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A Hard Day's Night: Optimizing Productivity of *Arabidopsis thaliana* in Hydroponic Systems

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ABSTRACT

The purpose of this study was to explore effects of the exogenous application of supplemental nutrients when differentiating both light and dark cycle requirements set by the plant's circadian mechanisms. *Arabidopsis thaliana*, a model organism for plant research, was grown hydroponically under standard environmental conditions. It was found that adding additional carbohydrates solely during the plant's light cycle displayed a 255% increase in root:shoot ratio compared to the 24h application of equivalent nutrients, suggesting this approach of differentiating nutrient and carbohydrate requirements has promising results for plant growth and productivity.

Literature Review

Plant Growth & Productivity

Plant growth occurs within a daily alternation between day and night. In the light, growth can be directly fueled by photosynthesis - the process by which atmospheric carbon dioxide (CO_2) is converted into organic compounds (photosynthate), utilizing the energy provided by sunlight [1, 2]. In the dark, when photosynthesis is not possible, it is well documented that plants must rely on stored carbohydrates accumulated during the previous light cycle [1,2]. Even though the growth rate varies through the light-dark (LD) cycle, plants reach a fine balance "between maximizing usage of photosynthate during the day and avoiding carbon starvation at night" [1,3]. Zeeman & Rees [4] of the Department of Plant Sciences at the University of Cambridge and Department of Plant Biochemistry at the Institute of Molecular Plant Biology, determined that *Arabidopsis thaliana* (Arabidopsis) - a model organism for plant research - accumulates approximately 50% of the carbon assimilated during the day as starch in their leaves. In many plants, including Arabidopsis, these carbohydrate reserves are stored in chloroplast starch granules which are degraded during the night cycle to produce sugars vitally important for continued growth and metabolism [2,5]. Published in *Plant, Cell & Environment*, Smith & Stitt [6] specifically derived that for normal plant productivity, the rate of starch degradation in Arabidopsis leaves is near-linear in that approximately 95% of the starch is used by dawn. This observation suggests a timing mechanism, namely the circadian clock, matches the usage of starch to the anticipated length of the night. These circadian mechanisms ensure a constant supply of sugars until dawn - which researchers at John Innes Centre emphasize as essential for the maintenance of normal growth at night [1,5].

Circadian Mechanisms

Interestingly, plants have a remarkable ability to regulate basic physiological processes and growth according to day-night cycles named circadian rhythms. In biology, circadian clocks are endogenous 24-h timers present in most living organisms - allowing them to schedule physiological processes to occur at specific, appropriate times within the LD cycle [7]. Within plant literature, there is a significant body of research characterizing and quantifying plant behaviour

in light and dark cycles. Plants demonstrate the ability to immediately adjust the rate of starch degradation under altered photoperiods such as an unexpected early-onset night [5,8]. Although growth is lower at night [3], Dodd et al. [9] of the Department of Plant Science at the University of Cambridge and Plant Biology Institute at the Hungarian Academy of Sciences propose the target for circadian control is carbohydrate accumulation during the light cycle. For the purpose of this literature review, two main experimental approaches of Dodd et al. [9] will be described: (1) wild-type plants (Columbia-0 Arabidopsis) were grown in 10-h light/10-h dark (T20), 12-h light/12-h dark (T24), and 14-h light/14-h dark (T28) cycles; (2) long- and short-circadian period mutants of Arabidopsis were grown in T20 and T28 cycles. Their results demonstrate that maximal plant productivity occurs when the period of the mutant genotypes' LD cycle matches their respective circadian clock [9]. More specifically, when the environment matched the anticipated dawn, Arabidopsis plants contained more chlorophyll, fixed more carbon, grew faster, and gained survival advantage. To confirm this, researchers of The Max Planck Institute for Molecular Plant Physiology examined the rate of starch degradation under an unexpected early-onset of night as well as Arabidopsis grown under abnormal day lengths [5]. They reported that plants subjected to a single early night immediately adjusted the rate of starch degradation so that reserves lasted until the next anticipated dawn [5]. Their results suggest that the ability to anticipate dawn - under circadian control - is essential for maintaining plant productivity [5]. Second, Graf et al. [5] discovered that in plants grown from germination in both shortened (17 h) and extended (28 h) LD cycles, starch was fully consumed approximately 24 h after the last dawn. The results imply that the rate of starch degradation is controlled by the anticipation of dawn through the plant's circadian mechanisms, regardless of the laboratory's environmental dawn [5]. In summary, the plethora of literature surrounding plant physiology illustrates a highly sophisticated and controlled process of day-night cycles required for plant growth and productivity.

Optimization of Plant Growth

Essential Nutrients

While the aforementioned science of plant growth is widely accepted in the field, also well documented is the knowledge that a minimum of 14 nutrients - in addition to oxygen, carbon dioxide, and water - are required for important functions essential for proper growth and development [10,11]. With negligible differences used by more recent research, the criteria formulated by Arnon & Stout [12] to determine the eligibility of an essential element still stands: (1) the plant cannot complete its life cycle with a deficiency of that element; (2) the deficiency is specifically for that element in question; and (3) the element is directly contributing to the nutrition of the plant. Based on this proposed criteria, the following elements can be identified as essential nutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), chlorine (Cl), boron (B), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), nickel (Ni), and molybdenum (Mo) [11]. These plant nutrients can be further subdivided into macro- and micronutrients loosely based on the element concentration in plant tissues. However, Mengel et al. [11] suggest the classification of nutrients based on biochemical behaviour and physiological functions is most appropriate. Regardless, a substantial body of research can support the statement that deficiency in any of the aforementioned nutrients results in reduced plant growth and crop yield [13]. For the purpose of this study, three essential macronutrients imperative for plant growth and development will be further described. Nitrogen (N), phosphorus (P), and potassium (K) will be investigated as they are "three important limiting factors frequently added as fertilizers in modern agricultural schemes" [10]. White & Brown [13], researchers associated with the Department of Plant Sciences at the University of California and the Scottish Crop Research Institute, have synthesized in a systematic review of the literature that in agricultural soil, there is rarely sufficient NPK available for early growth to sustain optimal productivity throughout the crop lifespan.

Nitrogen (N) is associated with crop production improvements as no higher organisms can survive unless the uptake of N from plant roots is present [10]. Given that N comprises 16% of proteins in animals, this uptake process in plants is biologically critical [14]. With Arnon & Stout's [12] criteria in agreement, Fischer et al. [15] of the Department of Biological Sciences at the University of Calgary and University of Alberta succinctly summarized that

nitrogen deficiency causes phenotypes such as chlorosis and leaf discoloration, increased root shoots in exploration for limited nutrients, impaired reproduction, and reduced biomass production. Although ultimately reaching a uniform consensus with Frink et al. [14] with regards to the essential role of nitrogen, Fischer et al. [15] further elaborate on arising limitations to the practice of adding nitrogen-based fertilizers to soil: not only does it increase costs of high-yielding crop production, but the environmental damage associated with the use of these fertilizers has become significant due to the excess of N remaining in soil and water. Almost ten years prior, Good et al. [16] published their concerns in *Trends in Plant Science*: two decades ago, approximately 85 - 90 million metric tons (MMt) of nitrogenous fertilizers were added to agricultural soil worldwide annually, and this is only expected to increase to 240 MMt by 2050. However, only 50 - 70% of applied N is lost from the plant-soil system [16]. Given their findings, they concluded that "the globalization of nitrogen deposition [is] beginning to have significant consequences for terrestrial ecosystems" [16]. For this reason, one of the main goals in current plant nutrition research is to optimize the usage and uptake of plant N fertilizer [10].

Phosphorus (P) is an important component of macromolecules, corresponding to approximately 0.2% of plants' dry weight [10]. Despite this small amount, P is essential for energy metabolism and transduction cell signalling pathways [10]. Lastly, potassium (K) is a crucial macronutrient involved in several cellular signalling pathways as well as metabolic adjustments in response to drought, soil salinity, high light intensity, and hormones during development and reproduction [10]. However, the aforementioned nutrients do not work alone in plant nutrition. Usherwood & Segars [17] of the Potash & Phosphate Institute of Canada explain that a deficiency of P or K, for example, can drastically alter the effectiveness of N within the plant. With the abundance of evidence establishing NPK as indispensable for plant growth and development, most nutrient solutions formulated to date have attempted to optimize these essential nutrients for maximal plant growth and productivity.

Optimal Nutrient Solutions

Over the past several decades, multiple studies have claimed to have developed the optimal nutrient solution for maximum plant productivity with specific parameters in mind. For example, Toquin et al. [18] published an original design and nutrient solution composition for hydroponically grown Arabidopsis plants suggesting that it may be "suitable for many experimental purposes" due to its flexibility, easy handling, fast maintenance, and low cost. Several years later, Conn et al. [19] of The Waite Research Institute and Australian Research Council Centre of Excellence in Plant Energy Biology presented an optimized hydroponic culture system and nutrient solution (with altered micronutrient formula). Having trialled previous systems described in the literature, they observed confounding factors that affected normal plant growth, such as algal contamination and hypoxia [19]. With the goals of (1) exclusion of light from the growth solution; (2) simplification of handling plants; and (3) easy implementation of different analyses, a new hydroponic system and nutrient solution was published with certain advantages including versatility, quick assembly, and low maintenance costs [19]. To further highlight the specificity of each system in the literature, Tocquin et al. [18] focused on technical parameters within the system and nutrient solution to ensure successful germination in addition to synchronized growth and development, whereas Conn et al. [19] generated a design in an attempt to solve previously noted issues affecting normal plant growth. This survey of methodology in the literature shows that the research goal(s) will dictate different approaches with respect to growth medium and nutrient solution composition.

Due to the variance of information regarding the effectiveness of nutrient solutions composition and concentration on Arabidopsis growth, van Delden et al. [20] of the Department of Horticulture at Wageningen University, the Netherlands, compared plant growth performance using common nutrient solutions including the aforementioned Tocquin et al. [18] and Conn et al. [19]. Using a systematic literature scan, five commonly used nutrient solutions on plant performance were identified for their main comparison in a deep-water culture system, and the electrical conductivity (EC[1]) was normalized to 1.1 dS m^{-1}. Their findings proposed that the best performing nutrient solutions for

[1] Proportional to the ion concentration of nutrients in solution - an important factor controlling product quality and plant health [20].

Arabidopsis (Col-0) plants grown in hydroponics were Conn et al. [19], Tocquin et al. [18], and half concentration of Hoagland & Arnon [21]. Interestingly, the poorest performing solution was Murashige & Skoog (MS) [22], resulting in severe growth retardation and stressed plants [20]. Yet, between 2015 and 2018, they discovered in that same systematic literature scan that 23 out of 90 studies using hydroponics for Arabidopsis research used MS medium as a nutrient solution [20]. Therefore, there are many areas of uncertainty within the field of plant nutrition.

Methods of Exploration

While we have a strong understanding of the essential nutrients that create favourable growth conditions, we have yet to fully unlock the plants' genetic potential. To the best of my knowledge, all studies on nutrient optimization have focused on a constant 24h regime. Yet, there is a neglected aspect of the field: night requirements for plants, which is half the story for optimization and productivity. The paucity of evidence to connect productivity whilst solely focusing on the night cycle demonstrates the need for additional research. Therefore, the question becomes, "Can plant productivity be increased by optimizing nutritional requirements during the dark cycle and differentiating it from the light cycle?". In order to investigate this overarching question based on the gap in the literature, hydroponics and Arabidopsis will be used.

Arabidopsis thaliana

Thirty-five years ago, Arabidopsis of the Brassicaceae family became the model organism for research in plant biology and currently stands alone as the most thoroughly studied and versatile flowering plant [23]. Published in *The Plant Journal*, Koornneef & Meinke [23] summarize the history and consensus of the "standard reference plant for all of biology" because of its important features such as a short generation time, prolific seed production through self-pollination, small size with limited growth requirements, and completion of the genome sequence. Recognizing this, *Genetics* previously announced that "Arabidopsis has joined the Security Council of Model Genetic Organisms. These favored few form the standard to which all other organisms are compared" [24]. Additionally, the picture emerging for Arabidopsis research is widely applicable; similar daily patterns of physiological processes can be translated to crops such as lettuce [15], corn [26], and soybean [27]. Not only can research be relevant to other plant species, but Arabidopsis also has medical implications: a team of American professors and researchers of molecular, computational and developmental plant biology discovered, through a systematic review of the literature, that the majority of human genes known or hypothesized to be a contributing factor in diseases had orthologs in Arabidopsis [28]. For the multitude of reasons previously stated, Arabidopsis will be the plant of choice for this research focus.

Hydroponics

With the broad question, "Can plant productivity be increased by optimizing nutritional requirements during the dark cycle?" in mind, hydroponics is decidedly the method of cultivation best suited for Arabidopsis research. Hydroponics refers to a cultivation method of growing plants using nutrient solutions in water instead of soil substrates [29]. Since hydroponic production techniques offer higher yields and crop quality, the Food and Agriculture Organization of the United Nations recommends hydroponic farming in geographical areas of the world lacking arable land and fresh water supplies [30]. Techniques date back to the 1920s, and most systems have been used for commercial vegetable production, i.e. tomatoes, beans, spinach, cucumbers, and lettuce, with continuous improvements of systems over time [29]. Regarding specific research related to plant nutrition, soil as a growing medium poses unnecessary difficulties such as root examination and impartial control of chemical substrate composition [20]. Summarized succinctly by Lee

& Lee [29], experts in the Department of Food Science and Technology at Ohio State University, further advantages include greater control of growth conditions (e.g. temperature, pH, humidity, volume and flow velocity of water, nutrients, duration of light, etc.), elimination of crop exposure to soil-borne diseases and their resulting pesticide usage, efficient water usage as it can be reused/recycled, and even distribution of nutrient solution to crops. Given the numerous benefits, hydroponic research and commercial implementation has significantly increased over the past several decades [29]. The total greenhouse vegetable production in Canada reached approximately 635,000 metric tonnes in 2017 alone [31]. Given the superior advantages offered by hydroponics, it is the natural choice for elucidating the research question.

Research Focus

There is an abundance of research surrounding the optimization of plant growth. The current approaches of improving plant productivity focus on the circadian mechanisms and photosynthetic efficiency during the day. Despite this, the extent to which the exogenous application of supplemental nutrients during the dark cycle can further productivity has not yet been explored. The overarching goal of the proposed research is to increase plant productivity while maintaining various hydroponic inputs (e.g. water and lighting) by focusing on the historically neglected aspect of the plant's LD cycle. In other words, can alternative nutrient solutions at night relative to the day cycle unlock the plant's genetic potential? Therefore, the question of study becomes: "How does the exogenous application of supplemental nutrients in a hydroponics system during the dark cycle affect the productivity of Arabidopsis?".

It was originally hypothesized that by providing the plant directly with supplemental nutrients at night, Arabidopsis may enhance the overall available utilization of carbohydrates for growth, therefore maintaining growth rates through the darkness and maximizing overall productivity. On the other hand, the additional carbohydrates may cause growth inhibition, overloading the plant. For the purpose of this study, productivity is defined as biomass: the fresh and dry weight of all plant tissue.

Results and Discussion

Wild type (WT) Arabidopsis (*Arabidopsis thaliana* ecotype Columbia L Heynh (Col-0)) was grown hydroponically in this quantitative experimental study. Nutrient solutions during the light and dark cycle varied in 7 treatment groups (12h light:12h dark); after 61 days, plants were harvested and measured to determine the physiological productivity of plants grown under varying supplemental nutrients in deep water culture hydroponic systems (Table 1; Appendix A).

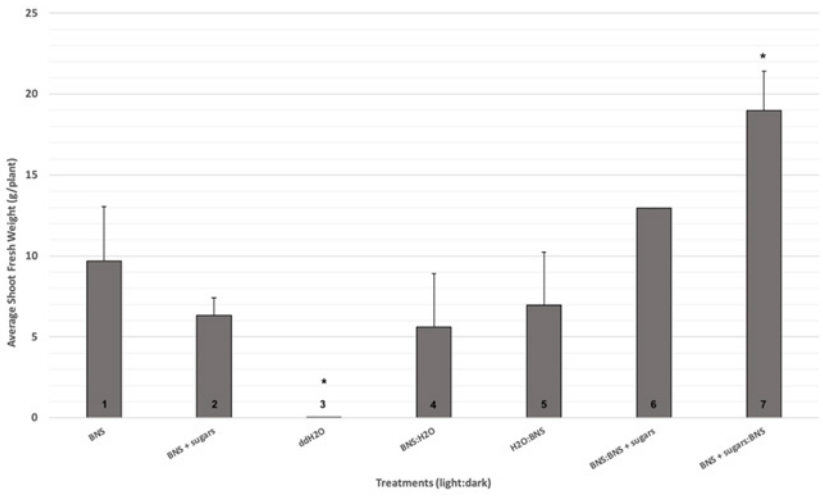

Figure 1. Effects of nutrient solution on shoot fresh weight of hydroponically grown *Arabidopsis thaliana* plants. The data are expressed as the mean ±SE of 1-10 plants. Asterisk indicates a significant difference in fresh shoot weight relative to all other conditions ($P < 0.005$).

Certain samples were excluded from analysis due to high root perturbation from over aeration leading to inhibition of root growth, discolouration of leaves, and premature death of certain plants causing inconsistency in sample sizes (Appendix B) [20, 32-33]. Among all treatment groups, 37% of plants were excluded. High numbers of dead plants could suggest an ineffective hydroponic system due to mechanical trauma, specifically frequent root perturbation from repeated lid removal (i.e. every 12 hours). Future research is advised that "merely changing the solution on a weekly basis provides enough air exchange to maintain adequate levels of oxygen while avoiding the accumulation of toxic levels of carbon dioxide" [32]. Root fresh weight was also excluded from analysis since water retention significantly varied based on the complexity and size of the root system.

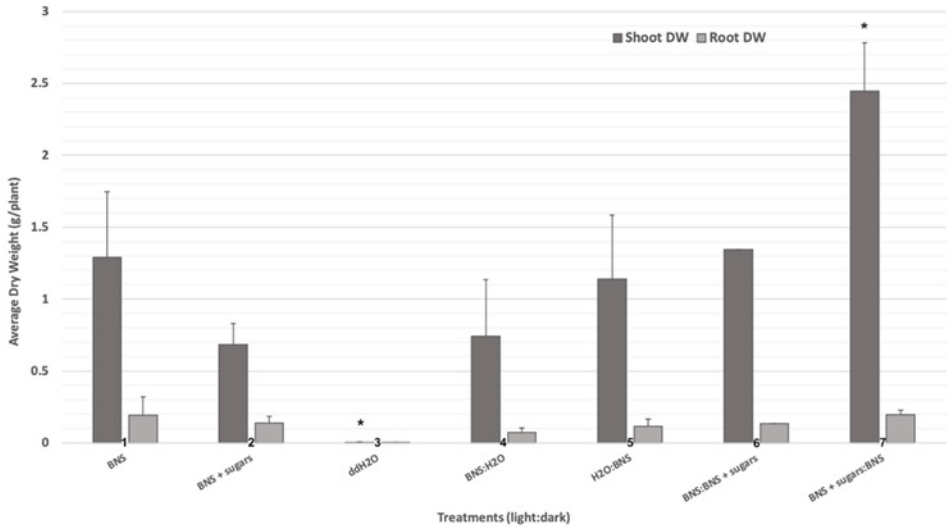

Figure 2. Effects of nutrient solution on shoot dry weight and root dry weight of hydroponically grown *Arabidopsis thaliana* plants. The data are expressed as the mean ±SE of 1-10 plants. Asterisk indicates a significant difference in shoot dry weight relative to all other conditions ($P < 0.005$).

Controls

As expected, plants grown in double distilled water had the lowest productivity; shoot fresh weight (SFW), shoot dry weight (SDW), and root dry weight (RDW) (Table 1) was significantly lower since there were no supplemental nutrients [10,13]. The root:shoot ratio (RSR) was also significantly lower compared to all other treatment groups (Table 1). A higher RSR value is indicative of a greater proportion of shoot to root biomass whereas a lower value signifies more root relative to shoot biomass. Typically, plants "subjected to nutrient limitations will grow additional roots to search for nutrients required for growth" [32].

Interestingly, when comparing treatment 1 (BNS control) to treatment 2 (BNS with sugars control), the constant exogenous application of supplemental carbohydrates decreased the overall productivity (Figures 1 and 2) and this was also reflected in a lower RSR value. More specifically, treatment 2 had a 1.5-fold decrease in SFW, a 1.9-fold decrease in SDW, and a 1.4-fold decrease in RDW to treatment 1 (Table 1), although not statistically significant ($P > 0.005$). This is contradictory to the original hypothesis that Arabidopsis productivity would be enhanced with

additional carbohydrates available to be utilized for continued growth through the night cycle. Under the chosen experimental conditions, the additional sugars caused growth inhibition. It was also observed the average time to flowering (TF) was delayed by approximately 3 days when supplemental sugars were added to the nutrient solution. This observation, though statistically indifferent, is consistent with the literature: glucose controls several aspects of growth, development, and metabolism and Arabidopsis plants supplemented with high concentrations of glucose have delayed flowering [34-36].

Table 1. Effect of nutrient solution on: shoot fresh weight (SFW) and dry weight (SDW), root dry weight (RDW), root:shoot ratio (RSR) at 61 days after sowing (DAS), and average time to flowering (TF).

	Treatment	SFW (g plant^{-1})	SDW (g plant^{-1})	RDW (g plant^{-1})	RSR	TF
1	BNS (light:dark)	9.699	1.291	0.193	6.698	28.7
2	BNS + sugars (light:dark)	6.328	0.683	0.14	4.887	31.4
3	ddH_2O (light:dark)	0.017	0.005	0.0025	2.12	30.5
4	BNS (light); ddH_2O (dark)	5.61	0.741	0.073	10.218	28.6
5	ddH_2O (light); BNS (dark)	6.967	1.141	0.114	10.006	27.4
6	BNS (light); BNS + sugars (dark)	12.97	1.343	0.137	9.803	40.8
7	BNS + sugars (light); BNS (dark)	18.981	2.447	0.196	12.485	33.2

Linking Additional Carbohydrates to Circadian Mechanisms

To answer the posed research question, additional sugars were added solely during the light or dark cycle (treatment 6 and 7). The most significant result was that treatment 7 (BNS with additional sugars during the light cycle) showed approximately a doubling and tripling of shoot weights compared to both treatment 1 and 2 respectively (P-value < 0.005), yet the RDW was statistically indifferent (Table 1). As a result, the RSR of treatment 7 displayed a 255% increase from the 24h exogenous application of the same sugars (treatment 2), indicating that sugars during the day were significantly utilized for growth. Comparatively, when sugars were only added during the dark cycle (treatment 6), there is only a 1.4-fold increase to treatment 1, suggesting the exogenous application of sugars was most efficient during the light cycle of the plant (i.e. treatment 7). Supplementing with additional sugars during the light cycle possibly enhances the endogenous pool of carbohydrates leading to increased growth, whereas addition of sugars during the dark cycle (treatment 2 and 6) possibly disrupts the circadian mechanisms for optimal growth. Knowing circadian mechanisms play an essential role in the rate of starch degradation at night [1], it is perhaps not surprising that significant differences are seen in plant productivity when carbohydrates are altered between day and night. Notably, the exogenous application of sugars at night (treatment 6) significantly increased the flowering time by approximately 12 and 9 days relative to treatment 1 and 2 respectively (Table 1). Previous research has established that addition of carbohydrates such as glucose will delay flowering time of Arabidopsis, however, differentiating the timing of application further advances this observation: the data revealed that adding sugars solely at night significantly inhibits flowering time (Table 1).

Limitations, Implications & Future Research

With roughly 83 million people being added to the world's population every year [37], the need for food is matched with declining amounts of arable land [17]. In addition, decreasing freshwater supplies threaten food security for 11% of the global population [38]. Given this ever-growing mismatch between global food supply and demand, the need for exploring methods in optimization of crop production is critical.

The experimental study discussed above is based on "Arabidopsis plants grown under highly controlled and invariant conditions" [1]. Although studies of this nature are beneficial in providing detailed understanding of uncharted concepts, similar research must take steps into the real world of large-scale hydroponic farming. As previously mentioned, Arabidopsis research is widely applicable to relevant crops such as lettuce [25], corn [26], and soybean [27]. To advance this new understanding, future research should adapt the method design for a greater sample size - to establish unambiguous trends - and vary concentrations of BNS or additional sugars in lettuce to observe the direct results on leaf area, size, and yield. Furthermore, given the small sample size of this study, starch content throughout the night cycle ought to be measured in order to test the potential finding that additional sugars disrupt the circadian mechanisms of plants during the dark cycle.

Conclusion

The urgency to explore methods in optimization of crop production is a direct result of the global demand for food in the midst of declining arable land and freshwater. Despite the abundance of existing research surrounding plant productivity, current approaches focus on a constant 24h nutrient regime. The predominant purpose of this study was to explore effects of the exogenous application of supplemental nutrients when differentiating both light and dark cycle requirements set by the plant's circadian mechanisms. *Arabidopsis thaliana*, a model organism for plant research, was grown hydroponically under a controlled environment. It was found that adding sugars during the light cycle (treatment 7) displayed a 255% increase in RSR compared to the control (treatment 2). This suggests the exogenous application of additional sugars during the light cycle was most effective for growth and overall productivity. This approach of differentiating nutrient and carbohydrate requirements has promising results.

Methods

Wild type (WT) Arabidopsis (*Arabidopsis thaliana* ecotype Columbia L Heynh (Col-0)) was grown hydroponically in this quantitative experimental study by the method outlined below; the general procedure is summarized in Figure 3. In order to explore the question of study, nutrient solutions during the light and dark cycle varied in 7 treatment groups (12h light:12h dark): (1) BNS:BNS; (2) BNS + sugars:BNS + sugars; (3) double distilled (dd) H_2O:dd H_2O; (4) BNS:dd H_2O; (5) ddH_2O:BNS swapped with treatment 4; (6) BNS:BNS + sugars; and (7) BNS + sugars:BNS swapped with treatment 6. All experiments were carried out simultaneously and climate room conditions were set to 12h light:12 h dark cycle with 37 ± 13% atmospheric humidity at a temperature of 22 ± 2.5℃ [19,32]. The average light intensity was maintained at approximately 200µmol m^{-2} s^{-1} supplied by fluorescent tubes and pH was initially set to 5.60. Consistent aeration of each hydroponic tank was accomplished via a common 12-outlet aquarium air pump (VIVOSUN 1750 GPH 110L/min) to a small aquarium air stone at the bottom of each tank.

Plant Material, Germination, and Seedling Growth

Germinating medium (GM) was prepared with 0.7% agar and autoclaved. Black 1.5mL microcentrifuge tubes (Bioplastics, Article B74010) were also autoclaved and sterilized prior to use. Once the GM was cooled to 55-60℃, each tube was placed in a microcentrifuge tube rack and filled with 250-300 µL GM with a micropipette - or until tubes are filled such that there is a dome of GM-agar - and left to solidify for 30 minutes. Two to three WT Arabidopsis seeds, generously supplied by the University of Calgary, were superficially placed on the GM surface (to maximize chances of germination) using a sterilized tweezer. Initial trials punctured a single hold in the centre of the microcentrifuge tube caps with a leather punch in advance of autoclave as directed by Conn et al. [19]. This small hole ensured limited light penetration into the culture medium - therefore eliminating the possibility of algal growth - and minimal water

loss through evaporation from the hydroponic tank [19]. However, this method quickly proved to be ineffective due to limited germination of seedlings; germination was successfully restarted in the microcentrifuge tubes without caps.

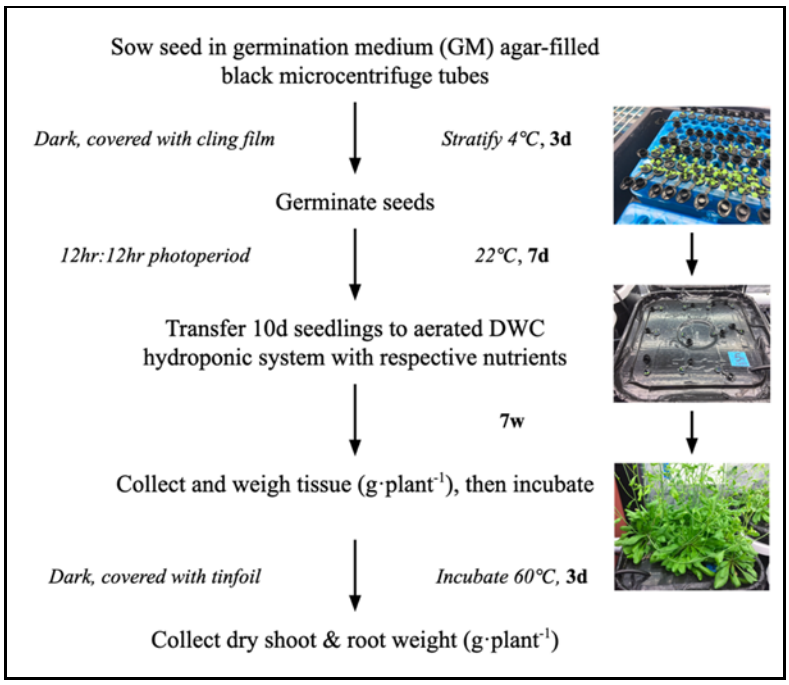

Figure 3. Simplified Arabidopsis hydroponic growth method. Flow chart outlining the timeline and key steps in the process. Timing (in bold) on the right side of arrows indicate time between steps (d: days). Images on right-hand panel showing set-up of seed germination, transfer into mature hydroponic tank, and mature plants before data collection.

To improve synchronization, microcentrifuge tube racks were wrapped in tinfoil and seeds were stratified at 4°C for 72 hours in the dark [39]. Upon removal, the microcentrifuge tubes were cut approximately 10mm from the base - a distance carefully chosen after an "optimization experiment testing longer and shorter tubes" [20]. Tubes were left in the microcentrifuge tube racks to adjust under standard conditions until 10 days after sowing (DAS) then 10 plants were transferred to the respective hydroponic system, essentially assigning each to one of the 7 treatment groups. However, not all 10 plants survived the full experiment and therefore were removed from their mature hydroponic tank.

Preparation of Deep Water Culture (DWC) Hydroponic System

To avoid potential algal contamination of the culture medium due to exposure to light, containers (Letica, 2QR) were covered with black Duct Tape. Consequently, this would "reduce nutrient uptake efficiency, plant growth, perturb the composition of the growth solution (nutrients, pH), [etc.] …for this reason alone it is important that hydroponic systems avoid illumination of the growth media if they are to be used in physiological studies" [20]. Using a hole-bit, 12 holes (3x4 pattern all evenly separated) were drilled per container lid - to support the lip of the microcentrifuge tube - and the cut edges were smoothed with a metal file to prevent further root damage [19].

Standard growth solutions with NPK all contained a modified ¼ Hoagland formula, hereafter referred to as BNS [19,21]. The nutrient solution, as outlined by Conn et al. [19], contained the following macronutrients: 5.6 mM K, 2.1 mM Ca, 2 mM Mg, 2 mM NH_4, 3.71 mM Cl, 9 mM NO_3, 2 mM SO_4, 0.6 mM PO_4, and 1.55 mM Na. Additional sugars, if applicable to the respective treatment group, were supplied by Bud Candy Organic™ OIM - a nutrient

solution frequently used in the hydroponic cannabis industry - after initial trials of pure glucose and sucrose resulted in bacterial and algal growth on roots. The solution contained the following sugars: 0.5% D-galactose, 7% D-ribose, 1% D-xylose, 4% glucose, 1.6% maltose.

Daily Procedure

Other than the three control groups, two tank lids were switched at 8am and 8pm daily between BNS and ddH_2O as well as BNS with sugars and BNS to simulate the changing of nutrients between the light and dark cycle. All 7 treatment groups were subject to the experiment for 61 DAS.

Plant Measurements & Statistical Analysis

61 DAS, plants were harvested: fresh root systems and shoots were separated and biomass was weighed in preparation for evaluation of the physiological productivity of each treatment group [19]. Dry weights were determined after roots and leaves were wrapped in tinfoil and kept in an incubator at 60℃ for 3 days [20]. Time to flowering - number of days from vegetative growth to flowering stage - was recorded by regular observation of the plants; the date was documented when the first floral bud of the plant was visible at the apex to analyze periods of general developmental stages [18,39]. The average fresh shoot weight, dry shoot weight, and dry root weight was calculated and standard deviation was recorded. Graphs were made using Microsoft Excel Version 16.47.

Abbreviations

LD: light-dark; DWC: deep water culture; NPK: nitrogen, phosphorus, and potassium; BNS: basal nutrient solution (¼ Hoagland nutrient solution); DAS: days after sowing; FSW: fresh shoot weight; DSW: dry shoot weight; DRW: dry root weight.

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Appendix

Appendix A

Raw data set of all 7 treatment groups; collected SFW, root fresh weight (RFW), SDW, and RDW per plant. Rows highlighted in orange were excluded from analysis.

	1.0 - BNS (night/day)			
	Shoot FW	Root FW	Shoot DW	Root DW
1.1	10.17	7.166	1.646	0.161
1.2	11.17	4.664	1.514	0.126
1.3	11.08	5.349	1.567	0.145
1.4	8.91	10.93	0.993	0.204
1.5	3	2.839	0.452	0.071
1.6	9.69	13.755	1.145	0.258
1.7	13.89	20.29	1.719	0.444
	2.0 - BNS + sugars (day/night)			
	Shoot FW	Root FW	Shoot DW	Root DW
2.1	5.76	3.966	0.532	0.095
2.2	6.92	5.14	0.696	0.125
2.3	1.65	1.489	0.187	0.044
2.4	1.05	1.579	0.125	0.04
2.5	5.73	6.91	0.699	0.178
2.6	6.63	3.824	0.712	0.136
2.7	4.76	2.99	0.5	0.086
2.8	6.43	5.651	0.743	0.167
2.9	5.97	3.676	0.604	0.117
2.10	8.42	9.357	0.978	0.214

	3.0 - double distilled H2O (day/night)			
	Shoot FW	Root FW	Shoot DW	Root DW
3.1	0.006	0.09	0.002	0
3.2	0.007	0.13	0.002	0
3.3	0.04	0.283	0.013	0.006
3.4	0.03	0.077	0.008	0.004
3.5	0.023	0.142	0.008	0.004
3.6	0.03	0.109	0.01	0.003
3.7	0.011	0.088	0.004	0.003
3.8	0.016	0.119	0.006	0.002
3.9	0.002	0.044	0	0.003
3.10	0.002	0.049	0	0
	4.0 - BNS (day); H2O (night)			
	Shoot FW	Root FW	Shoot DW	Root DW
4.1	0.19	0.087	0.027	0.002
4.2	0.09	0.122	0.017	0.003
4.3	0.4	0.664	0.049	0.018
4.4	1.98	0.934	0.138	0.027
4.5	2.29	1.664	0.415	0.046
4.6	7.32	4.054	0.941	0.102
4.7	10.68	5.599	1.301	0.109
4.8	2.79	1.418	0.409	0.04
4.9	3.4	1.998	0.369	0.052
4.10	7.18	3.364	1.01	0.086

	5.0 - H2O (day); BNS (night)			
	Shoot FW	Root FW	Shoot DW	Root DW
5.1	0.11	0.013	0.015	0
5.2	0.17	0	0.017	0
5.3	0.72	0.597	0.165	0.015
5.4	0.09	0.148	0.017	0.005
5.5	0.04	0.109	0.01	0.011
5.6	3.35	2.125	0.679	0.058
5.7	0.58	0.784	0.12	0.015
5.8	7.88	5.853	1.183	0.123
5.9	9.67	6.123	1.56	0.161
	6.0 - BNS (day); BNS + sugars (night)			
	Shoot FW	Root FW	Shoot DW	Root DW
6.1	0.049	0.066	0.008	0.003
6.2	0.17	0.124	0.02	0.005
6.3	0.105	0.053	0.022	0.006
6.4	0.335	0.094	0.065	0.008
6.5	0.117	0.107	0.016	0.005
6.6	0.082	0.068	0.017	0.005
6.7	0.171	0.064	0.032	0.005
6.8	12.97	4.286	1.343	0.137

	7.0 - BNS + sugars (day); BNS (night)			
	Shoot FW	Root FW	Shoot DW	Root DW
7.1	16.43	4.95	2.179	0.166
7.2	0.229	0.199	0.034	0.009
7.3	0.105	0.038	0.018	0.004
7.4	0.107	0.015	0.022	0
7.5	21.285	7.987	2.822	0.23
7.6	19.228	6.272	2.34	0.192

Appendix B

All plants with severe growth inhibition and discolouration of leaves were excluded from statistical analysis (5 plants on right side and left corner of lid in close proximity to aeration tube and blue label).