Nebraska Soybean Board
FINAL Research Report Form

1/6/2022, 1:39:05 pm

Note: Submit this report no later than 90 days after the NSB-funded project officially terminates.
This post-project 90-day time-frame will allow the Lead PI time to complete any final data analysis and a final technical report, plus the drafting of any articles for submission to scientific journals. Note that this completed report will be provided to the National Soybean Checkoff Research Database, (soybeanresearchdata.com).

Project # and Title: #703 Effects of Growth Conditions on Anti-Inflammatory Bioactivity

Principal Investigator: Toshihiro Obata

Co-PI's & Institutions: Kaustav Majumdar (UNL)

Project Date (Including Extension): 10/01/2020 ***to*** 09/30/2021 ***(For example: mm/dd/yyyy to mm/dd/yyyy)***

Total Budget for Project: $ 75,880.00

1. Briefly State the Rational for the Research:

The research team had discovered the anti-inflammatory bioactivity of soybean sprouts using the simulated gastrointestinal digestion and bioactivity assays in cultured human intestinal cells in the FY19 project. In the FY20 project, we confirmed that a diet containing soybean sprout could improve chronic inflammatory disorders in mice. These results indicate that soybean sprouts are promising health-beneficial food, which has great potential to mitigate chronic inflammation in humans. Chronic inflammation in the intestine can induce various metabolic disorders, including chronic gastrointestinal diseases, cardiovascular diseases, and obesity. To avoid culture conditions unfavorable to anti-inflammatory activity and continuously produce soybean sprouts with high anti-inflammatory activities, we investigated the effects of growth conditions of the parental plants on the sprouts' bioactivities in this FY21 project.

2. Research Objectives: (copy from project, but keep in a brief bullet format)

In this FY21 project, alteration of chemical compositions in soybeans and their sprouts would be determined in the plants that experienced heat and drought stress at the grain filling stage (R5-R6) with the special focus on the bioactive compounds. The chemical profiles of the soybean and its sprout will be analyzed in relation to the germination rates, growth rate to produce sprout, and anti-inflammatory activity. Results of this research will be used to assess the plasticity of the production of bioactive compounds in soybeans and establish growth conditions to maintain high anti-inflammatory activities in sprouted soybeans.

Objective 1: Effects of heat and drought stresses during the grain filling stage on the chemical composition, germination rates, and the properties of sprouts in the offspring.

Objective 2: Characterize the anti-inflammatory activity in the digested sprouts derived from the stressed soybean plants

3. General Approach Used and (if applicable) the Nebraska Test Locations:

Objective1: Food grade soybean obtained from Laura soybean (Iowa) and Williams 82 were grown in the greenhouse at the UNL East Campus (27-29°C/21-24°C day/night temperature, watered every day to 100% soil capacity) until the grain filling stage (R5). Twenty-four plants of each cultivar were divided into four groups and treated with the following conditions for two weeks; control, drought (water daily to 30% soil capacity), heat (10 °C temperature elevation), and simultaneous drought and heat (heat/drought). Following the treatments, all plants were grown under the control condition until physiological maturity. Yield parameters including the pod number per plant, seed number in individual pods, and seed weight per individual pod were determined. Seeds were sprouted for four days to measure the length and weight of sprouts and germination rates. Seeds and sprouts were immediately frozen in liquid nitrogen and ground for the chemical composition analyses by gas chromatography-mass spectrometry (GC-MS).

Objective 2: Soybean sprout materials were digested by mimicking human gastrointestinal (GI) digestion using digestive enzymes. The chemical composition in the digests will be determined by the method described above. Additionally, the profile of the small peptides will be analyzed by LC-QTOF. The anti-inflammatory activity will be tested in Caco2 GI epithelial cells to determine the anti-inflammatory activity of the digested soybean sprouts. In GI epithelial cells, the inflammation will be induced by pre-treatment of Interleukin (IL)-1.□The expression of pro-inflammatory cytokines (Interleukin: IL-8 and IL-17) and Cyclooxygenase-2 (COX-2) will be measured by commercially available human-specific enzyme-linked immunosorbent (ELISA) assays and Western-immunoblotting, respectively.

4. Describe Deliverables & Significance Attained for Each Research Objective:

Objective1: Heat stress severely affected seed filling and germination rates.
The stress treatments were applied to the food soybean variety in May, and the seeds were harvested in July. Williams 82 cultivar took longer to flower and were treated in June. As we could harvest the seeds of this cultivar in late November, all data shown here is for the food soybean variety. Although these treatments did not affect the number of pods and the number of seeds in the pods, heat and heat/drought treatments significantly reduced the seed weight. The seed abortion rate significantly increased in heat/drought treatment. These results indicate that the heat treatment reduces seed filling and causes seed abortion, especially when it occurs simultaneously with drought stress. While nearly all seeds from the control plants sprouted (sprouting rate 98.3%), the seeds from the heat/drought treated plants very poorly germinated with a sprouting rate of 7.5%. Although sole drought stress did not affect the sprouting rate (89.2%), heat stress reduced the sprouting rate to 44.2%. The results indicate that heat stress has adverse effects on sprout production, most likely due to the reduced seed filling. The soybeans that experienced simultaneous heat and drought stresses may not be suitable for the production of sprouts due to very poor germination. Therefore, we decided to exclude the heat/drought condition for the analyses of sprouts.
The seed and sprout samples were harvested and processed for the biochemical and anti-inflammatory activity assays. However, we could not complete the metabolite analysis due to the unusual shortage of machine time. The GC-MS equipment in the PI Obata's lab experienced major defects twice in this reporting period, and we could not analyze samples for longer than three months. The analysis will be completed in early 2022, and the results will be analyzed in relation to the variation in anti-inflammatory activities. Additionally, we will also examine the Williams 82 samples to gain insight into genetic variations in metabolite contents and anti-inflammatory activities, as well as the effects of environmental stresses on them.

4. Describe Deliverables & Significance Attained for Each Research Objective (continued)

Objective2: Two anti-inflammatory activity assay systems were established for high-throughput and health-relevant analyses.
The anti-inflammatory activity assay workflow with the Caco2 cell line after simulated GI digestion is an excellent system to investigate food digestibility and the possible biological effects of the digested compounds on the intestinal cells. However, it is time-consuming and can process only up to five-six samples within a week. Thus, we have developed a small-scale semi-high throughput digestion procedure to process up to 24 samples/day to test a large number of samples within a reasonable amount of time. This workflow enables us the analysis of larger sample sets such as a genetic association panel to identify genomic components associated with the anti-inflammatory activity. Another problem of the previous system is that Caco2 is a cancer-derived cell line and does not always reflect the physiology of normal intestinal cells. Thus, we developed a novel system (Co-PI Majumder's lab collaborating with Definigen, Cambridge, UK) of using human intestinal organoids. The organoids are more physiologically relevant to the human population to access the bioactivity of the digested compounds and their potential impact on human intestinal health. The system will also allow us to access the amounts of the compounds that can be absorbed into the bloodstream in the digested soybean sprouts and their impacts on overall human health.

5. List where the Project Research Results/Findings were Publicized:

(a) The Soybean Research & Information Network website introduced our research project. https://soybeanresearchinfo.com/research-highlight/nebraska-scientist-explores-soybean-anti-inflammatory-properties-to-aid-human-digestion/

(b) The scientific manuscript describing the beneficial health effects of soybean sprouts is under preparation. This will be published in a scientific journal such as "Journal of Functional Foods", "Food and Function", "Journal of Agricultural food chemistry", "Plos One", etc.

(c) The results will be distributed to the general public via UNL websites and local news.

Note: The above boxes will automatically accomodate for your text inputs; HOWEVER, the Final Report comprised of the above listed items must be kept to THREE PAGES. A Technical Report of no more than TEN PAGES (preferably fewer) can be appended to this report.
Submit both reports as a single PDF with this file name format: #XXX > FINAL > Project Title > PI last name

Please email this completed form to the Agriculture Research Division (jmcmahon10@unl.edu) based on the reporting schedule given to you. If you have any questions, please call Jen McMahon at the ARD at 2-7082.

Technical Report

Soybean plants were treated with drought, heat, and simultaneous drought/heat stresses at the early grain filling stage (R5-6) for two weeks. Williams 82 and a food soybean cultivar from Laura Soybean, Iowa, were cultivated on the pots filled with 4 kg of pasteurized soil. Twenty-four plants were grown under the control condition (27-29°C/21-24°C day/night temperature, watered every day to 100% soil capacity) until the R5 stage. Then plants were divided into four groups with six plants each and cultured for two weeks in control, drought, heat, or simultaneous drought/heat conditions. Drought stress was applied by reducing daily water supply to 30% soil capacity determined by the changes of the total weight of individual pots. Heat stress was applied by moving the plants to another greenhouse with 35.5-37.5°C/ 31.6-34.4°C day/night temperature. Following two weeks of stress period, all plants were moved back to the control condition and grown until physiological maturity. Individual pods were harvested from each plant, and the number of pods per plant, seed number per pod, seed weight per pod, the number of aborted seeds were determined. The following results are those of the food soybean cultivar as Williams 82 cultivar reached physiological maturity at the end of November, and the analysis is ongoing.

No significant impact of stress treatments was observed on the pod number and seed number. Seed weight was significantly reduced in heat-stressed plants, and the combination of heat and drought further reduced the seed weight (Fig A). The percentage of aborted seeds showed an increasing tendency in the plants treated by stress combination, although the difference was not statistically significant (Fig. B). These results indicated that the stress treatment did not affect seed setting but seed filling. Drought stress applied in this experiment was not significant to solely induce yield loss, but it reduced seed weight when it was applied simultaneously with heat stress.

Obtained seeds and sprouts from this experiment will be used for chemical composition analysis to assess the effects of these treatments on the nutritional quality of the soybean. The sprouts will be tested for the anti-inflammatory activities following the simulated gastrointestinal digestion.

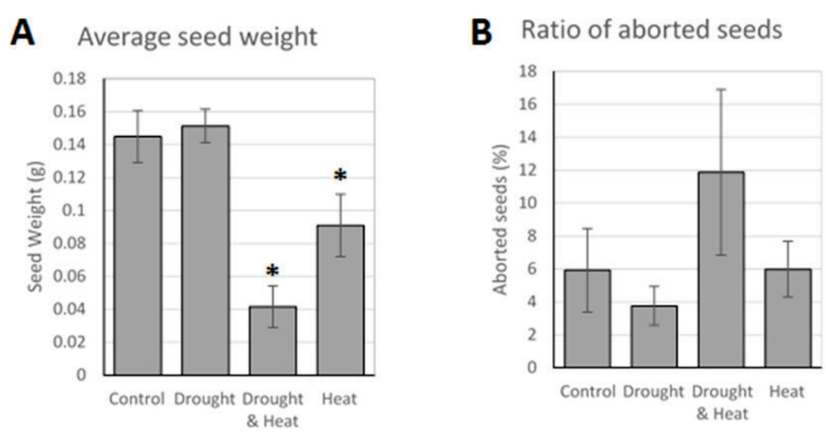

Fig. The seed weight (A) and the ratio of aborted seeds (B) in the soybean plants treated with control, drought, heat, and simultaneous drought/heat conditions during the grain filling stage. The values were the means of six plants with SEM. The asterisks indicate the conditions which showed significant differences from the control by Students' t-test ($p<0.05$).