

Grant Code: AW7061

Title: Development of In-plant Sap Sensing for Aluminum Toxicity in Wheat and Lime Treatment evaluation

Personnel: Johnny Li, Assistant Professor in Precision Agriculture, Soil & Water Sys. Daniel Strawn, Professor in Environmental Soil Chemistry; Kurt Schroeder, Associate Professor & Cropping Systems Agronomist

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Justification/Rationale: Globally, about 50% of all arable soils are classified as acidic. A meta-analysis from 1570 observations from 121 field-based studies worldwide observed that liming of agricultural acid soils increases crop yield by 36.3%, on average, which results in a global increase of upland crop yields by 7.70×10^8 Mg, rice yields by 0.56×10^8 Mg, and grass production by 5.90×10^8 Mg every year. Based on FAO stats data of the average food demand per capita over the world (Wang 2021), the estimated liming-induced increment in crop and biomass production has the potential to feed nearly 1261 million people in the future.

Development of in-plant sap sensing will empower long-term, high-resolution, cost-effective monitoring of soil health, plant-soil-microbial interactions across a range of soil properties and improve the crop development and productivity impacted by soil acidity.

In-situ plant Aluminum (Al) sap sensing for real-time soil acidity and Al toxicity in plant monitoring and plant improvement has not been reported in the available literature. Currently, available Al for plant uptake is assessed by extraction of soil with KCl or CaCl_2 solutions (Yang 2022). However, the actual plant uptake may be quite different than the soil extract predicted bioavailability and depends on the crop cultivar genotype and soil physicochemical conditions. Standard soil test, plant leaf tissue analysis and plant sap test are destructive, labor and time intensive, and require periodic leaf samplings over growing season to determine actual uptake amounts that affect plant growth (Hochmuth 2022). Hyperspectral imaging was explored for detecting cadmium stress in two leafy green crops and accelerate soil remediation efforts (Zea 2021). Current leaf sap tests are too difficult to make temporal measurements needed to characterize Al uptake and effects of weather events or impacts of in-season lime application. Therefore, it is critical to develop in-plant Al sap sensing to acquire new knowledge and preliminary data related to plant sap Al content as affected by soil acidity and lime treatments. Important information needed to assess in-situ Al toxicity analysis and eventual development of wireless communication and remotely sensed plant conditions.

Objectives: This project will address these specific needs in

Northwest Palouse region by advancing in-plant Al sap sensing and hyperspectral imaging for wheat plant Al toxicity and lime treatment evaluation in acid soils. Our three objectives are: (1) Develop and validate an in-plant Al sap sensor based on Hydrogel Ionic Gated MXene-Graphene FET under different Al chemical forms/concentration and pH levels in the laboratory; 2) calibrate in-plant sap sensing using extracted sap samples from wheat grown

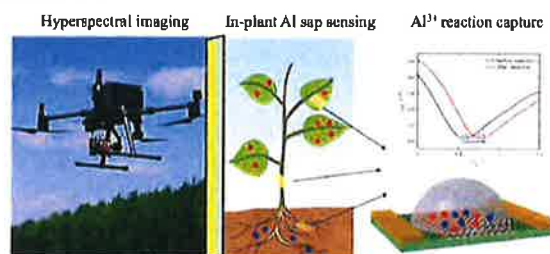


Fig.1 In-plant Al sap sensing and in-field hyperspectral imaging

in various soluble Al concentrations in the greenhouse and compare results to standard soil and plant tests used to predict Al toxicity; and 3) validate the field deployment feasibility of in-plant Al sap sensing and hyperspectral imaging for in-field Al toxicity monitoring in an existing soil acidity and lime treatment research trial in Potlatch, ID and Parker farm. The new in-plant sap sensing will allow for real-time field measurement of Al in plant sap, leading to in-season lime treatment evaluation and correction of Al toxicity in an efficient precision manner.

Methods/Plan of work: The long-term goal of this project is to increase agricultural crop productivity in acid soil and reduce aluminum (Al) toxicity by optimizing the lime treatment impact in plants through a novel in-plant Al sap sensor to real-time monitor soil acidity and Al toxicity in plant continuously and efficiently. The hydro-gel-ionic-gated MXene-Graphene FET based plant Al sap sensor makes direct measurement of signal change (Dirac point shift) caused by the reactions between OH^- , soluble Al species (Al^{m+}) in the sap and the corresponding hydrogel ionic gated agents Ti_3C_2 and $(\text{Ti}_3\text{C}_2\text{O})^-$ in the sensor respectively (Fig.1). Tasks for **objective 1** includes Characterization of Al chemical forms and its reaction. The concentrations of Al^{3+} species and the total soluble Al in soil, will suffice to predict toxicity. These species increase the solubility of total Al in solution, and their concentrations varied at different pH levels. We will characterize and quantify signal change (Dirac point shift) of total Al and its key forms in the plant sap and using controlled standard Al solutions with various hydrolysis species (pH 4.5-7.5) and chelated Al species made with citric acid and oxalic acid as representative model plant sap species. The sensitivity of the corresponding signal change in the plant Al sap sensor and its chemical reaction agent will be monitored in the different solutions. The total Al will be detected by the in-plant sap sensor and signal readout and concentration will be calibrated for low pH soil (4-5), and mid-range (5.5 to 6) and a high pH (6.5-7.5) acid soil. Tasks for **objective 2** includes Sensor calibration of Signal change (Dirac Point Shift). The chemical agent in the in-plant sap will react with the OH^- at certain pH level and the different Al forms (Al_m^+), will cause constant voltage and current (V_D and V_G , I_{DS}) change with the change of charge density on MXene-graphene surface. Moreover, changes in the relative amount of positive and negative charges could cause Dirac point shifting. Dirac point shift will be characterized by the total Al and its key chemical forms concentration at different pH levels for the in-plant Al sap sensor. Based on the signal change, we can infer the concentration of the target chemicals, which can be compared and correlated with the standard soil test and plant sap analysis result for real-time soil acidity and Al toxicity in plant assessment. Point shift (i.e., the valley of the “V-shape” curve) for wheat plant sap and the Al toxicity detection in different pH conditions will be calibrated by investigating the measurement error between the controlled solution and the actual extracted wheat plant sap. The calibration factor/coefficient of the total Al and Al species concentration reading in ppm based on the standard soil test and plant sap test will be measured by Co-PI Strawn on ICP-AES (MDL ~ 0.001 mg/L). Tasks for **objective 3** is Validation of In-situ plant Al plant sap sensing in the two lime field trials in Idaho directed by Co-PI Schroeder. The first lime trial was established in the fall of 2016 in Potlatch, ID. The site includes 0, 1, 2 and 3 ton/A lime rates with each treatment being replicated four times. Individual plots measure 8 ft wide x 100 ft long. A second site will be established near Moscow in 2023 using the same experimental design. The soil samples from our trial sites and testing for soil pH, soluble aluminum, and a number of other measurements at 3-inch increments to a depth of 12 inch will be collected to validate the effectiveness of the developed in-plant Al sap sensing. The agronomic measurements such as plant height and tiller

count (for cereal crops) and will harvest to obtain grain yield, test weight, and protein content to evaluate the lime treatment performance. The in-situ plant Al sensor will be deployed to the field for testing the optimal power consumption, data collection frequency (diurnal pattern from every 1 second to 1 hour) and the spatial distribution (three pH zones with 5 replicates) and other environmental factors. Thus, the in-plant Al sap sensor will be validated with the in-field lime trial in different soil acidity conditions and will provide insights for Al toxicity in plant and its effects on the crop yield and quality and potential in-season site-specific precision amendment.

Duration: 3 years: 2023-2026. Year 2 of 3

Cooperation/Complementation: The in-plant Al sap sensor prototype will be evaluated in the greenhouses coordinated by Co-PI Dr. Daniel Strawn at University of Idaho and field trials at the two liming sites in Idaho led by Co-PI Dr. Kurt Schroeder. Soils at these sites have become acidified from years of ammoniacal fertilizer application. The in-plant sap sensor prototype will collaborate with Nanosensor Scientist Dr. Chenglin Wu at Texas A&M.

Anticipated Benefits/Expected Outcomes: Currently there are no sap sensor for Aluminum Toxicity in plant assessment. The existing commercial nitrate pocket tester cost ~\$1,000, Sample (every two weeks over the growing season) and Ship in 24 hour for in-lab analysis cost more than \$70 / sample (labor cost not included) and \$96 per sap test consultation. The proposed in-plant sap sensor will be targeted to be within hundred-dollar range and can be customized to unattendance monitoring any nutrient profile and even pesticide/disease diagnose. After the calibrated in-plant Al sap sensor validated with the lime trial research at Idaho in different soil acidity conditions, the in-situ plant Al sensing data and its spatially-correlated total Al over time can be fused with weather and soil data, multi- and hyperspectral remote sensing data, agronomic data and final yield data in the same filed, which will provide insights for Al toxicity in plant and its effects on the crop yield and quality and its potential in-season site-specific precision lime amendment on approximately 50% of the world's arable land. The research outcomes from this study will benefit agronomy, plant biology and the precision agriculture research.

Transfer of Information/Technology: Results will be integrated into education, training and outreach activities as described below to maximize communication of the information obtained from this project nationally and internationally, including 1) written publication in IWC weekly newsletter, Focus on Research, and/or Idaho Grain magazine on the in-plant sap sensor concept and field trial results; 2) written publications (at least 2 manuscripts) to peer-reviewed journals; 3) presentations to be made at technical symposia and professional conferences; 4) Example as PI's course ASM305 Precision Agriculture and ASM240 Computer Applications; 5) field demonstrations at Dryland field days (DFD) for farmer with new tools for wheat breeding, wheat diseases and Plant Diagnostic. This project will allow for development and advancement of new plant sensor and precision Ag technology for growers.

Literature Review: Soil health is soil's capacity to perform agronomic functions including sustainable production of crops and animals while maintaining and improving the environment (Lal 2021). Al is the most abundant metal in earth crust and comprises 7% of it. Under acidic conditions ($\text{pH} < 5.0$), Al is solubilized into a toxic trivalent cation, Al^{3+} (Taylor, 1991). The predominant and most toxic form in the soil solution is Al^{3+} . In many agriculturally important

plant species, the presence of only micromolar concentrations of Al^{3+} can result in the inhibition of root growth within minutes or hours (Kochian, 1995). Al toxicity inhibit Root growth inhibition and hinder the water and nutrient uptake in plant (Ca, Mg, P, K, etc.) and thus reduce the crop yield (Sade 2016). Thus, continuous monitoring of nutrient availability in soil responsive to environmental variations and the uptake efficiency during growth of plants is critical for soil amendments and crop management including soil acidity monitoring and remediating plant Al toxicity. The microfluidic and microsystem with integrated sensors has been explored for in-situ soil nitrogen sensing (Garland 2018, Ali 2019), and in planta nitrate Sensor (Ibrahim 2022) respectively. However, there are soil acidity induced Al toxicity in plants detection for nutrient uptake and plant tolerance impact study has not been reported yet. It is essential to gain the knowledges of soil acidity and Al toxicity in plant before it's 'subclinical' deficiencies or visual symptom occurs for potential precise correction of Al toxicity.

References

- Ali, M. A. et al. Novel All-Solid-State Soil Nutrient Sensor Using Nanocomposite of Poly(3-Octyl-Thiophene) and Molybdenum Sulfate," 2019 20th International Transducers & Eurosensors XXXIII), 2019, pp. 170-173, doi: 10.1109/TRANSDUCERS.2019.8808341.
- Garland, Nate & McIlmore, Eric & Cavallaro, Nicholas & Mendivelso-Perez, Deyny & Smith, Emily & Jing, Dapeng & Claussen, Jonathan. (2018). Flexible Laser-Induced Graphene for Nitrogen Sensing in Soil. *ACS Applied Materials & Interfaces*. 10. 10.1021/acsami.8b10991.
- Hochmuth, George & Hochmuth, Robert. (2022). Plant Petiole Sap-Testing for Vegetable Crops: CV004/CIR1144, rev. 5/2022. EDIS. 2022. 10.32473/edis-cv004-2022.
- Jiang, Huawei & Ali, Md Azahar & Jiao, Yueyi & Yang, Bing & Dong, Liang. (2017). In-situ, real-time monitoring of nutrient uptake on plant chip integrated with nutrient sensor. 289-292. 10.1109/TRANSDUCERS.2017.7994045.
- Kochian, L.V. Cellular mechanisms of aluminum toxicity and resistance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 46 (1995), pp. 237-260
- Lal, R. (Ed.). (2021). *Soil Organic Matter and Feeding the Future: Environmental and Agronomic Impacts* (1st ed.). CRC Press. <https://doi.org/10.1201/9781003102762>
- Lal, R. (Ed.). (2021). *Soil Organic Matter and Feeding the Future: Environmental and Agronomic Impacts* (1st ed.). CRC Press. <https://doi.org/10.1201/9781003102762>
- Sade, H., Meriga, B., Surapu, V., Gadi, J., Sunita, M. S. L., Suravajhala, P., & Kishor, P. K. (2016). Toxicity and tolerance of aluminum in plants: tailoring plants to suit to acid soils. *Biometals*, 29(2), 187-210.
- Taylor, G.J. Current views of the aluminum stress response: the physiological basis of tolerance. *Curr. Top. Plant Biochem. Physiol.*, 10 (1991), pp. 57-93
- Wang, Y, Yao, Z, Zhan, Y, Zheng, X, Zhou, M, Yan, G, Wang, L, Werner C, Butterbach-Bahl K. Potential benefits of liming to acid soils on climate change mitigation and food security. *Glob Chang Biol*. 2021 Jun;27(12):2807-2821. doi: 10.1111/gcb.15607. Epub 2021 Apr 9. PMID: 33742490.
- Xiao, Feng, Zhang, Baojie, Lee, Chery. (2008). Effects of low Temperature on Aluminum(iii) Hydrolysis: Theoretical and Experimental Studies. *Journal of environmental sciences (China)*. 20. 907-14. 10.1016/S1001-0742(08)62185-3.
- Yang, J. & Sun, Lin & Shen, Xing & Dai, Min & Ali, Imran & Peng, Changsheng & Naz, Iffat. (2022). An overview of the methods for analyzing the chemical forms of metals in plants. *International Journal of Phytoremediation*. 10.1080/15226514.2022.2033687.
- Zea, Maria & Magalhaes de Souza, Augusto & Yang, Yang & Lee, Linda & Nemali, Krishna & Hoagland, Lori. (2021). Leveraging high-throughput hyperspectral imaging technology to detect cadmium stress in two leafy green crops and accelerate soil remediation efforts. *Environmental Pollution*. 292. 118405. 10.1016/j.envpol.2021.118405.

**FY2025
COMMODITY COMMISSION BUDGET
Principal Investigator: Johnny Li**

Allocated by _____ during FY2023	\$	-
(Commission/Organization)		
Allocated by <u>Idaho</u> Wheat Commission during FY2024	\$	30,360
(Commission/Organization)		

REQUESTED SUPPORT:		<u>Awarded for FY2024</u>	<u>Requested for FY2025</u>
Budget Categories			
(10) Salary (<i>staff, post-docs, et</i> NOTE: Faculty salary/fringe NOT allowed)	\$	16,250	\$ 15,600
(12) Temporary Help/IH	\$	1,800	\$ 3,000
(11) Fringe Benefits	\$	742	\$ 639
(20) Travel	\$	472	\$ 655
(30) Other Expenses	\$	3,200	\$ 5,396
(40) Capital Outlay >\$5k	\$	-	\$ -
(45) Capital Outlay <\$5k	\$	-	\$ -
(70) Graduate Student			
Tuition/Fees	\$	7,896	\$ 8,260
TOTALS	\$	30,360	\$ 33,550

TOTAL BUDGET REQUESTED FOR FY2025:	\$ 33,550
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BREAKDOWN FOR MULTIPLE INDEXES:					
Budget Categories	Johnny Li	Dan Strawn	Kurt Schroeder	(Insert Co-PI Name)	
(10) Salary (<i>staff, post-docs, et</i> \$	15,600	\$ -	\$ -	\$ -	-
(12) Temporary Help \$	-	\$ 1,800	\$ 1,200	\$ -	-
(11) Fringe Benefits \$	390	\$ 149	\$ 100	\$ -	-
(20) Travel \$	655	\$ -	\$ -	\$ -	-
(30) Other Expenses \$	3,396	\$ 2,000	\$ -	\$ -	-
(40) Capital Outlay >\$5k \$	-	\$ -	\$ -	\$ -	-
(45) Capital Outlay <\$5k \$	-	\$ -	\$ -	\$ -	-
(70) Graduate Student					
Tuition/Fees \$	8,260	\$ -	\$ -	\$ -	-
TOTALS \$	28,301	\$ 3,949	\$ 1,300	\$ -	-
Total Sub-budgets				\$	33,550

ANNUAL REPORT

Grant Code: AW7061

TITLE: Development of In-plant Sap Sensing for Aluminum Toxicity in Wheat and Lime Treatment evaluation

PERSONNEL: Johnny Li, Assistant Professor in Precision Agriculture, Dept. of SWS
Daniel Strawn, Professor in Environmental Soil Chemistry Dept. of SWS
Kurt Schroeder, Associate Professor & Cropping Systems Agronomist

ADDRESS: Dept. of SWS, University of Idaho, 875 Perimeter Dr., Moscow, ID 83843; 208-885-1015; liujunl@uidaho.edu

ABSTRACT

Globally, about 50% of all arable soils are classified as acidic. Aluminum toxicity in acid soils, adversely abets the plant root growth, and limits the nutrient/water uptake impacting crop productivity. The soil PH in Palouse region has continued to decline from 7 to 5.5 or even 5.0. It is essential to gain the knowledges of soil acidity and aluminum toxicity tolerance in the plant before it's 'subclinical' deficiencies or visual symptom occurs. Standard soil test, plant leaf tissue analysis and plant sap test were destructive, labor and time-intensive and conducted in the analytics lab. This research is aiming to develop an in-plant sensing system based on a Hydrogel Ionic Gated MXene-Graphene FET platform to continuously monitor the plant aluminum concentration and validated by the hyperspectral imaging and the in-season plant sap testing result before and after lime/nitrogen application. In this report period, we prepared six concentration levels of 250 mL AlCl_3 solution at 0, 2.7, $2.7\text{e-}1$, $2.7\text{e-}2$, $2.7\text{e-}3$, $2.7\text{e-}4$ ppm with aluminum chloride hexahydrate in water acidified with 1.0 M hydrochloric acid (pH = 4.0). The initial I-V curve shows a significant shift in the signal change of the customized surface reaction agent (5%, which is more than 10 times the noise level at 0.5%) with a PBS base with an extremely low concentration at 1 fg/ml of Al component. Gradual increase of Al^{3+} concentration shows an almost linear response increase. It approved the feasibility of the proposed sensing platform and potential for plant Al sensing and liming performance evaluation. We tested the sap extraction protocol in the wheat plant in greenhouse and field condition and conducted aerial hyperspectral imaging on the lime trial field. We expect to prototype the plant sap sensing system and test the desired soil acidity and Al toxicity in plant sensing for the 2024-2025 season.

OBJECTIVE

Our three objectives are: (1) Develop and validate an in-plant Al sap sensor based on Hydrogel Ionic Gated MXene-Graphene FET under different Al chemical forms/concentration and pH levels in the laboratory; (2) calibrate in-plant sap sensor using extracted sap samples from wheat grown in various soluble Al concentrations in the greenhouse and compare results to standard soil and plant tests used to predict Al toxicity; and (3) validate the field deployment feasibility of in-plant Al sap sensor for in-field Al toxicity monitoring in an existing soil acidity and lime treatment research trial northwest Palouse region. The new in-plant sap sensor will allow for real-time field measurement of Al in plant sap, leading to in-season lime treatment evaluation and correction of Al toxicity in an efficient precision manner.

ACCOMPLISHMENTS

This project is to address these specific needs in Northwest Palouse region by advancing in-plant Al sap sensor development and testing of wheat plant Al toxicity and lime treatment performance assessment in acid soils. In this report period, we were able to develop a design of in-plant Al sap sensor concept based on Hydrogel Ionic Gated MXene-Graphene FET platform for detection of aluminum toxicity of wheat plant and lime treatment evaluation (Fig. 1). The prototyping has been delayed due to our collaborator's lab relocation and delay acquiring the necessary semiconductor manufacturing equipment due to the CoVID-19 and supply chain challenges. We expect to customize surface functionalization to achieve the desired soil acidity and Al toxicity in plant sensing for the 2024-2025 season.

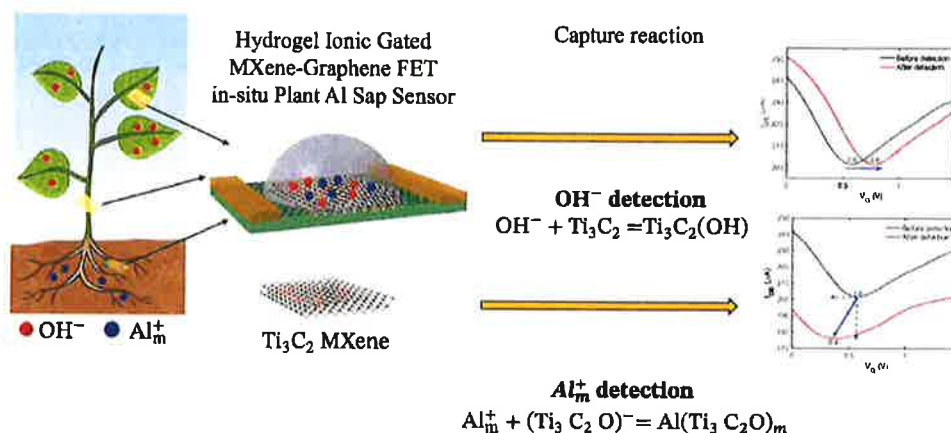


Fig. 1. In-plant Al sap sensor design based on Hydrogel Ionic Gated MXene-Graphene FET sensing platform with customized surface function reaction agent for Al detection

Sap Extraction Method Testing: In order to validate the proposed in-situ plant sap sensing, we tested two sap extraction method including centrifugation and cotton absorption as in Fig.2 in this project.



Fig. 2. Sap extraction method comparison (a) Centrifugation, (b) Cotton absorption in greenhouse, (c) Cotton absorption for sap extraction in wheat field.

For centrifugation-based sap extraction, 2-3" stem cuttings were placed xylem end down in 50 mL centrifuge tubes (one plant in the heading stage can fill about two tubes) and then went through centrifugation at 10,000 rpm for 30 min. Full tube weight ranged from ~25-28 g in each pair of balanced tubes). The stems reabsorb/adhere to extracted xylem after centrifugation, so no measurable amount was collected. Additionally, we tested a special filter tube designed for protein purification/concentration) for better sap separation. Finally, it was able to separate approximately 50-75 μL , which is practical for cross-validation between our in-plant sap sensing and the traditional plant ap testing result.

For cotton absorption, immediately after harvesting wheat stems, 1.5 mL Eppendorf tubes with cotton and straw pieces were placed upside down on the cut ends of stems (Fig.2b and c). The tubes were then secured to the stems using parafilm (This is also done to minimize any evaporative losses). After 24 hours, the parafilm was removed and tubes were capped and stored in a cooler until centrifugation. Tubes were placed in a centrifuge set for 15,000 rpm for 3 minutes. After centrifugation, cotton and straws were removed and tubes were visually assessed for sap volume collected. Out of 69 tubes, only ~3.5 μL total sap was collected. Most tubes had no sap and very few tubes (15/69) had less than 1 μL .

Preparation of AlCl_3 Solution for Sap Sensor Testing: To characterize and quantify Signal change (Dirac point shift) of total Al and key Al forms in the plant sap, we had prepared controlled standard Al solutions with various hydrolysis species and chelated Al species made with citric acid and oxalic acid as representative model plant sap species. Series of concentration levels of AlCl_3 solution at 250mL from 0, 2.7, $2.7\text{e-}1$, $2.7\text{e-}2$, $2.7\text{e-}3$, $2.7\text{e-}4$ ppm was prepared with aluminum chloride hexahydrate in water acidified with 1.0 M hydrochloric acid (pH = 4.0) (Table 1). We started by making the 10^{-4} M AlCl_3 solution by adjusting the solution pH to just below 4.0 (~3.8-3.9) by adding drops of 1.0 M HCl, and then adjusting the solution pH to 4.0 (+/- 0.02) by adding drops of 1.0 M NaOH accordingly and then perform a series of 10:1 dilution using the stock 10^{-4} M AlCl_3 standard solution (refer to Table 1.). We transfer each solution to a 250 mL bottle, respectively and adjust the pH of each solution by adding 1.0 or 0.1 M NaOH or HCl accordingly. Aim for pH 4.0 (+/- 0.02).

Table 1. Volume of 10^{-4} M AlCl_3 standard solution required for 10^{-5} M - 10^{-8} M dilutions

Dilution Concentration	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M
Volume of 10^{-4} M HCl needed	25.0 mL	2.50 mL	250 μL	25 μL

In-plant Al sap sensor calibration with the prepared AlCl_3 Solution: The sensitivity of the corresponding signal change in the hydro-gel-ionic-gated MXene-Graphene FET based plant Al sap sensor and its chemical reaction agent was monitored with the above prepared solutions. We have worked with our consultant Dr. Chenglin Wu at the Nano Characterization and Synthesis Lab at Texas A&M University and obtained preliminary results of FET sensing of synthetic AlCl_3 solutions with varying concentrations as in Fig.3: (a) step change in signal by introducing Al solution; (b) real-time signal change versus varying Al concentration; (c) normalized responses versus Al concentration. The initial I-V curve shows a significant shift (5%, which is more than 10 times the noise level at 0.5%) with a PBS base with an extremely low concentration at 1 fg/ml of Al component as shown in Fig. 3(a)(b). Gradual increase of Al^{3+} concentration also shows an almost linear response increase as shown in Fig. 3(b)(c). These indicate the feasibility of the proposed sensing platform and potential for plant Al sensing.

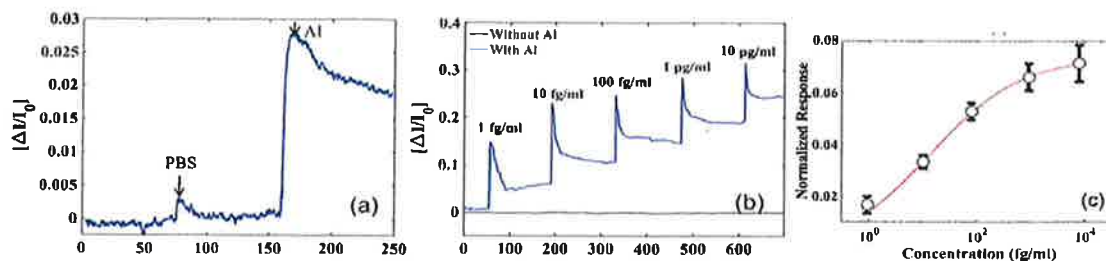


Fig.3 Al detection of the customized surface reaction agent of the proposed in-plant sap sensor preliminary result

Hyperspectral imaging of liming treatment field trial: We conducted two aerial hyperspectral imaging flights on the lime trial field in Potlatch, ID and Parker farm in Moscow, ID in late growth stage to assess the field variation with the lime treatment. We will extract the plot-based spectral information for the strips with 0(control), 1, 2, 3 ton/acre lime rate and other soil/plant ground measurement that Dr. Schroeder has conducted (Table 2). We also conduct the multispectral imaging and ASD hyperspectral scanning for a commercial farm which another collaborator was taking the sap tests before and after the foliar applied fertilizer. We just received their sap testing result. We are working on correlating with the multispectral/hyperspectral indices with the field treatment condition.

Table 2 Potlatch liming treatment field trial (Directed by Co-PI Dr. Schroeder)

Plot#	TRT #	Lime Rate
101	1	Control
102	4	3 ton
103	2	1 ton
104	3	2 ton
201	3	2 ton
202	4	3 ton
203	1	Control
204	2	1 ton
301	4	3 ton
302	3	2 ton
303	2	1 ton
304	1	Control
401	3	2 ton
402	2	1 ton
403	1	Control
404	4	3 ton

OUTREACH / APPLICATIONS / ADOPTION The project has developed an in-plant Al sap sensing protocol based on IoT sensor and hyperspectral imaging for the aluminum toxicity of wheat plant and its potential liming treatment in acid soil, which can provide insights and provide guidance on the liming treatment and its economy decision making. Our in-plant sap extraction

protocol including centrifugation and cotton absorption can provide a comparison analysis for the proposed Al sap sensor and hyperspectral imaging approach. Our plant sap sensing and hyperspectral imaging analysis was invited to present to the Idaho water resource seminar series, which is open to all interested professionals, legislators, local government representatives, attorneys, students and interested public. We also convey our research findings to the 2023 ASA, CSSA, SSSA International annual meeting and 2023 Hermiston Farm Fair on Nov 29-20, 2023, by Oregon State University. We will present our comprehensive research result in the upcoming 2024 ASABE Annual International Meeting, July 28 - 31, 2024 in Anaheim, California so that it might be adopted by the agriculture community widely.

NEXT STEPS / PROJECTIONS

The goal of this project is to provide farmers/growers with novel continuous monitoring of aluminum toxicity in wheat plant and evaluate the liming treatment performance through the state-of-the-art in-plant Al sap sensing and hyperspectral imaging data analysis algorithm. For 2024-2025 season, we will 1) cultivate wheat plant in two different acid soil condition and 2) acquire a pair of 5mm Dynamax sap flow sensor to attach to the thin wheat stem to measure sap flow rate and its diurnal pattern at different growth stage, which can cross validate with our in-plant Al sap sensor, 3) continue to develop the in-plant Al sap sensor prototype and test it in greenhouse with the above cultivated wheat plant grown in different acid soil condition, 4) conduct typical soil and plant analysis as well as the destructive ICP-AES instrumental analysis. In addition to the centrifugation and cotton absorption sap extraction method, we might try a third option of collecting xylem using cotton in centrifuge tubes to increase the volume of sap extraction, 5) We will also conduct the hyperspectral/multispectral imaging of the wheat plants response under acid soil and lime treatment in greenhouse and in-field lime trials Dr. Schroeder directed to investigate the in-plant sap sensing and its potential scaleup with imaging approach.

Research findings and in-plant Al sap sensing from this research will help growers better estimate their subfield aluminum toxicity and liming performance and optimize their liming scheduling and rate so that precision amendment practices can be adopted to greatly increase crop productivity in acidic soil and improving global food security in the near future.

PUBLICATIONS AND PRESENTATIONS:

1. Chen, G, Li, L(2023). Aerial Nondestructive Testing and Evaluation (aNDT&E). Materials Evaluation 81 (1): 67–73 <https://doi.org/10.32548/2023.me-04300>
2. Kwaku Opoku-Ware, Carson Sass, Johnny Li. In-Situ Plant Sap Sampling and Multispectral Imaging for Plant Nutrient Management. 2023 ASA, CSSA, SSSA INTERNATIONAL ANNUAL MEETING. ASA Virtual Session # 155603, October 29-November 1, 2023, St Louis, MO.
3. Invited Talk "Advance Robotics Sensing, Control and Computing for Smart Agriculture" by 2023 School of Mechanical and Materials Engineering Seminar Series at Washington State University, September 21, 2023
4. Invited Talk "Drone remote sensing and AI for Precision Agriculture" by 2023 Hermiston Farm Fair on Nov 29-20, 2023, Oregon State University.
5. Kwaku Opoku-Ware, Huilin Cai, Johnny Li. Hyperspectral Imaging for Aluminum Toxicity and Lime Treatment Performance Evaluation. 2024 ASABE Annual International Meeting, July 28 - 31, 2024 in Anaheim, California (To be submitted)

Appendix:

A. Calculations for preparation of 1.0 M HCl and 0.1 M HCl

- $\text{HCl} = 1\text{H}^+_{(1.008 \text{ g/mol})} + 1\text{Cl}^-_{(35.453 \text{ g/mol})} = 36.46 \text{ g/mol} = \text{FW}$
- Density of 37% HCl = 1.2 kg/L
- 37% HCl = 37 g HCl/100 g solution

Determine the molarity of the concentrated HCl:

$$\frac{37 \text{ g HCl} \times \frac{1 \text{ mol HCl}}{36.46 \text{ g}}}{100 \text{ g solution} \times \frac{1 \text{ L}}{1.20 \times 10^3 \text{ g}}} = \frac{12.63 \text{ mol HCl}}{\text{L}} = 12.63 \text{ M HCl}$$

To prepare 1.0 M HCl:

- $(12.63 \text{ M} \times X \text{ mL}) = (1.0 \text{ M} \times 250 \text{ mL})$
- $X \text{ mL} = 19.79 \text{ mL}$ (added 230.21 mL H_2O)

Dilute 1.0 M HCl to 0.1 M:

- $(1.0 \text{ M} \times X \text{ mL}) = (0.1 \text{ M} \times 250 \text{ mL})$
- $X \text{ mL} = 25 \text{ mL}$ (added to 225 mL H_2O)

B. Determine how much 0.1 M HCl to add to 1 L H_2O to achieve a solution with pH = 4.0:

- $[\text{H}^+] = 10^{-4} = 0.0001 \text{ M HCl}$
- $(1 \text{ L} \times 0.0001 \text{ M HCl}) = (X \text{ L} \times 0.1 \text{ M HCl})$
- $X \text{ L} = 0.001 \text{ L HCl} = 1.0 \text{ mL} = 1000 \mu\text{L} \rightarrow 1.0 \mu\text{L/mL}$

C. Calculations for preparing 1.0 M NaOH and 0.1 M NaOH

- $\text{NaOH} = \text{Na}^+_{(22.990 \text{ g/mol})} + \text{OH}^-_{(17.007 \text{ g/mol})} = 39.997 \text{ g/mol} = \text{FW}$

To prepare 1.0 M NaOH:

- $(1 \text{ mol/L})(40.00 \text{ g/mol})(1 \text{ L}/1000 \text{ mL}) = 0.040 \text{ g/mL}$
- $(0.040 \text{ g/mL})(250 \text{ mL}) = 10 \text{ g}$

To prepare 0.1 M NaOH:

- $(0.1 \text{ mol/L})(40.00 \text{ g/mol})(1 \text{ L}/1000 \text{ mL}) = 0.0040 \text{ g/mL}$
- $(0.0040 \text{ g/mL})(250 \text{ mL}) = 1.0 \text{ g}$

D. Calculations for preparing 1×10^{-4} M AlCl_3 solution:

- $(1 \times 10^{-4} \text{ mol/L})(133.33 \text{ g/mol}) = 0.013333 \text{ g/L} = 13.333 \text{ mg/L}$