A NEW HYDROPONIC SYSTEM FOR TESTING MINERAL NUTREINT DEFICIENCIES AND ITS APPLICATION TO SOYBEANS

Austen M. Lambert, David L. Cole, Sophia M. Anderson, Amanda Haderlie, Caden J. Seely, & Bryan G. Hopkins
Brigham Young University, Provo UT
alam99@byu.edu (801)-674-4339

ABSTRACT

Correlating plant tissue nutrient concentrations with visual symptoms is valuable in combating mineral nutrient deficiencies and toxicities. Due to changing climates and decreasing water supplies throughout the world, agricultural lands need to improve nutrient and water management in crops, including soybeans (*Glycine max* L.). Because nutrient concentrations can be easily controlled, hydroponics effectively demonstrate isolated specific nutrient related symptoms. However, many hydroponic systems present challenges in creating isolated nutrient deficiencies because nutrients are often added as salts with cationic and anionic pairs. For example, if potassium sulfate is used as the potassium (K) source, altering the K level will also impact the sulfur (S) concentrations. This creates the possibility of a dual deficiency and other potential interactions. As a result, a system was developed to create mineral nutrient deficiencies using the following single mineral nutrient sources: ammonium nitrate; nitric, phosphoric, sulfuric, hydrochloric, and boric acids; potassium, calcium, magnesium, zinc, and copper carbonates; manganese acetate; sodium molybdate; iron chelate 6% (EDDHA), along with HEDTA as a chelate. This solution, previously tested in an environmentally controlled growth chamber and now adjusted and improved, was effective in growing plants to maturity and creating multiple nutrient deficiencies in soybeans. Stem size, plant height, and shoot and root biomass was significantly impacted for several nutrients, especially for those with low concentrations of nitrogen (N), phosphorus (P), and K. Unfortunately, some adjustments made to the hydroponic system (based on work from previous studies) were too deficient and healthy plants were not able to grow. Additional nutrient rate adjustments and fine tuning will be required to create all visual nutrient deficiencies. This information, once complete, will be beneficial for farmers and their advisors managing soybean crops, as well as scientists studying these species.

MATERIALS AND METHODS

Growing Environment

Using an environmentally controlled growth chamber, soybean was grown from May to August 2021 at Brigham Young University in Provo, UT, USA (40.245,-111.650, 4550 ft above sea level). Growth chamber lighting was provided by a combination of metal halide and high-pressure Na lamps, with 1800 and 1000 µmol (photons) m−2 s−1 of photosynthetically active radiation [AccuPAR LP-80, Meter Group, Inc. (formerly Decagon Devices, Inc.), Pullman, WA, USA] at 12 inch and 43 inch, respectively, from the light source. Plants were grown in a 16/8 h light/dark photoperiod. Temperatures

were 70 ± 2 ◦F for the dark photoperiod, and 73 ± 2 ◦F during the first 4 h, and 77 ± 2 ◦F for the remainder of the light photoperiod.

Each experimental unit consisted of soybean grown in a 3.7 gal plastic container (11.4 inch inside diameter, 10.6 inch height) filled with a hydroponic solution. The containers were placed into opaque wooden boxes and covered with opaque plastic lids 0.4 inch thick. Each lid had four (2.0 inch diameter) holes for plants that were completely covered by fittings and a smaller diameter access hole in the center that had an oxygen tube running through it which was then completely covered with opaque tape to exclude light from the roots and nutrient solution. The fittings for holding plants were plastic cups (2.0 inch inside diameter at the top, 1.1 inch diameter at the bottom) sitting flush with the top of the plastic lids, and they hang down 1.5 inch into the buckets, with the bottom 0.25 inch sitting in the hydroponic solution. One layer of white nylon matte mesh (0.08 inch × 0.15 inch) netting material was placed into a fitting cup with another fitting cup placed on top of it. Plastic beads (0.15 in diameter) were then placed within the fitting cup, filling 1/3 of fitting cavity. The system in this study was similar to that shown in photographs by Cole et al. (2020), with differences explained above.

Three soybean seeds (“NK S46-A1”, Syngenta, Basel, Switzerland), were germinated in each fitting by placing them on top of the beads. The fitting cup was then covered with an opaque plastic that was then covered with opaque tape, so that the seeds were near the surface of the hydroponic solution, receiving moisture, and they were protected from light. The hydroponic solution level was checked daily and refilled as needed so that the seeds were receiving adequate moisture without being saturated. 5 d after planting, slits were cut in the form of an ‘X’ on the tape and plastic over the fittings, allowing the seeds to grow into the light.

Oxygen was supplied to the solution through PVC tubing passed through the access hole in the center of the opaque lids and then into the nutrient solution. The tubing included a plastic T connector at the bottom of the bucket that split the oxygen supply into two lines. Cylindrical bubbler air stones (0.5 inch diameter with 1 inch length), commonly used in aquariums, were attached to the end of the tubing to diffuse the size of air bubbles. The system was checked for soluble nutrients to avoid contamination.

Treatment and Block Design

Fourteen treatments were established just prior to planting in a randomized complete block design (RCBD) with three replicated blocks. A positive control (Table 1) contained what was estimated to be optimal concentrations of all nutrients based initially on Cole et al. (2020) also considering modifications discussed in Cole et al. (2021). Each of the other 13 treatments had the same concentration of all nutrients as the positive control, apart from a reduced concentration of: N, P, K, S, Ca, Mg, Zn, Mn, Fe, Cu, B, Mo, or Cl. The reduced treatments included 40% of the concentration found in the positive control for N, P, K; 10% of the concentration for S, Ca, Mg; and 0% for all micronutrients. The N, P, and K nutrients were added throughout the growing season, with the amounts in Table 1 added at the initial time of planting, and then again at 25 d after planting. Additionally, all treatments received a total replenishing of their original nutrient concentrations at 58 d after planting.

Table 1. *Hydroponic Nutrient Concentrations (μM) for the positive control*

Macronutrients						
N	P	K	S	Ca	Mg	
15,000	750	6,000	2,150	3,960	1,500	
Micronutrients						
Zn	Fe	Mn	Cu	B	Cl	Mo
4.2	26.8	8.1	1.5	18.1	39.8	0.2
Chelate and Buffer						
		HEDTA	EDDHA	MES		
		63.8	26.8	4,000		

The nutrient solution was composed of the following: ammonium nitrate; nitric, sulfuric, phosphoric, hydrochloric, and boric acids; potassium, calcium, magnesium, zinc, and copper carbonates; manganese acetate; sodium molybdate; iron 6% chelate (EDDHA); and HEDTA chelate. Each nutrient was added to deionized water (container ~80% full) and stirred in the order of N, K, Ca, Mg, Zn, Mn, Cu, B, Mo, P, S, Cl, Fe, and MES and then brought to volume.

Growth and Harvesting

After seed planting and germination, the number of healthy plants was thinned down to one plant per fitting (resulting in four plants per container as each container had four fittings) at 19 d after planting and then to two plants per container at 25 d after planting. At 35 d after planting, the containers were thinned to 1 plant per container.

Beginning 28 d after planting, visual ratings of plants were taken each week until the final harvest. The visual ratings were done using a scale of 0 to 5, with 0 being a dead plant and 5 being the relatively healthiest plant in the study at that time. Pictures were taken of the plants at 33 d after planting, as well as 65 d after planting. At the time of pictures being taken, both the sitting height and the length of the longest shoot were recorded for each remaining plant. The plants that were thinned at 25 d and 35 d after planting were harvested, and the final harvest of all remaining plants occurred 88 d after planting. The harvesting of the soybeans began with harvesting the bean pods off the plants. Plants were then harvested by separating shoots and roots by cutting the base of the stem immediately above the fitting and cutting the roots off just below the fitting (the minimal plant material interwoven into the mesh was not included in the dry weight measurements as it was impossible to separate the plant tissue from the netting, etc.). Roots were rapidly rinsed by plunging three times in deionized water to remove any loose surface-bound nutrients. Bean pods, shoots, and roots were each placed into separate paper bags and air dried. Dry biomass was determined gravimetrically.

RESULTS AND DISCUSSION

The objective of successfully growing soybean to maturity using this nutrient solution was achieved. However, the measured parameters show that the theorized and adjusted “optimum” nutrient concentrations were not optimum for every nutrient, which will require adjustment in future experiments, as discussed below.

As a general result, the treatments that appeared to be deficient when compared to our positive control were showing signs of deficiency throughout the entirety of the growing process. The visual ratings at the time of the 35 d thinning and the 88 d final harvest showed similar results, indicating that the deficiencies that were successfully induced, were held throughout the entire growing period. Our hydroponic solution was effective at inducing nutrient deficiencies throughout several treatments, though successful deficiencies were not achieved for all nutrients.

Nutrient analysis is not yet complete. There were essentially no differences in visual, shoot length, and biomass for some nutrients (K, S, Ca, Cl, Mo, and B), suggesting that further adjustments are needed in the nutrient solution in order to study these nutrients in soybean. However, there were significant biomass and plant height reductions for N, P, Mg, Zn, Mn, Cu, and Fe compared to the full nutrient solution (positive control), which is an indicator of a successfully inducing nutrient deficiencies (Figure 1).

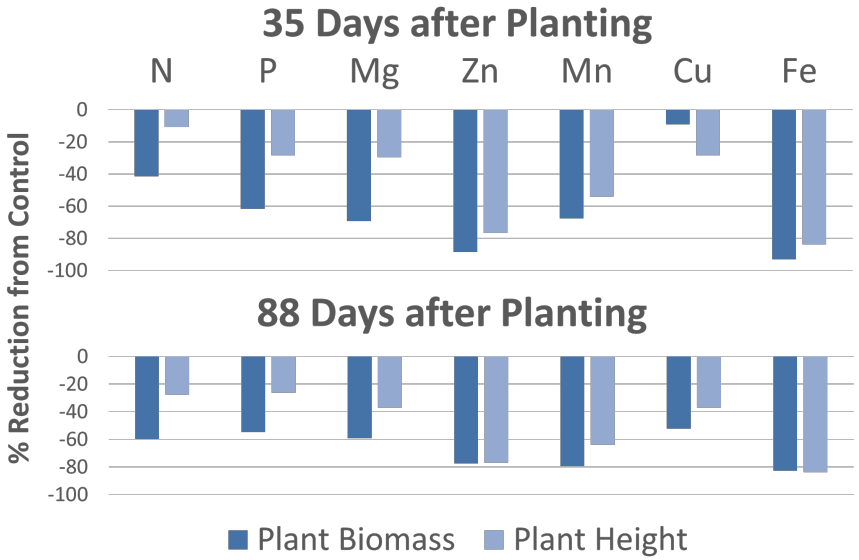

Figure 1. *The percent reduction of plant biomass and plant height in comparison to the control treatment, all percent reductions shown were statistically significant compared to the positive control.*

One of the objectives of the study was to observe the visual characterization of nutrient deficiencies within soybean. Throughout the trial, the treatments shown in Figure 1 all showed significant visual symptoms of deficiency, including, but not limited to, reduced height, wilting, chlorosis, reduced canopy density, and/or reduced greenness. The purpose of photographing the soybean throughout the study was to then connect these visual symptoms with the nutrient deficiency. There is not space here to show all of these, but the N treatment is shown as an example (see Figure 2). Within treatments of reduced N, P, Mg, Zn, Mn, Cu, and Fe, we were able to get clear visual deficiency symptoms, with the prior stated treatments ranging between 1.1-3.3 on average visual ratings, compared to the control treatments which had an average visual rating of 4.1 throughout the study.

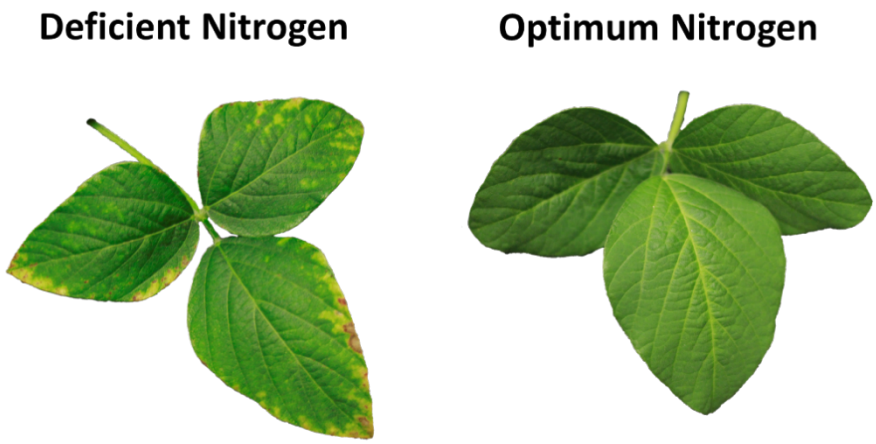

Figure 2. *Comparison of an optimum nutritional leaf and a nitrogen deficient leaf.*

There are several hypotheses connected to why we may not have seen nutrient deficiencies within certain treatments. Compared to previous studies, this is the first time that we have struggled to create a nutrient deficiency for K. Tissue analyses performed from experiments using a previous version of this solution showed possible low levels of K within the positive control and so we raised the total K concentration from 4000 µM to 6000 µM within the positive control. This adjustment, in addition to changing the process for adding the primary macronutrients from providing the full concentration at the time of planting, to an in-season supply of primary macronutrients, could account for the lack of K deficiency within the visual symptoms of the K deficient treatment.

This trial included no adjustments to the concentrations of S or Ca from previous studies by Cole et al. (2020 and 2021). When using this solution with quinoa, Cole et al. (2020) saw that plants visually showed slight deficiencies in biomass, though not enough to be statistically significant, the tissue concentrations could still show nutrient deficiencies. Because we have not completed tissue analysis, we cannot make conclusions on whether treatments that did not show significant biomass differences from the positive control had clear nutrient deficiencies in this study.

For the micronutrients Cl, Mo, and B, tissue analysis is likely to be required to judge whether a deficiency was achieved. We are doubtful of a deficiency within the B and Cl treatments because of biomass and plant height data, which was higher than the positive control, though not statistically significant, at both the 35 d thinning and the final harvest. Previous analysis of the B tissue concentrations showed signs of potential toxicity, and slightly increased height and biomass of the reduced B plants may support that hypothesis by Cole et al. 2021, further tissue analysis on B concentrations will be needed to further understand the potential of B toxicity. The reduced Mo treatment did show an average 30% reduction in biomass from the control at the 35 d harvest and the final harvest, but plant height was statistically the same as the positive control and so these results are not conclusive and further study is warranted.

The reduced Fe treatment was an interesting case. We are confident that an Fe deficiency was induced, though the results are likely insufficient for our study because the deficiency led to significant necrosis throughout all Fe deficient plants and the deficiency did not allow the plants to successfully grow to maturity. In the future, we

recommend adding at least a low concentration of Fe into the reduced Fe treatment so that the plants can grow to maturity and show symptoms of Fe deficiency. A similar problem may have been observed within the reduced Zn treatments.

The stability of the pH of the hydroponic solution also remained a concern throughout this study, like that discussed in Cole et al. (2021). The pH of the solution remained primarily between 8 and 9, despite the addition of MES as a buffering agent. Throughout the trial, the pH would fluctuate between 7 and 9 rather rapidly among each treatment, and NaOH and Acetic Acid at small concentrations would be used to try and equalize the pH of the study each week. Despite efforts to create a uniform pH across the study, the pH continued to fluctuate, which is not representative of in-field soil conditions. We are not concerned about the high pH of the study, but further studies are needed to experiment with MES as an effective solution buffer without becoming toxic to the health of the plants.

SUMMARY

The results of this study are a mixed success. The adjusted Hopkins single nutrient source hydroponic solution was still effective at growing healthy soybean plants. Additionally, despite unfinished tissue nutrient analysis, we did see significant reductions in plant height and biomass due to suspected nutrient deficiencies for reduced N, P, Mg, Zn, Mn, Cu, and Fe compared to the positive control. Some nutrients (K, S, Ca, Cl, Mo, and B) likely did not have a nutrient deficiency in their respective reduced treatments, though some nutrients, like B, may have potential toxicity at its concentration within the positive control. Further use of this hydroponic solution will need to consider adjusting the concentrations of the nutrients that did not see clear deficiencies, in addition to adding slight amounts of Zn and Fe to the deficient treatments to ensure mature plants can be grown. Finally, further research is needed to understand the potential of MES as an effective buffer of the Hopkins hydroponics solution without becoming toxic to soybean plants, and still effectively buffering the pH of the solution between a small interval.

REFERENCES

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