  
Pubudu Handakumbura  
Kylee Tate  
Gerard X. Lomas  
Jennifer Ramirez

Prepared for  
the U.S. Department of Energy  
under Contract DE-AC05-76RL01830

Pacific Northwest National Laboratory  
Richland, Washington 99354

## Abstract

Sorghum (*Sorghum bicolor*) is a C4 grass species that is a major food and bioenergy crop grown worldwide. Understanding the response of sorghum to climate change is therefore critical to future sustainable food and bioenergy production. Major advancements in genome sequencing have provided a wealth of genomic data for DOE-relevant plant species, but the functions of many of these genes remain unknown. Genotype to phenotype characterization of sorghum and other bioenergy crops is needed to identify key genes responsible for plant resilience to drought and other environmental stressors. Proteases, which modulate protein degradation and cellular signaling processes by catalyzing the hydrolysis of proteins into smaller proteins, peptides, or amino acids, are highly implicated in plant drought stress responses. We developed a method for using activity-based protein profiling (ABPP) of selected protease activities (serine and cysteine proteases) to characterize drought stress responses in sorghum roots and leaves. Analysis of these ABPP data identified several known serine and cysteine proteases as well as uncharacterized sorghum proteins which may also have protease activities that can be explored in future validation studies.

## Acknowledgments

This research was supported by the **Earth and Biological Sciences Investment**, under the Laboratory Directed Research and Development (LDRD) Program at Pacific Northwest National Laboratory (PNNL). PNNL is a multi-program national laboratory operated for the U.S. Department of Energy (DOE) by Battelle Memorial Institute under Contract No. DE-AC05-76RL01830.

## Contents

Abstract.....	ii
Summary .....	<b>Error! Bookmark not defined.</b>
Acknowledgments.....	iii
1.0 Introduction.....	1
2.0 Methods .....	3
3.0 Results and Discussion .....	6
4.0 References.....	11

## Figures

Figure 1.	Structures of activity-based probes (ABPs) and overview of plant ABPP sample preparation workflow. (A) FP2 and alkyne E-64d were synthesized according to previously published routes. (B) Sorghum root and leaf tissues were harvested and labeled with ABPs using vacuum infiltration, followed by protein extraction and click chemistry functionalization for SDS-PAGE or streptavidin enrichment. ....	4
Figure 2.	Addition of detergent (0.01% DDM) improves <i>in vivo</i> labeling of waxy sorghum leaves with probe by reducing surface tension and enabling full submersion of all leaves.....	6
Figure 3.	Sorghum physical measurements at time of harvest (n = 12-16 for root and leaf weights; n = 4-5 for number of leaves). ....	6
Figure 4.	Appearance of drought and control sorghum plants at time of harvest (6-7 weeks growth).....	7
Figure 4.	Fluorescent SDS-PAGE gel of serine and cysteine ABP-labeled proteins in sorghum roots. ....	8
Figure 6.	Volcano plot of proteins identified in sorghum root samples using cysteine and serine protease ABPs. ....	10

## Tables

Table 1.	Proteins identified as statistically different between drought vs. control plants of both genotypes using serine and cysteine protease ABPs in sorghum root tissues.....	9
----------	--	---

## 1.0 Introduction

Functional annotation of bioenergy crops has lagged behind genome sequencing, yielding large numbers of genes of unknown function and posing a major challenge to understanding the fundamental mechanisms impacting stress responses. Drought stress triggers diverse, tissue-specific changes in sorghum intercellular signaling pathways; proteolytic activity has been recognized as a key function in drought stress response (Wang et al. 2016, Abdel-Ghany et al. 2020, Goche et al. 2020, Lyon et al. 2016), but we lack a detailed understanding of which specific proteases are responsible for drought resistance and recovery in above and below ground tissues within live, intact plants.

Advancing our mechanistic understanding of how plants respond and adapt to environmental stresses is vital to our development of a sustainable biofuel economy. The molecular mechanisms responsible for drought tolerance in bioenergy crops such as sorghum have not been fully elucidated. Recent transcriptomic and proteomic analyses of sorghum under drought stress have identified differences in protease and protease inhibitor expression between drought resistant and drought sensitive lines (Abdel-Ghany et al. 2020, Goche et al. 2020). These findings suggest proteases play a critical role in drought stress response and modulation of specific protease activities may be a determinant for sorghum drought tolerance.

However, while proteomic and transcriptomic analyses can report on the identities and abundances of protease gene products at a given timepoint, the extent to which enzymes of interest are functionally active or inactive at the time of sampling cannot be confirmed. The functions of many plant proteases remain unknown (Van der Hoorn 2008). Plants use multiple mechanisms for regulating protease activity within specific tissues, including up- or downregulation of proteases or protease inhibitors, which may be small proteins, peptides, or small molecules (van der Hoorn 2008, Have et al. 2018). Protease activity may also be controlled at the spatial level; proteases and protease inhibitors may be sequestered in specific regions of cells or tissues and released in response to biochemical signals or through physical damage. Bulk proteomic identification of proteases in homogenized plants may therefore not reflect actual protease activities within intact, spatially organized tissues. To understand proteases in plant drought stress responses, we must develop approaches that can accurately report on protein function in intact plant samples. This project leverages PNNL's expertise in chemical biology, plant sciences, and proteomics to advance our understanding of protease activities in tissue specific drought response and demonstrate the utility of ABPP in gene to function characterization in bioenergy crops.

In this project, we developed a workflow for *in vivo* activity-based protein profiling (ABPP) to explore protease activities in bioenergy grasses under drought conditions. By using ABPs that react selectively with enzymes based on their mechanism of action (Cravatt, Wright, & Kozarich 2008, Willems, Overkleeft, & Kasteren 2014, van der Hoorn et al. 2011, van der Hoorn et al. 2004, Morimoto & van der Hoorn 2016), we can rapidly characterize diverse proteins with specific functions in complex samples without requiring prior knowledge of protein identities. ABPP therefore has the potential to enhance metagenome functional annotation and enable predictive biology for bioenergy crops. Very few studies have applied ABPP to plants for exploring signatures of stress and the molecular mechanisms of plant stress responses, and to our knowledge, ABPP has never been applied to bioenergy crops. Our approach using ABPs, coupled with the extensive proteomics capabilities at EMSL, provides new functional information for protease activities in sorghum root and leaf tissues. This work also sets the stage for future

experimental opportunities to achieve functional annotation for uncharacterized proteins and genes of unknown function in bioenergy crops using ABPP.



## 2.0 Methods

### 2.1 Probe synthesis

FP2 was synthesized according to Wiedner et al. 2012 and alkyne E64-d was synthesized according to Kaschani et al. 2009 and Hang et al. 2006.

### 2.2 Plant growth and harvest

Tall pots (Deepot Tree Pots) were prepared by covering pot bottoms with parafilm and adding 3 g SoilMoist (soaked overnight in 100 mL water prior to planting) to the bottom of the pot, followed by 1400 g sand (Mosser Lee Desert Sand). Pots were watered with 100 mL water prior to planting seeds at ½ inch depth for two sorghum genotypes, RTx430 (pre-flowering drought tolerant) and BTx642 (post-flowering drought tolerant). Pots were misted with water and covered with parafilm until seeds germinated in a walk-in growth chamber. Plants were grown under water replete conditions with fertilizer (applied once per week) for 4-5 weeks, receiving 50-100 mL water every 2-3 days.

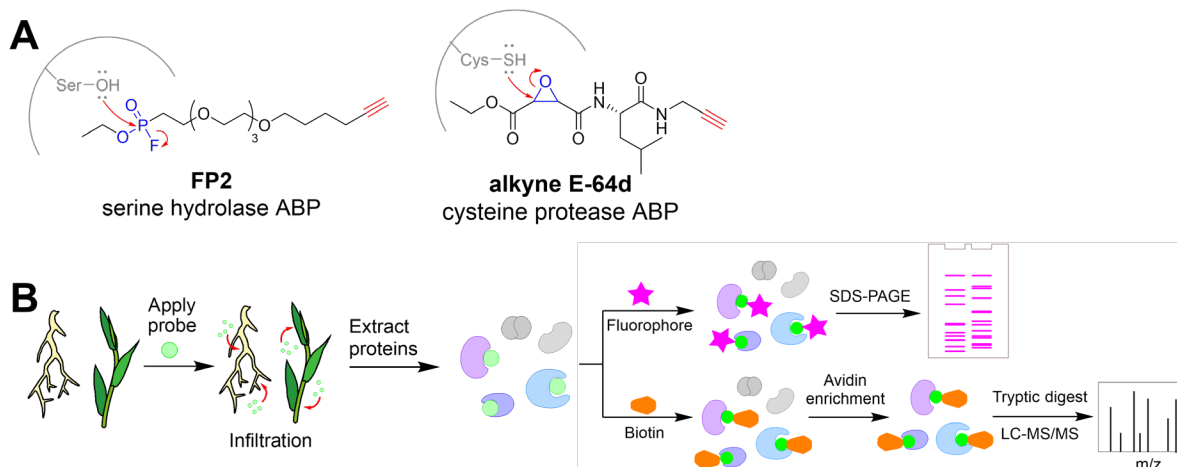
Drought conditions were started for plants by applying 50 mL on the first day of drought. Drought plants received 25 mL water every 2 days for 1 week, followed by 1 week with no water. Control plants received the same amount of water as during the first 4-5 weeks of growth. All plants were harvested at 6-7 weeks total age.

On the day of harvest, plants were measured (height, leaf count). Dead or shriveled leaves were discarded. Above ground tissues were removed and leaves separated from stalks at the collar with scissors; leaf tissues were then weighed for each plant. Sand containing roots was emptied from pots, and the roots were rinsed with water to remove excess sand. Root wet weights were also recorded for each plant.

Root and leaf tissues (1.5-2.0 g) were trimmed as needed with scissors and weighed into 50 mL Falcon tubes for probe labeling. Leaf tissues were pooled across multiple plants as needed to achieve sufficient biomass for each sample.

### 2.3 Probe labeling, tissue processing, and homogenate preparation

MilliQ water (25 mL) with 0.01% n-dodecyl-β-D-maltoside (DDM) was added to each Falcon tube, ensuring all tissues could be submerged in this volume. DMSO (vehicle control) was added to no probe samples. FP2 and alkyne E64-d (5 μL of each stock solution at 50 mM in DMSO) were added to each probed sample to achieve a final concentration of 10 μM of each probe (Fig. 1A). Tube caps were loosely placed on top of each tube.



**Figure 1.** Structures of activity-based probes (ABPs) and overview of plant ABPP sample preparation workflow. (A) FP2 and alkyne E-64d were synthesized according to previously published routes. (B) Sorghum root and leaf tissues were harvested and labeled with ABPs using vacuum infiltration, followed by protein extraction and click chemistry functionalization for SDS-PAGE or streptavidin enrichment.

Samples were vacuum infiltrated in vacuum desiccators for 3 x 5 min cycles under house vacuum, agitating between cycles to disrupt air bubbles, and then allowed to incubate with probe for 2 hr at room temperature (Fig. 1B). Probe labeling solution was decanted from each tube, tissues removed with tweezers and patted dry with paper towels and transferred to Covaris TT2 tissueTUBE bags. Bags were sealed with Teflon caps, flash frozen, and stored at -80 °C for further processing.

Plant tissues were pulverized repeatedly with a plastic-coated hammer or using a Covaris CP02 cryoPREP automated pulverizer (setting 5) until pulverized to a fine powder. Pulverized tissue powders (~1.25 mL volume) were then transferred to 2.0 mL mechanically resistant tubes for bead beating. Ceramic beads (2.7 mm, 0.3 mL) and glass beads (100 μm, 0.1 mL) were added to each tube followed by 1 mL homogenization buffer (1% SDS, 0.1 M Tris-HCl pH 6.8, 2 mM EDTA; freshly prepared 20 mM DTT, 2 mM PMSF). Samples were homogenized using an Omni bead beater (2 rounds, 30 s at 6,000 rpm). Samples were centrifuged for 10 min at 4 °C, 15,000 x g and the supernatant transferred to a fresh 2 mL tube.

Proteins were precipitated using a 1:1 volume homogenate to cold 20% TCA in acetone. Samples were incubated on ice for 1 hr, then centrifuged for 10 min at 4 °C, 15,000 x g. The supernatant was then discarded. The protein pellet was washed with cold acetone, dried for 5 min, and then resuspended in 300 μL 1% SDS in PBS + 1 mM DTT. Samples were heated to 85 °C for 2 min, vortexed, and then transferred to a 1.5 mL tube. The tube was washed with an additional 300 μL PBS, which was transferred to the 1.5 mL tube to achieve ~1.2 mL of sample in 0.5% SDS and 0.5 mM DTT. These probe-labeled proteomes were centrifuged at 15,000 x g for 4 min to pellet any undissolved particles, and protein concentration was determined using a BCA assay.

## 2.4 Click chemistry and enrichment

Plant tissue homogenates were normalized to 1 mg/mL and subjected to click chemistry attachment of either TAMRA azide for qualitative fluorescent gel analysis or biotin azide for streptavidin enrichment.

For gel analyses, to plant tissue homogenate (40 μL at 1 mg/mL) samples, TAMRA azide (final concentration 30 μM), sodium ascorbate (final concentration 500 μM), THPTA (final

concentration 2 mM), and copper sulfate (final concentration 4 mM) were added. Samples were rotated or agitated at r.t. for 1 hr in the dark. 4X LDS sample loading buffer (16  $\mu$ L) and 10X reducing agent (6  $\mu$ L) were then added to each tube, and samples were heat denatured at 85 °C for 2 min. Samples were then run immediately on 4-12% Bis-Tris gels with MOPS running buffer. Gels were imaged for fluorescence with a Typhoon FLA 9500 laser scanning imager, then fixed in SYPRO fix for 30 min, washed with DI water, and stained with GelCode Blue for 1 hr. Gels were destained overnight in DI water and then total protein was visualized using a GelDoc imager.

For ABPP proteomics sample preparation, to plant tissue homogenates (1 mg/mL, 1 mL), we added biotin azide (30.8  $\mu$ M final concentration), sodium ascorbate (2.5 mM final concentration), THPTA (1 mM final concentration), and copper sulfate (2 mM final concentration). Samples were rotated or agitated at r.t. for 1 hr. After completion of the click chemistry reaction, proteins were precipitated by adding 1 mL cold 20% TCA in acetone to each tube, vortexed briefly, and chilled on ice for 5 min. Proteins were pelleted by centrifuging at 14,000 x g and 4 °C for 10 min. The supernatant was decanted or pipetted off and discarded. The pellet was washed with 1 mL cold acetone, centrifuged again as described above, and decanted. The protein pellet was dried for 5 min and then reconstituted in 400  $\mu$ L 1.2% SDS in 1X PBS, vortexed and heated to 95 °C for 2 min. Samples were sonicated for 2 rounds of 9 x 1 s pulses, 60% amplitude. Samples were then heated at 95 °C for 2 min, followed by centrifugation at 14,000 x g for 10 min to pellet any undissolved particulates. Protein concentration for each sample was determined using a BCA assay, and samples were normalized to the same concentration for enrichment (1.0 mg/mL).

Samples were enriched using 100  $\mu$ L streptavidin agarose beads which had been rinsed with 2 x 1 mL 0.5% SDS in 1X PBS, 2 x 1 mL 6 M urea in 25 mM ammonium bicarbonate (ABC) pH 8 buffer, and 4 x 1 mL 1X PBS. Normalized proteomes in 1.2% SDS were transferred to cryovials containing rinsed beads with 2 mL 1X PBS for a final concentration of ~0.2% SDS. Samples were rotated at 37 °C for 1 hr. Beads were then washed with 2 x 0.5% SDS, 2 x 1 mL 6 M urea in ABC buffer, 2 x 1 mL MilliQ water, 2 x 1 mL PBS, and 8 x 1 mL ABC buffer. Beads were transferred to low-binding tubes for reduction and alkylation.

Samples were reduced with 5 mM TCEP hydrochloride in 6 M urea in ABC buffer at 37 °C for 30 min, 1200 rpm. Samples were then alkylated with 10 mM iodoacetamide for 45 min at 50 °C. Beads were rinsed with 8 x 1 mL PBS and 4 x 1 mL ABC buffer. Supernatant was removed, 200  $\mu$ L ABC buffer added to each sample, and 1:4000 trypsin:protein added. Samples were digested at 37 °C, 1200 rpm, for 10 hr.

After digestion, peptides were transferred to a fresh tube and dried in a Savant SC110 SpeedVac. Peptides were reconstituted in 40  $\mu$ L ABC buffer with 0.01% DDM, heated to 37 °C for 5 min, and then ultracentrifuged at 53,000 rpm, 4 °C for 20 min. 25  $\mu$ L of each sample was vial up and stored at -20 °C until ready for LC-MS/MS analysis.

Samples were analyzed using the standard proteomics LC-MS/MS pipeline (QExactive). Existing data processing software (MSGF+, Mage suite) were used to generate and export peptide data. Data were processed by summing redundant peptides, peptide roll up, removal of reverse hits and contaminants, and statistical analysis to output lists of statistically significant enriched proteins. Missing value imputation was applied for statistical analyses (two-tailed t-test).

### 3.0 Results and Discussion

We optimized our existing ABPP workflow for sorghum samples by submerging excised tissues in probe solution to retain protease activities under native conditions. Previous studies of ABPP in plants showed that *in vivo* labeling of tissues resulted in less loss of certain protease activities compared to ABPP of homogenized tissues (Kaschani et al. 2009). Initial experiments yielded inconsistent results for leaf tissues; we observed that the waxy cuticle of sorghum leaves prevented full submersion of leaf tissues during the probe labeling step. Addition of 0.01% DDM to the probe labeling solution reduced surface tension around leaves and led to improved uptake of the probe labeling solution by vacuum infiltration (Fig. 2).

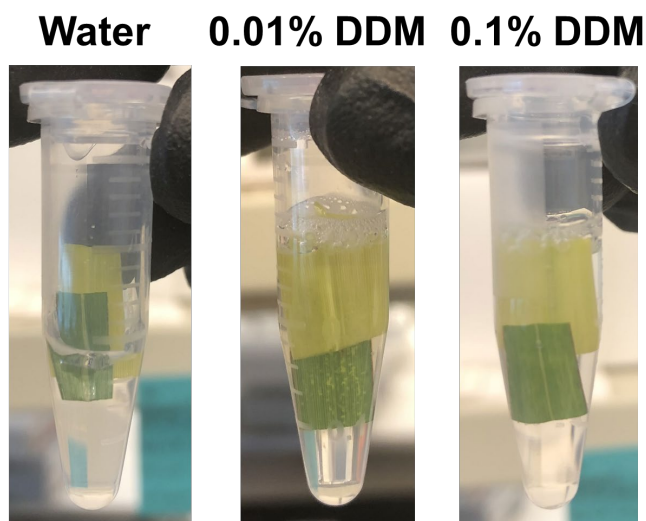


Figure 2. Addition of detergent (0.01% DDM) improves *in vivo* labeling of waxy sorghum leaves with probe by reducing surface tension and enabling full submersion of all leaves.

Drought conditions resulted in significant stress for both sorghum genotypes. Root wet weight, leaf wet weight, and total number of leaves for drought plants were decreased for drought plants compared to controls (Fig. 3). Visually, plants under drought were smaller and had more yellowing of leaves (chlorosis) (Fig 4).

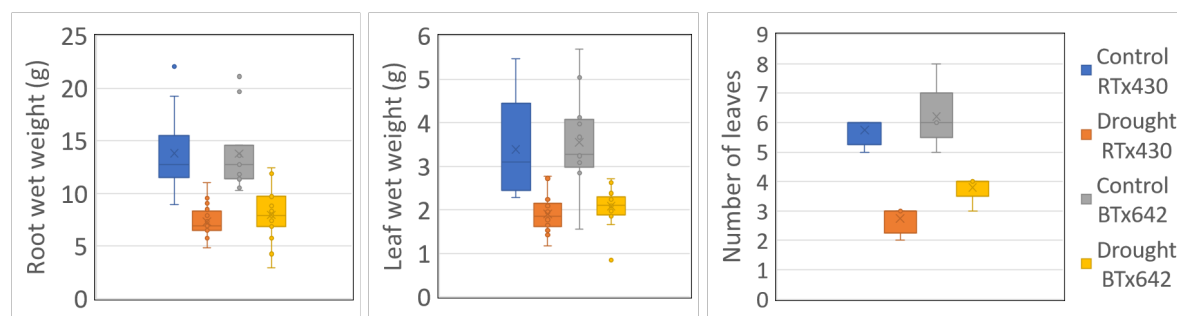


Figure 3. Sorghum physical measurements at time of harvest (n = 12-16 for root and leaf weights; n = 4-5 for number of leaves).

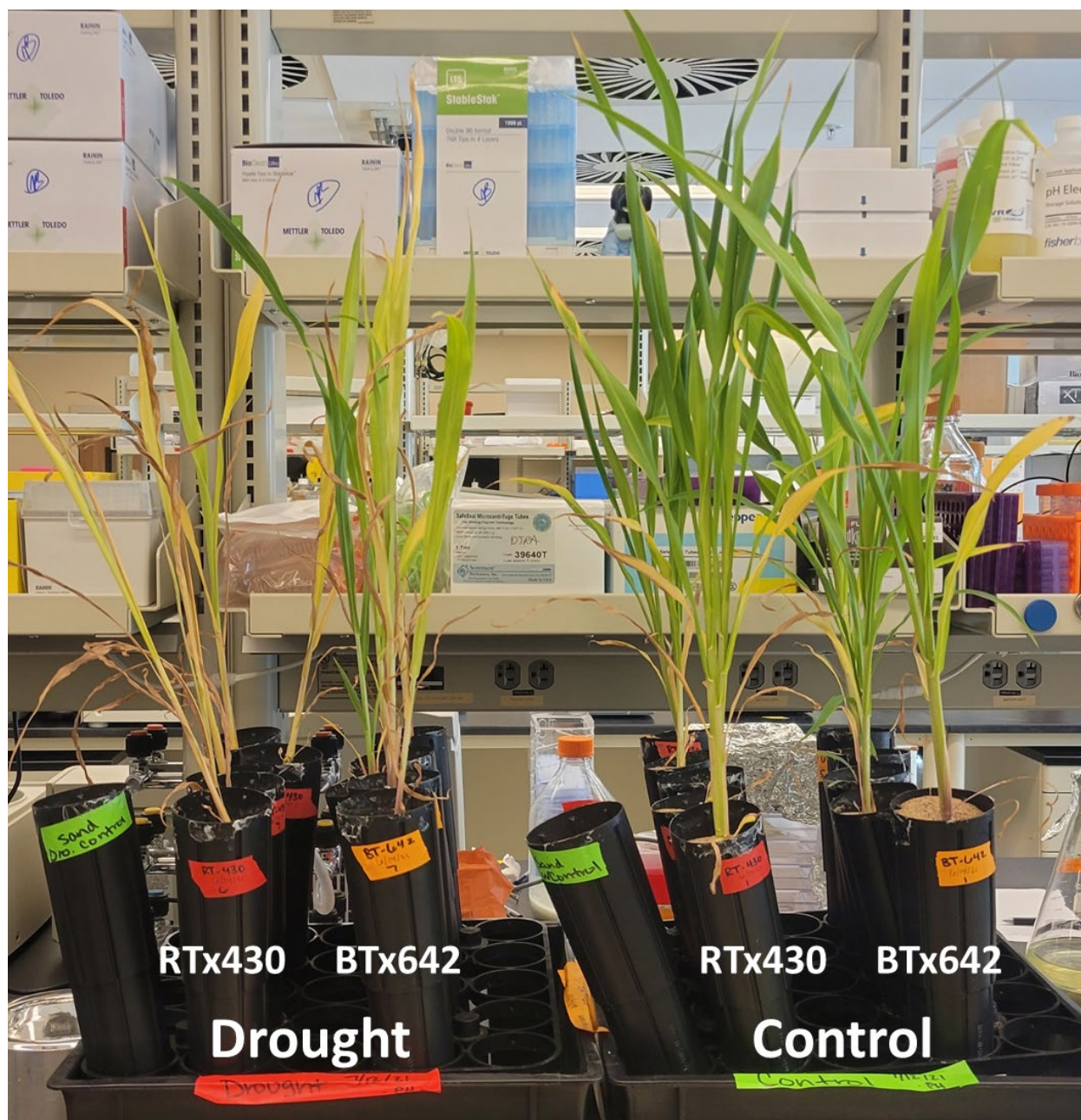


Figure 4. Appearance of drought and control sorghum plants at time of harvest (6-7 weeks growth).

Gel analysis of cysteine and serine protease ABP-labeled sorghum roots showed two major bands at ~30 and ~50 kDa (Fig. 4), while no probe (NP) lanes showed little to no fluorescence, suggesting our ABPP workflow was suitable for ABPP proteomics sample preparation. Initial gel results for sorghum leaves (vacuum infiltration performed with probe in water, without DDM) were inconsistent, potentially due to challenges associated with infiltration of the probe solution due to the leaf cuticle, as described above. ABP-labeled leaf tissues that were vacuum infiltrated with 0.01% DDM were collected and have been reserved for future analysis.

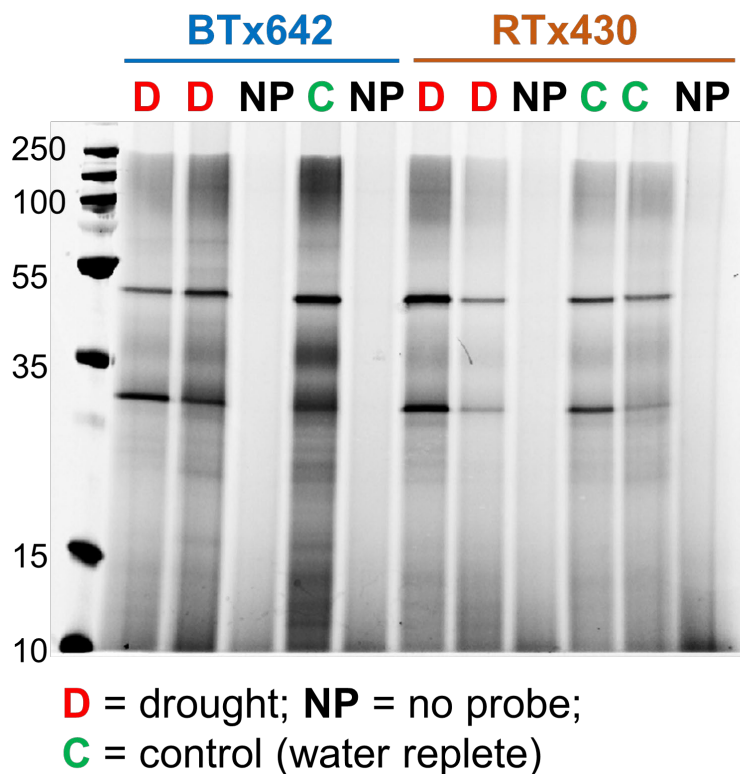


Figure 5. Fluorescent SDS-PAGE gel of serine and cysteine ABP-labeled proteins in sorghum roots.

Due to a limited number of samples, statistical analysis for ABPP proteomics could only be performed for comparison of root tissue samples across both genotypes for either the NP vs. probed samples or the drought vs. control conditions ( $n = 3-4$  per group). Additional samples for each condition and genotype (drought and control, BTx642 and RTx430) were collected and reserved for future processing.

These preliminary results yielded 8 proteins with serine or cysteine protease functional annotations and 3 uncharacterized proteins that were enriched in control compared to drought root tissues (Table 1). Comparison of the NP vs. probed samples as shown in the Fig. 6 volcano plot yielded a larger number of proteins that had known serine or cysteine protease activities, including alpha/beta hydrolases, subtilases, and various esterases, as well as additional proteins of unknown function.

These initial findings indicate our *in vivo* sorghum tissue labeling approach can be used to identify functional cysteine and serine proteases in roots, while a modified approach using detergents in the labeling solutions may be needed to obtain more consistent results for leaves. Future analysis of reserved both leaf and root tissue samples will provide additional data for more robust statistical analysis of drought vs. control plants.

Table 1. Proteins identified as statistically different between drought vs. control plants of both genotypes using serine and cysteine protease ABPs in sorghum root tissues.

Protein	Description	p-value
Sobic.007G053300.1	defline=similar to Chromosome chr9 scaffold_7, whole genome shot	0.00016
Sobic.006G030100.3	defline=similar to Acetyl-coenzyme A carboxylase; arabi-defline=	0.00126
Sobic.003G367300.1	defline=similar to Actin-1; arabi-defline=actin 7; rice-defline=	0.00216
Sobic.010G232100.1	defline=similar to AT4g05320/C17L7_240; arabi-defline=polyubiqui	0.00303
Sobic.005G107900.1	defline=similar to Herbicide safener binding protein; arabi-defl	0.00369
Sobic.008G178800.1	defline=similar to Methionine synthase 2 enzyme; arabi-defline=C	0.00716
Sobic.003G350100.1	defline=similar to Histone H2B.4; arabi-defline=Histone superfam	0.00829
Sobic.008G178800.1	defline=similar to Methionine synthase 2 enzyme; arabi-defline=C	0.00944
Sobic.009G240600.1	defline=none; arabi-defline=alpha/beta-Hydrolases superfamily pr	0.01106
Sobic.003G367300.1	defline=similar to Actin-1; arabi-defline=actin 7; rice-defline=	0.01231
Sobic.002G301200.1	defline=similar to Serine carboxypeptidase-like precursor; arabi	0.01892
Sobic.010G224900.1	defline=similar to Tubulin beta-2/beta-3 chain; arabi-defline=tu	0.01965
Sobic.010G020100.1	defline=similar to Putative uncharacterized protein; arabi-defli	0.01990
Sobic.008G114300.1	defline=similar to Putative acetyl-CoA carboxylase; arabi-deflin	0.02188
Sobic.009G240600.1	defline=none; arabi-defline=alpha/beta-Hydrolases superfamily pr	0.02548
Sobic.006G030100.3	defline=similar to Acetyl-coenzyme A carboxylase; arabi-defline=	0.03011
Sobic.001G103800.2	defline=none; arabi-defline=alpha/beta-Hydrolases superfamily pr	0.03394
Sobic.009G240600.1	defline=none; arabi-defline=alpha/beta-Hydrolases superfamily pr	0.03438
Sobic.009G006100.1	defline=similar to Actin-11; arabi-defline=actin 7; rice-defline	0.03441
Sobic.010G250000.1	defline=none; arabi-defline=Ribosomal protein L2 family; rice-de	0.03713
Sobic.001G171300.1	defline=similar to Putative uncharacterized protein; arabi-defli	0.03748
Sobic.001G265500.1	defline=similar to Putative uncharacterized protein; arabi-defli	0.04295
Sobic.004G300100.1	defline=similar to Os02g0649400 protein; arabi-defline=esterase/	0.04319
Sobic.009G240600.1	defline=none; arabi-defline=alpha/beta-Hydrolases superfamily pr	0.04448
Sobic.004G259200.1	defline=similar to ADP,ATP carrier protein 1, mitochondrial prec	0.04559
Sobic.003G261500.2	defline=similar to Os01g0688200 protein; arabi-defline=alpha/bet	0.04627

Serine and cysteine hydrolases are indicated in red. Uncharacterized proteins are indicated in blue.

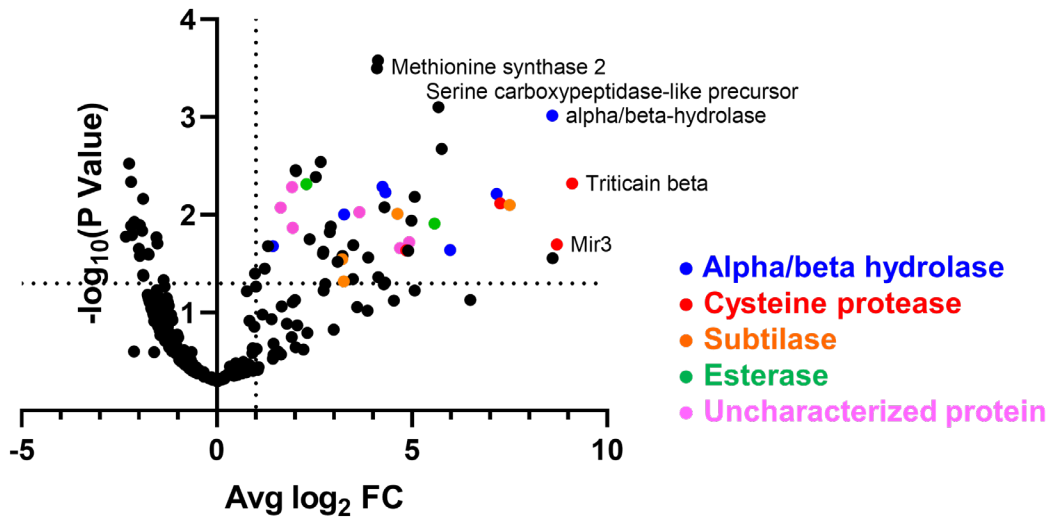


Figure 6. Volcano plot of proteins identified in sorghum root samples using cysteine and serine protease ABPs.



## 4.0 References

- Abdel-Ghany, S. E., F. Ullah, A. Ben-Hur, and A. S. N. Reddy. "Transcriptome Analysis of Drought-Resistant and Drought-Sensitive Sorghum (*Sorghum Bicolor*) Genotypes in Response to Peg-Induced Drought Stress." *Int J Mol Sci* 21, no. 3 (Jan 24 2020). <https://doi.org/10.3390/ijms21030772>. <https://www.ncbi.nlm.nih.gov/pubmed/31991584>.
- Cravatt, B. F., A. T. Wright, and J. W. Kozarich. "Activity-Based Protein Profiling: From Enzyme Chemistry to Proteomic Chemistry." *Annu Rev Biochem* 77 (2008): 383-414. <https://doi.org/10.1146/annurev.biochem.75.101304.124125>. <https://www.ncbi.nlm.nih.gov/pubmed/18366325>.
- Goche, T., N. G. Shargie, I. Cummins, A. P. Brown, S. Chivasa, and R. Ngarra. "Comparative Physiological and Root Proteome Analyses of Two Sorghum Varieties Responding to Water Limitation." *Sci Rep* 10, no. 1 (Jul 16 2020): 11835. <https://doi.org/10.1038/s41598-020-68735-3>. <https://www.ncbi.nlm.nih.gov/pubmed/32678202>.
- Hang, H. C., J. Loureiro, E. Spooner, A. W. van der Velden, Y. M. Kim, A. M. Pollington, R. Maehr, M. N. Starnbach, and H. L. Ploegh. "Mechanism-Based Probe for the Analysis of Cathepsin Cysteine Proteases in Living Cells." *ACS Chem Biol* 1, no. 11 (Dec 20 2006): 713-23. <https://doi.org/10.1021/cb600431a>. <https://www.ncbi.nlm.nih.gov/pubmed/17184136>.
- Have, M., T. Balliau, B. Cottyn-Boitte, E. Derond, G. Cueff, F. Soulay, A. Lornac, *et al.* "Increases in Activity of Proteasome and Papain-Like Cysteine Protease in Arabidopsis Autophagy Mutants: Back-up Compensatory Effect or Cell-Death Promoting Effect?" *J Exp Bot* 69, no. 6 (Mar 14 2018): 1369-85. <https://doi.org/10.1093/jxb/erx482>. <https://www.ncbi.nlm.nih.gov/pubmed/29281085>.
- Kaschani, F., S. H. Verhelst, P. F. van Swieten, M. Verdoes, C. S. Wong, Z. Wang, M. Kaiser, *et al.* "Minitags for Small Molecules: Detecting Targets of Reactive Small Molecules in Living Plant Tissues Using 'Click Chemistry'." *Plant J* 57, no. 2 (Jan 2009): 373-85. <https://doi.org/10.1111/j.1365-313X.2008.03683.x>. <https://www.ncbi.nlm.nih.gov/pubmed/18786180>.
- Lyon, D., M. A. Castillejo, V. Mehmeti-Tershani, C. Staudinger, C. Kleemaier, and S. Wienkoop. "Drought and Recovery: Independently Regulated Processes Highlighting the Importance of Protein Turnover Dynamics and Translational Regulation in *Medicago Truncatula*." *Mol Cell Proteomics* 15, no. 6 (Jun 2016): 1921-37. <https://doi.org/10.1074/mcp.M115.049205>. <https://www.ncbi.nlm.nih.gov/pubmed/27001437>.
- Morimoto, K., and R. A. van der Hooft. "The Increasing Impact of Activity-Based Protein Profiling in Plant Science." *Plant Cell Physiol* 57, no. 3 (Mar 2016): 446-61. <https://doi.org/10.1093/pcp/pcw003>. <https://www.ncbi.nlm.nih.gov/pubmed/26872839>.
- van der Hooft, R. A. "Plant Proteases: From Phenotypes to Molecular Mechanisms." *Annu Rev Plant Biol* 59 (2008): 191-223. <https://doi.org/10.1146/annurev.arplant.59.032607.092835>. <https://www.ncbi.nlm.nih.gov/pubmed/18257708>.
- van der Hooft, R. A., T. Colby, S. Nickel, K. H. Richau, J. Schmidt, and M. Kaiser. "Mining the Active Proteome of *Arabidopsis Thaliana*." *Front Plant Sci* 2 (2011): 89. <https://doi.org/10.3389/fpls.2011.00089>. <https://www.ncbi.nlm.nih.gov/pubmed/22639616>.
- van der Hooft, R. A., M. A. Leeuwenburgh, M. Bogyo, M. H. Joosten, and S. C. Peck. "Activity Profiling of Papain-Like Cysteine Proteases in Plants." *Plant Physiol* 135, no. 3 (Jul

- 2004): 1170-8. <https://doi.org/10.1104/pp.104.041467>.  
<https://www.ncbi.nlm.nih.gov/pubmed/15266051>.
- Wang, X., X. Cai, C. Xu, Q. Wang, and S. Dai. "Drought-Responsive Mechanisms in Plant Leaves Revealed by Proteomics." *Int J Mol Sci* 17, no. 10 (Oct 18 2016).  
<https://doi.org/10.3390/ijms17101706>. <https://www.ncbi.nlm.nih.gov/pubmed/27763546>.
- Wiedner, S. D., K. E. Burnum, L. M. Pederson, L. N. Anderson, S. Fortuin, L. M. Chauvigne-Hines, A. K. Shukla, *et al.* "Multiplexed Activity-Based Protein Profiling of the Human Pathogen *Aspergillus Fumigatus* Reveals Large Functional Changes Upon Exposure to Human Serum." *J Biol Chem* 287, no. 40 (Sep 28 2012): 33447-59.  
<https://doi.org/10.1074/jbc.M112.394106>.  
<https://www.ncbi.nlm.nih.gov/pubmed/22865858>.
- Willems, L. I., H. S. Overkleeft, and S. I. van Kasteren. "Current Developments in Activity-Based Protein Profiling." *Bioconjug Chem* 25, no. 7 (Jul 16 2014): 1181-91.  
<https://doi.org/10.1021/bc500208y>. <https://www.ncbi.nlm.nih.gov/pubmed/24946272>.

# **Pacific Northwest National Laboratory**

902 Battelle Boulevard  
P.O. Box 999  
Richland, WA 99354

1-888-375-PNNL (7665)

***[www.pnnl.gov](http://www.pnnl.gov)***