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Hybrid Genetic Programming and Multiverse-based Optimization of Pre-Harvest Growth Factors of Aquaponic Lettuce Based on Chlorophyll Concentration

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***Abstract*— Optimizing photosynthesis is vital in maintaining quality farm produce in the agricultural food production sector. The nonlinear behavior of the interaction of crop pre-harvest growth factors can promote or retard its growth. This study employed multigene symbolic regression genetic programming (MSRGP) in developing the chlorophyll-*a* fitness function allied with bioinspired algorithms, namely genetic algorithm (GA), particle swarm optimization (PSO), and multiverse optimization (MVO), in determining the ideal combination of carbon dioxide, light intensity, air temperature, and humidity that will induce photosynthesis based on aquaponic lettuce (*Lactuca sativa var. Altima*) leaf chlorophyll-*a* concentration. Light spectra were characterized through the floating leaf disk technique, which resulted in white spectra as the most photosynthetic conducive based on the light reaction and dark respiration. Leaf spectro-textural-morphological signatures were extracted for non-destructive MSRGP chlorophyll-*a* concentration measurement. Carbon dioxide and humidity have a strong positive impact on chlorophyll-*a* concentration. Photosynthesis is impeded by Vis/IR above 7.817. The hybrid MSRGP-MVO generated the ideal global solution of 880.744 ppm of CO_2, 543.147 µmol m^{-2} s^{-1} of the visible white light spectrum, 22.238 °C air temperature, and 67.742% humidity which resulted in 651.144 mg g^{-1} of Chl-*a*, 0.934 leaf weight ratio, 0.066 roots to shoot ratio, 141 xylem vessels mm^{-2} 127.389 stomata mm^{-2}, and more prominent intracellular chloroplast concentration for the harvest stage lettuce. The established standard for fresh weight, chlorophylls *a* and *b*, and vitamin C concentrations are essential for developing an adaptive nutrient management system to maintain the expected growth signatures of lettuce at the end of the 6-week cultivation cycle.**

***Keywords*—Chlorophyll pigment; genetic programming; lettuce; multiverse optimization; photosynthetic rate.**

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I. INTRODUCTION

Optimizing photosynthesis is vital in maintaining acceptable and marketable farm produce. Agricultural yield is dominantly driven by the combination of photosynthetic growth factors, namely carbon dioxide concentration, light intensity, temperature level [1], [2], photoperiod [3], vapor pressure [4], and the nutrients coming from soil and water resources that include nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S) as the macronutrients [5]. NPK, iron, and salinity concentration were previously optimized based on germination rate and root morphology for lettuce and rice [6]-[8]. Crops, such as corn, rice, tomato, and lettuce, are vulnerable to climate change resulting in lower biomass production [5], [9], [10]. The initial tendency to improve crop quality is through adjusting the irrigation and fertigation [11], air velocity [12], and spectral filters [13]. When the temperature rises to 30 °C in long term scenario, many leafy vegetables experience retardation in growth, and the worst is they will naturally speed up the senescence stage. The controlled condition of closed environment agriculture (CEA) can maintain the suitable growth and quality stressors for specific crops. Plant growth is manifested through their organs, wherein the leaf is the easiest one to analyze [14]. It can also be the leaf canopy area, greenness due to the presence of high chlorophyll concentration, and leaf textures. Plant quality is expressed in molecular phenes such as vitamin, moisture, and pigment concentrations [15]. On the other hand, toxic elements such as arsenic (As) retards plant growth and worsen its quality for long-term intake [16]. Food production essentially needs an optimized and maintained environmental condition to generate expected crop growth and quality at the end of the cultivation season. Hence, phenotypic growth and quality parameters must optimize environmental factors since they are directly affected by any changes in the cultivation condition.

Lettuce is a highly vulnerable crop with an increased temperature that exhibits tip burning, withering, and chlorosis on its leaf canopy [17]. The combination of low relative humidity and light intensity varies the cortical cylinder thickness and stele diameter, improving water transport [4]. Surface response analysis (SRA) was employed to optimize the temperature inside a greenhouse [18]. The morpho-anatomical traits of crops can be visual determinants concerning their environmental response at the microscopic level.

The primary photosynthetic pigments are chlorophylls *a* and *b*, and the auxiliary pigments are anthocyanin, carotenoids, and xanthophyll. These pigments also inhibit photosynthesis when sufficient energy levels are contained in its cells inside the leaf chloroplasts. Thus, leaf pigments are good visible indicators of the impact of abiotic stressors on plants concerning photosynthetic reactions. Leaf anthocyanin concentration is highest when low relative humidity and high irradiance [3], [4]. The use of a Sunlike light-emitting diode (LED) that mimics the natural solar spectrum (400 nm to 700 nm) proves that red and blue light of 9:1 ratio corresponds to better biomass production [19]. Far-red (FR) light has a positive impact on the photomorphogenesis of greenhouse plants [20], monochromatic red and blue lights result in to decrease in stomatal density [21], and red-blue and yellow spectrums affect the air temperature and humidity [22]. The combination of red-blue light (4:1) and 15 hours photoperiod has been tested effective for spinach [23]. The photoperiod of consecutive 15 light hours and 22 hours for dark-period respiration suggested an increase in photosynthetic rate as expressed by low starch content in the leaf [24].

Conventional destructive pigment extract quantification is through chromatography [25]. Non-destructive chlorophyll concentration of leaf has been measured based on the extracted leaf biomass and measured area [26] and through the use of an emerging device like atLEAF+ [27]. It was reported that the intracellular chloroplast position (ICP) is an anatomical factor that affects photosynthesis [28]. The ICP determines leaf morphology due to chloroplasts moving to the cell wall due to attraction on weak blue light (accumulation response) and moving away from strong blue light positioning to the anticlinal cell wall (avoidance response).

Current optimization approaches make use of crop features such as fresh weight and leaf shape index [29], dry biomass [30], carotenoid [31], stomatal conductance, size, and density [32], leaf starch content [24], root anatomy [33], and vitamin C and nitrate content [15]. Image phenotyping has been made possible through computer vision (CV) [5], [9]. The use of adaptive neuro-fuzzy inference systems (ANFIS) [1] and genetic algorithm (GA) [2] have been proven effective in optimizing nonlinear relationships of environmental stressors to canopy areas. The genetic algorithm is based on the survival of the fittest individual, naturally selecting the best one and combining them with a certain constraint based on chromosome reproduction. Other complex algorithms such as particle swarm optimization (PSO) and multiverse optimization (MVO) pose suitability for good exploration and exploitation of the best solutions. PSO is based on the individual and social thinking of a swarm of birds. MVO is based on the concept of cosmology that deals with white hole, black hole, and wormhole interactions [34].

Despite the abovementioned trends and technologies in optimizing nonlinear pre-harvest growth factors based on crop growth, no explicit technique has been developed using computational intelligence to aid in determining the exact values for lettuce cultivated in an aquaponics setup. ANFIS and GA have been employed only with the basis of visual canopy area that is one of the most obvious varying growth traits for hydroponic lettuce [1], [2]. However, not all lettuce cultivars have the same leaf canopy area response to environmental stimulus. In this paper, the use of fuzzy logic-based algorithms is deemed unfit for utilization due to limited samples. Most optimization techniques were aided by laboratory and manual determination, resulting in discrete environmental values that are still distant from the optimum global value.

This study aims to analyze and optimize the combined nonlinear effect of carbon dioxide, light intensity, air temperature, and humidity on the chlorophyll-*a* concentration of aquaponic green loose-leaf lettuce using hybrid genetic programming and a multiverse algorithm. Other bioinspired optimization models were also explored for pre-harvest growth factors optimization, such as genetic algorithm and particle swarm optimization. Standard leaf disk floatation was performed to determine the optimal light spectrum for faster photosynthetic and respiration rates. The chlorophyll-*a* concentration, fresh and dry weights of shoot and root systems, stem xylem vessels, stomatal density, and intracellular chloroplast formation were also analytically quantified and discriminated for unoptimized and optimized conditions. The developed model in this study is part of the 10-module vision-phenotype-lettuce (VIPHLET) model used for adaptive nutrient management in a smart lettuce farm.

II. Materials and Methods

This study constitutes the consecutive process of measuring light intensity and infrared radiation, leaf disk floatation to determine the best light spectrum for aquaponic lettuce, leaf canopy image capturing for computer vision, measurement of chlorophyll concentration, development of bioinspired optimization models, and microscopic analysis of lettuce leaf and stem to verify if the optimized environment condition induces lettuce growth (Fig. 1). For the optimization model, the inputs are the unoptimized pre-harvest growth factors that can be controlled in closed environment agriculture (CEA) chamber.

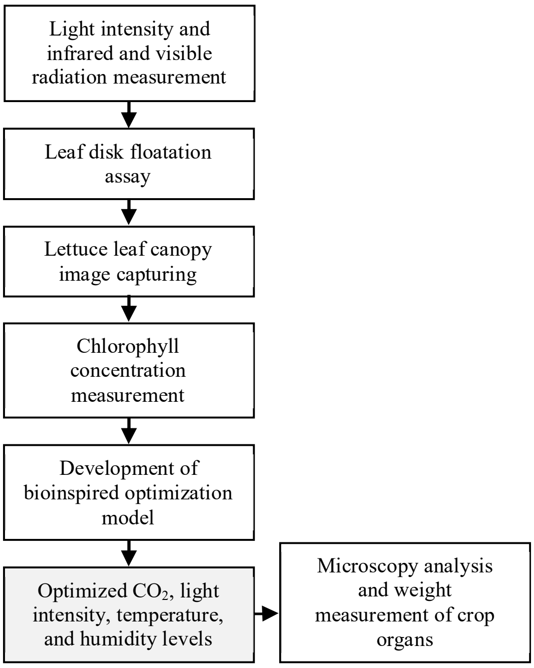

Fig. 1 Developmental architecture of optimizing pre-harvest growth factors of aquaponic lettuce (carbon dioxide, light intensity, air temperature, and humidity levels) using hybrid bioinspired algorithms

A. Plant Material and Controlled Environment Condition

Green loose-leaf lettuce (*Lactuca Sativa var. Altima*) was utilized as the cultivar because an abrupt change in environmental conditions easily impacts it. It is also the core crop being cultivated in the smart lettuce farm in Morong, Rizal, Philippines (14.5181° N, 121.2390° E), where this optimization model was applied. Lettuce seeds (Ramgo, Philippines) were placed at the chiller unpacked to undergo cold stratification for at least two weeks to break its dormancy. Nutrient film technique was used for the grow bed in both unoptimized and optimized cultivation with maintained nitrate of 124.70 mg L^{-1}, phosphate of 65.23 mg L^{-1}, and potassium of 168.74 mg L^{-1} for six weeks from which the pH and electrical conductivity are controlled between 6 ± 0.5 and 1 ± 0.2 mS cm^{-1} [6]. For cultivating with an unoptimized environment, diffused gas by equilibrating the inside chamber from the outside chamber. The air temperature varies in 29.5 ± 3.7 °C and humidity in 53.05 ± 17.77%. For the optimized cultivation, the conditions were set based on the recommended results of the selected bioinspired optimization algorithm.

B. Light Intensity, and Infrared and Visible Radiation Measurement

A closed chamber with 67449.16 cm^3 (21 x 14 x 14 in) was constructed using a black plastic tray. To reflect the light coming from the artificial photosynthetic light source, aluminum foil (Reynolds Wrap Heavy Duty, Canada) was covered on the inside wall surface of the microchamber. One visible monochromatic light source was attached, used, and detached at a time, maintaining the 12 in, 8 in, and 4 in a distance of the light source to the bottom of the plastic container (Fig. 2a). Red, orange, yellow, white, green, blue, and violet light spectrums with 32 W, 190 ± 5 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) (Firefly, Ace Hardware Corp., Philippines) was used (Fig. 2b). Three light intensity sensors (Adafruit TSL2561, China) were placed on the 4 in, 8 in, and 12 in segments to measure the light intensity and the infrared and visible radiations differentiated by each monochromatic spectrum and varied by the light source proximity. These spectral data were furtherly used in the optimization analysis.

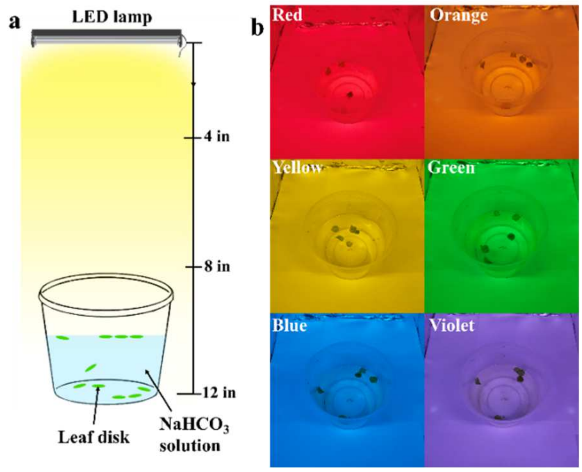

Fig. 2 (a) Distance of the bottom of container used in leaf disk floatation from the artificial photosynthetic light source and (b) the actual resemblance of monochromatic red, orange, yellow, green, blue, and violet light spectrums on the experiment photosynthetic chamber captured using a mobile phone camera

C. Leaf Disk Floatation Assay

The leaf disk floatation was used in this study to determine what is the monochromatic light spectrum that can provide the highest photosynthetic rate. It was performed by preparing a 0.2 % sodium bicarbonate ($NaHCO_3$) solution by mixing 300 mL of distilled water with 0.425 g of $NaHCO_3$ (Arm & Hammer, Philippines). A drop of liquid soap was added to wet the hydrophobic leaf surface. 10 uniform leaf disks from head development (the fifth week after sowing, WAS 5) and harvest (WAS 6) aquaponic lettuce leaves were cut for each trial using stout plastic straw of 1 cm diameter. Midrib and leaf veins were avoided. Next, leaf disks were placed into the barrel of a 10 cm^3 syringe (Mercury Drug, Philippines) and filled with 5 cm^3 of $NaHCO_3$ solution. A small vacuum inside the syringe was made to infiltrate the leaf disks with the solution. When the disks were sunk on the bottom, the plunger was removed, and the leaf disks were removed and placed into the clear plastic floatation container containing the same concentration of bicarbonate solution (Fig. 2). The container was placed under the fluorescent lamp with 12 in proximity for light-dependent photosynthetic reaction, and the timer started. The number of floating leaf disks was recorded every minute and continued until all the leaf disks floated. Immediately, the dark-period aerobic respiration was performed by placing the floatation container inside a box without a light source. Every minute, the number of remaining floating leaf disks was recorded. Overall, there were 42 trials (7 monochromatic lights x 2 lettuce leaf maturities x 3 light source proximities) for photosynthesis and 42 trials for the corresponding dak-period respiration. The photosynthetic rate during the light period ($1/ET_{50\text{-light}}$, min^{-1}) is calculated by getting the inverse of the time when 50% of the leaf disks are floating ($t_{50\%\text{-l}}$) (1). The respiration rate during the dark period ($1/ET_{50\text{-dark}}$, min^{-1}) is calculated by getting the inverse of the time when 50% of the remaining leaf disks are still floating ($t_{50\%-d}$) (2). The relative gross rate of photosynthesis ($1/ET_{50}$, min^{-1}) is the sum relative rates of photosynthesis and respiration (3).

$$1/ET_{50-light} = \frac{1}{t_{50\%-l}} \quad (1)$$

$$1/ET_{50-dark} = \frac{1}{t_{50\%-d}} \quad (2)$$

$$1/ET_{50} = \frac{1}{t_{50\%-l}} + \frac{1}{t_{50\%-d}} \quad (3)$$

D. Lettuce Leaf Canopy Imaging

The imaging system used is equipped with an IP camera 1080p (Logitech, China) that was installed on a downward projection at the height of 10 inches to capture the totality of the lettuce leaf canopy. The camera is connected to an Acer Nitro 5 laptop with Intel Core i7 8th generation processor and Matlab 2020a platform for control and processing. The captured images have 3000 x 3000 pixels (1:1 aspect ratio). The generated images were used for non-destructive chlorophyll concentration prediction.

E. Chlorophyll Concentration Measurement

This phase employed a non-destructive vision-based chlorophyll-*a* concentration prediction technique. The non-destructive approach was fully adapted from our previous work using multigene genetic regression programming (MSRGP) model configured with 100 initial population size and generation size, a tournament size of 50 with Pareto rate of 0.2, an elite fraction of 0.1, maximum genes of 10, and tree depth of 5 [6]. This developed model is based on the spectrophotometry experiment (Shimadzu UV-Vis 1900) on light absorption in the 663 nm and 645 nm optical densities of fresh leaf pigment mixed with 75% acetone. Using Matlab R2020a, the combination of independent variables, namely, spectral (red, green, and blue reflectance), Haralick texture (contrast and energy), and morphological (whole leaf canopy area) signatures were extracted from each single leaf image and evaluated by the MSRGP chlorophyll-*a* concentration model.

F. Development of Bioinspired Pre-Harvest Growth Factors Optimization Model

This phase is concerned with developing an effective bioinspired maximization optimization model in determining the best combination of nonlinear pre-harvest growth factors affecting the growth of aquaponic lettuce in terms of chlorophyll concentration. In this study, the objective function (Chl-*a*, mg g^{-1}) resembles the chlorophyll-*a* concentration of in vivo lettuce leaf, which is a function of the selected pre-harvest growth factors, namely, carbon dioxide concentration (α, ppm), light intensity (β, μmol m^{-2} s^{-1}), air temperature (θ, °C), and humidity (μ, %) (4). The Chl-*a* regression model was generated using multigene symbolic regression genetic programming. MSRGP is an evolutionary algorithm (EA) in the GPTIPS2 application of Matlab that evolves on a genetic search approach for mathematical topology and genetic parameters [35]. It was configured with 500 population size, 100 maximum generations, tournament size of 100, 0.1 elite fraction with lexicographic selection pressure that prefers simpler tree depth when individual fitness values are equal, 0.2 probability pareto tournament, maximum of 10 genes, maximum tree depth of 5, crossover rate of 0.84, mutation rate of 0.14, and functional set of {'times', 'minus', 'plus', 'sqrt', 'square', 'sin', 'cos', 'add3', 'mult3', 'log', 'cube', 'neg', 'abs'} (Fig. 3). MSRGP terminates when the change in fitness value < 0.001. This developed MSRGP model generated a R^2 of 0.98 for Chl-*a* objective function (4).

$$Chl - a = f(\alpha,\beta,\theta,\mu) = 0.0436\alpha^2 - 0.123\beta - 99.1\cos(\beta^2) - 20.06\alpha - 23.2\theta - 0.0124\alpha\mu^2 - 0.0118 \quad (4)$$

Three bioinspired algorithms were explored, namely, genetic algorithm (GA), particle swarm optimization (PSO), and multiverse optimization (MVO). These three algorithms have proven track on optimizing nonlinear conditions [1, 34, 36]. The evolutionary nature of GA, PSO, and MVO makes it possible to search the global best conditions to induce the healthy growth of lettuce. Hence, they differ in the mechanism of evolving the solution.

The genetic algorithm starts with initializing 100 random populations as the first generation (Fig. 3). The pre-harvest growth factors (α, β, θ, μ) are encoded in separate genes for each chromosome. A chromosome is a string of real numbers stored as a vector of four floats in this study. Technically, the chromosome resembles the chlorophyll-*a* concentration. Then, the fitness function (4) is evaluated that determines the desirability of the solution. In certain cases that severe exploration of chromosomes and the genes exceeded the boundary value, a penalty of -100 was imposed, which affects the individual's fitness. The 20 fittest individuals are selected (selection rate of 0.1) through a tournament (tournament size of 4) to be the parents of the next solution. To induce uniqueness in the population, a single-point crossover (crossover rate of 0.8) was employed to each chromosome that experiences the exchange of one of the genetic traits from its parent. It is followed by mutating two genes (mutation rate of 0.01) in an individual chromosome based on Gaussian mutation to form new individuals: the mean and standard deviation of the genes of the whole current population. The genetic algorithm converged after having 500 generations.

The particle swarm optimization starts with initializing 100 random particles with different positions (x) and velocity (v) (Fig. 3). Then, the fitness of each particle is evaluated. If the acquired fitness value of the particle is higher than the p_{best}, then the p_{best} is updated, and if the acquired fitness value of the p_{best} is greater than the g_{best}, then g_{best} is updated as the best solution at the current iteration and disseminated to all the swarm of particles. It is followed by updating the velocity and position of each particle in the swarm based on (5-6) where inertial weight (w, 0.4) reduces velocity impact to particle movements and increase exploitation, cognitive coefficient (ϕ_p, 2) accelerates the influence of p_{best} in the solution, and social coefficient (ϕ_g, 1.8) accelerates the influence of g_{best} in the solution. Hence, the particle velocity and position at time t+1 is affected by the current motion (first term), particle memory influence (second term), and swarm influence (third term) (5-6). The updated instantaneous velocity and position of the particle is added to the current position and follows the cyclic fitness evaluation. PSO converged after having 500 iterations.

$$v(t+1) = wv(t) + \phi_p r_p\left(p_{best} - x(t)\right) + \phi_g r_g\left(g_{best} - x(t)\right) \quad (5)$$

$$x(t+1) = x(t) + v(t+1) \quad (6)$$

The multiverse optimization starts with initializing 100 random 4-dimensional universes and 100 objects for time equals 1 (Fig. 3). It is followed by evaluating the inflation rate of each universe over the search space. In MVO, objects from the local universe move from higher to lower inflation rate universes through white holes. Black holes are created in the universes with lower inflation rates making them possible to receive objects from high inflation rate universes. The teleportation of objects is made through the presence of wormholes that are present randomly in any universe. Next is the calculation of wormhole existence probability (WEP, 0.2 to 1) (7), which directly affects the exploitation in a region and the traveling distance rate (TDR) (8) that defines the variation that an object can be teleported around the best universe. In the WEP and TDR equations (7-8), l is the current time, L is the maximum time, and p (value is 8) is the accuracy of exploitation concerning time. Next, the inflation rate of all universes is normalized (NI). The higher the NI, the higher the probability of sending objects to other universes. Then, all universes are sorted based on NI. The position of each universe is updated based on roulette wheel selection. The best universe exhibiting the best combination of pre-harvest growth factors is selected based on the generated fitness value. MVO converged after having 500 cyclic times of moving objects through wormholes.

$$WEP = WEP_{min} + l * \left(\frac{WEP_{max} - WEP_{min}}{L}\right) \quad (7)$$

$$TDR = 1 - \frac{l^{1/p}}{L^{1/p}} \quad (8)$$

The configured system boundaries for GA, PSO, and MVO are the following: 100 ppm < α < 1000 ppm, 16 µmol m^{-2} m^{-1} < β < 2000 µmol m^{-2} m^{-1}, 20 °C < θ < 32°C, and 10% < μ < 100%. There were 30 cyclic generations employed for each algorithm to ensure the reliability of the generated optimized pre-harvest growth factors. The result of this optimization phase is the best solution or the optimal combination of pre-harvest growth factors that will induce lettuce growth based on leaf chlorophyll-*a* concentration.

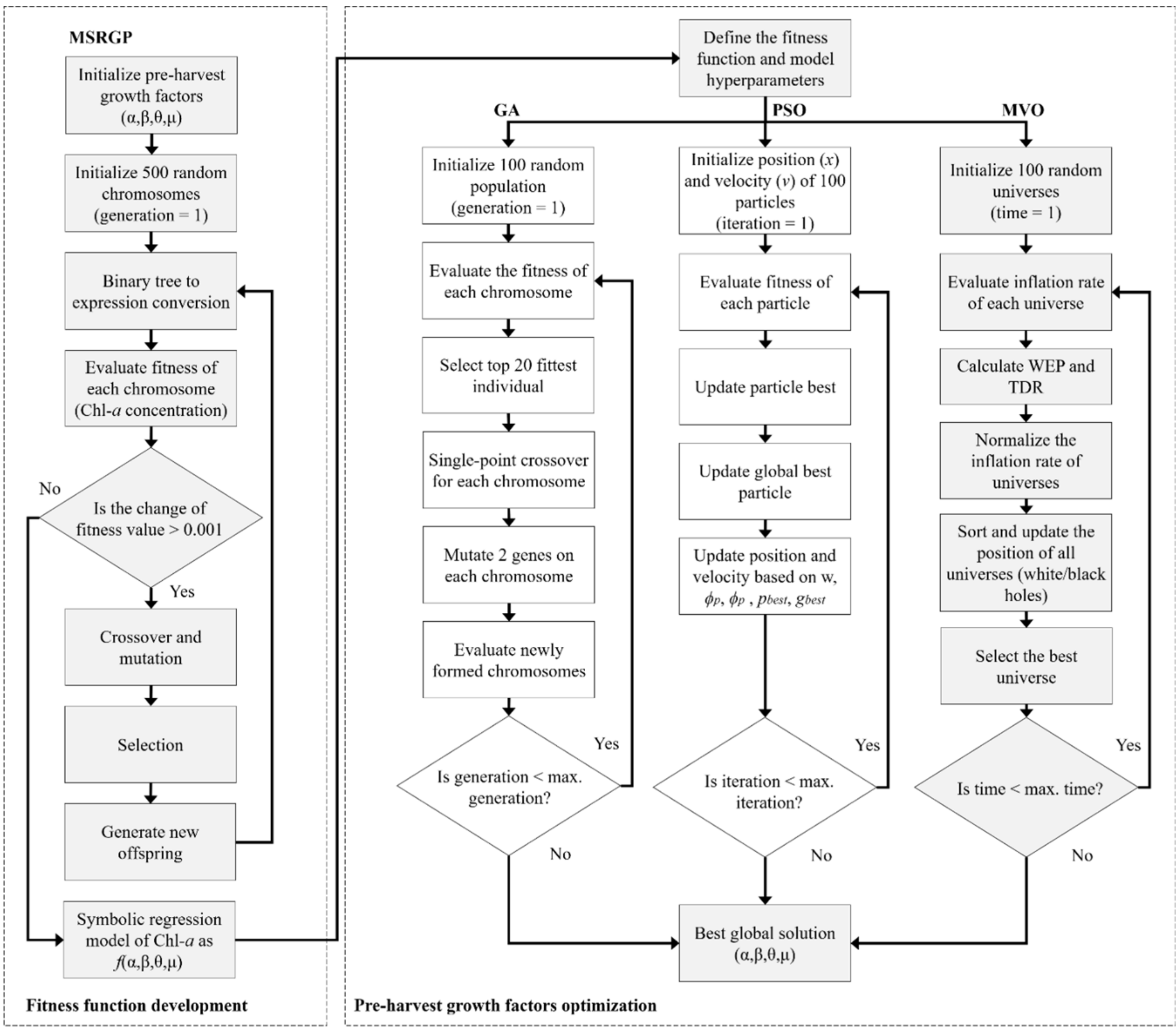

Fig. 3 System process flow of hybrid multigene symbolic regression genetic programming and multiverse optimization (MSRGP-MVO) (highlighted in gray), genetic algorithm and particle swarm optimization for chlorophyll-*a* concentration maximization approach based on the fitness function that is composed of carbon dioxide (α), light intensity (β), air temperature (θ), and humidity (μ) interactions

G. Microscopy Analysis and Crop Organ Weight Measurement

Microimaging, crop organ weight, phytopigment, and nutrient measurements were employed to verify the induced growth due to optimized pre-harvest growth stressors. For the microimaging, leaf chloroplast, phloem, and xylem were observed. The xylem vessels were observed in the stem section. Matured lettuce leaf and stem were visualized using a trinocular compound microscope (Howell Medical Supply, Philippines) and ToupView software. The topmost leaf was chosen to be dissected as it is dominantly exposed to environmental stressors. The intracellular chloroplast position was visualized by cutting holes of leaf tissues away from the midrib region, deaerating, and permeating it with water using the syringe method, and facing the abaxial surface down on the microscope light source (100x oil-immersion objective). Thin vascular stem tissues were excised from the transition zone near the first leaf shoot for the stem section. A few drops of safranin ($C_{20}H_{19}ClN_4$, from Thomas Scientific, Indonesia) were poured onto these lettuce tissues for staining, then washed with water. Fresh and oven-dried (40 °C) weight of shoot and root systems were measured using an electronic balance with 0.001 sensitivity at the end of the life cycle. Our previously developed method measured the chlorophyll-a, chlorophyll-b, and vitamin C concentrations [6]. The vitamin C concentration is based on using metaphosphoric acid (HPO_3) and glacial acetic acid (CH_3COOH) solution.

III. Results and Discussion

A. Lettuce Photosynthetic Pre-Harvest Growth Factors and Chlorophyll Concentration

In this study, the interaction of carbon dioxide concentration, light intensity, air temperature, and humidity in a partially diffused unoptimized environment served as the basis for analyzing the plant photosynthetic response in terms of chlorophyll-*a* concentration since it is the primary plant pigment responsible for oxygenic photosynthesis. In higher plants like lettuce, the higher chlorophyll-a concentration means high energy production, biomass growth, and a high photosynthetic rate [15, 19, 26]. By applying Pearson correlation analysis with 95% confidence to pre-harvest growth factors and chlorophyll-*a* concentration, it was found out that this primary photosynthetic pigment has a strong positive correlation to carbon dioxide and humidity for both head development (WAS 5) and harvest (WAS 6) stages (Table 1). Considering all other factors constant, chlorophyll-*a* concentration increases at 0.738 mg L^{-1} µmol m^{-2} s^{-1} of white light, 0.791 mg L^{-1} ppm^{-1} of CO_2, and 7.533 mg L^{-1} $\%^{-1}$ of air humidity (Fig. 4a and 4b). On the other hand, chlorophyll-*a* increases when there is a substantial decrease in temperature (Table 1), which agrees with [19, 26]. The humidity is strongly affected by the carbon dioxide concentration inside the microclimatic chamber though light intensity has a negligible impact on air temperature (Table 1). There is a temperature increase of 0.48 °C ppm^{-1} of CO_2 (Fig. 4c), and chlorophyll-*a* concentration significantly decays above 28 °C and below 300 µmol m^{-2} s^{-1} of white light (Fig. 4d). A 32 °C air temperature for lettuce is very damaging as a result of retarded photosynthetic rate. These complex interactions suggest that head development and harvest stage lettuces are identical, and optimization is rational in a controlled environment for higher production yield.

TABLE I
PEARSON CORRELATION VALUES OF PRE-HARVEST GROWTH FACTORS FOR AQUAPONIC GREEN LOOSE-LEAF LETTUCE

Growth Stage	Chl-a CO_2	Chl-a Light Int.	Chl-a Temp.	Chl-a Hum.	CO_2 Hum.	Light Int. Temp.
Head Dev.	0.850	0.360	-0.850	0.857	1.000	-0.012
Harvest	0.887	0.046	-0.812	0.823	0.998	0.004

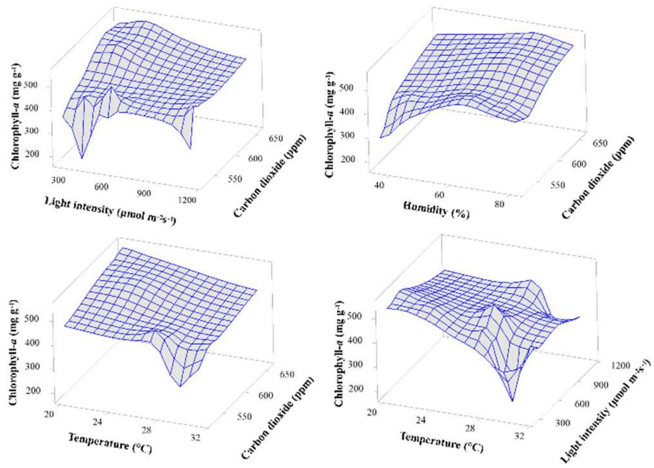

Fig. 4 Interaction of carbon dioxide, light intensity, air temperature, and humidity to chlorophyll-*a* concentration

B. Relationship of Light Spectrum and Radiation to Leaf Canopy Proximity

Differences in light intensity and the ratio of visible and infrared radiation show the photosynthetic inducing capacity of each visible light spectrum concerning the proximity of the leaf canopy to the light source (Fig. 5). The current study found that white light produces the highest intensity of 1131.480 µmol m^{-2} s^{-1}, 544.663 µmol m^{-2} s^{-1}, and 380.956 µmol m^{-2} s^{-1} on the proximal distance of 4 in, 8 in, and 12 in respectively among other visible light spectra. It is interesting that blue light only has the 38.026% strength of the white light intensity. It exhibits the highest visible to infrared radiation ratio (Vis/IR) for all proximal distances (Fig. 5). The results of this study indicated that the closer the proximity of the crop bed to the light source (d = 4 in) is, the lower is the Vis/IR (8.254) that will strike to crop leaf canopy. When the light source is 12 in away from the crop, the Vis/IR increases to 8.751. This means that when plants are exposed to 4 in proximity to a light source emitting significant infrared, heat energy may cause it to exhibit leaf discoloration, spreading of nodes, and the worst is killing the plant. This measured incident radiation from the artificial light source is different from the far-infrared radiation (FIR) suitable for plant leaves as it is reported to yield no tissue irritation due to lower energy composition [13, 19-20, 22]. Violet spectrum consistently exhibited the lowest intensity and Vis/IR for all proximities. Thus, without proper light calibration of artificial photosynthetic light sources may impede the growth of crops. This light characterization is necessary for the efficiency of artificial light sources that will be used to optimize the photosynthetic rate.

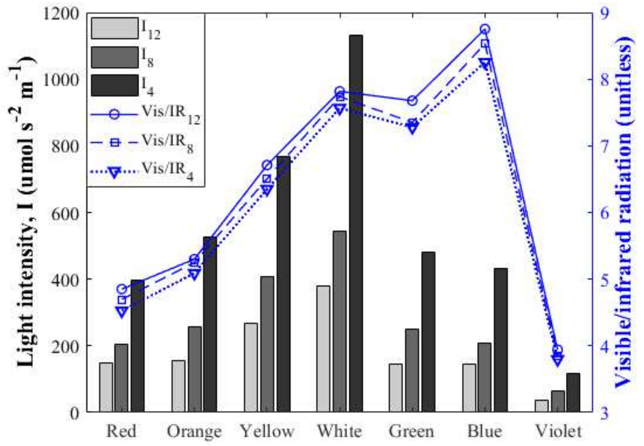

Fig. 5 Characterization of light intensity (I) and the visible and infrared radiation (Vis/IR) ratio of artificial photosynthetic lights based on spectrum and crop platform proximity to the light source. The numerical subscript represents the distance of crop platform to light source: 4 in, 8 in, and 12 in.

C. Photosynthetic and Respiration Rates Assay

In this study, photosynthetic and respiration rates were quantified destructively and indirectly by counting the floating lettuce leaf disks in the bicarbonate solution in light and dark reactions (Fig. 6a). At the start ($t_{0\%}$), deaerated leaf disks sunk at the bottom of the container due to permeation of water (increase in leaf disk density) in the spongy mesophyll tissue containing the photosynthetic cells (Fig. 6a). Photosynthesis materializes on the leaf disk when exposed to light as the carbon molecules interact with the bicarbonate solution. Oxygen is generated in the leaf interior, causing it to float ($t_{50\%}$ and $t_{100\%}$). This experiment showed evidence that white artificial light induces the highest relative photosynthetic rate ($1/ET_{50\text{-light}}$) for head development and harvest lettuce leaf disks in the 4 in, 8 in, and 12 in light proximity with the average of 0.111 disks min^{-1}, 0.162 disks min^{-1}, and 0.162 disks min^{-1} respectively (Fig. 6b). There is a 0.0182 floating leaf disk min^{-1} in^{-1} increase in proximity. The lower photosynthetic rate in closer proximity is caused by lower Vis/IR (Fig. 5).

Higher exposure to infrared radiation is incident to leaf disks against the photosynthetically active visible radiation. Greenlight exhibited the lowest photosynthetic rate except for head development leaf disks in 4 in proximity (Fig. 6b). For the relative respiration rate ($1/ET_{50\text{-dark}}$), leaf disks that were exposed to white light in the light-dependent period have consistent highest respiration rate of 0.029 min^{-1}, 0.027 min^{-1}, and 0.030 min^{-1} in the 4 in, 8 in, and 12 light proximity respectively compare to other leaf disks that were exposed to other visible light spectrums (Fig. 6c). Red, violet, and blue spectra deliberately followed the strength of white light in the dark period. It was also observed that leaf disks exposed to the green spectrum with Vis/IR of 8.531 are characterized by a significant decrease in respiration rate, which signifies low oxygen assimilation. A low dark respiration rate also results in lower utilization of produced sugar in light-dependent photosynthesis; thus, the growth of different plant organs is impeded. Hence, another important finding was that the highest relative gross rate of photosynthesis for both head development and harvest leaf stages occurred using the white spectrum (average of 0.174 min-1) followed by violet, red, blue, yellow, orange, and orange green. This partially supports the high photosynthetic yield of using Sunlike spectrum and RB light [19, 32] as white light was measured to be more conducive. Specifically, the 12 in the proximal distance with 380.956 μmol m^{-2} s^{-1} intensity is characterized with both the highest relative gross rate of photosynthesis (0.193 min^{-1}) and the ratio of gross rate of photosynthesis and respiration rate (6.516). This means that the plant can produce more than six and half times carbohydrates faster than it was utilizing energy for aerobic respiration. A very close distance to the light source directly breaks down the expected photosynthetic reaction. That strongly supports the reason why chlorophyll-*a* concentration has a strong negative correlation ($-0.812 \leq R^2 \leq -0.85$, Fig. 4) to the temperature, which is directly contributed by the infrared radiated by the light source [19, 21]. Above Vis/IR of 7.817 may induce more heat on the leaf surface and retards its expected growth. Thus, white light is considered as the best spectrum that may induce a faster gross photosynthetic reaction. The 8 and 12 in proximities of white light provide promising impact to green lettuce (Figs. 5 and 6). However, determining the exact optimal value for light intensity is necessary for precision engineering. The use of bioinspired algorithms was used to furtherly localize the optimal white light intensity.

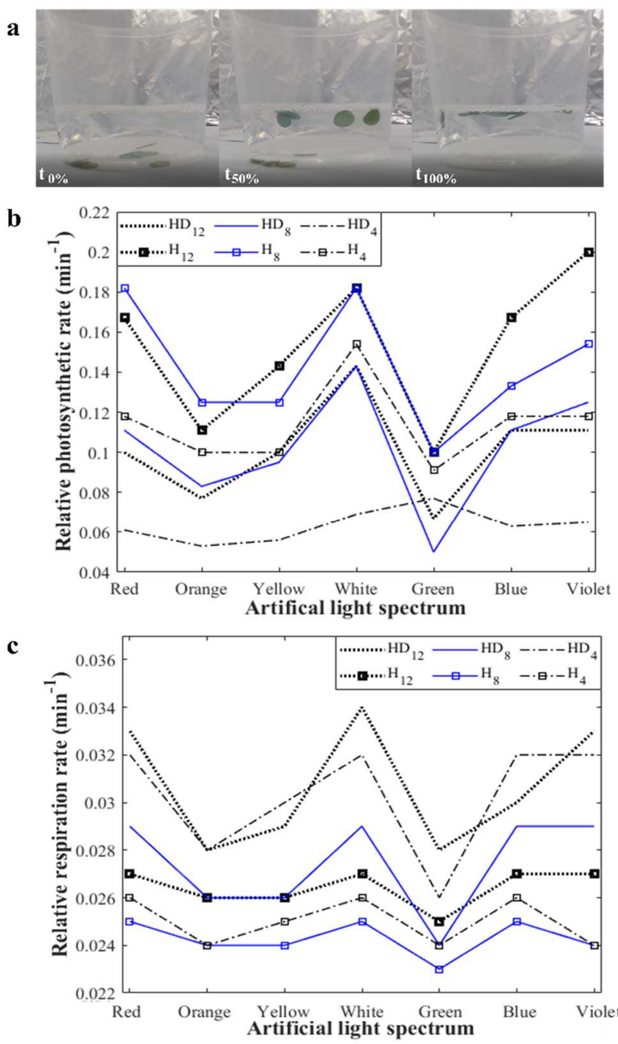

Fig. 6 (a) Leaf disk floatation experiment showing the time segments when 0%, 50%, and 100% of the leaf disks are floating on the surface of the sodium bicarbonate solution, and the resulting (b) relative photosynthetic rate during the light period using different light spectra and (c) the corresponding relative respiration rate during the dark period using the same leaf tissue samples.

D. Optimized Pre-Harvest Growth Factors

After several explorations on maximization techniques in optimizing the pre-harvest growth factors of aquaponic lettuce based on the multigene genetic programming-derived fitness function (4), these three selected bioinspired optimization algorithms, namely genetic algorithm (GA), particle swarm optimization (PSO), and multiverse optimization (MVO), successfully converged after 500 iterations (Fig. 7). PSO exhibited an abrupt increase in the objective chlorophyll-*a* concentration or fitness function value from 1800 to 8847 in iteration 136 to 138. The GA and MVO optimization curves have a similar trajectory except that MVO has an early onset of convergence (time = 261) while GA is on generation = 370 (Fig. 7).

Based on the convergence curves (Fig. 7), MVO has the highest fitness value for chlorophyll-*a* concentration. The optimization results of this study showed that GA, PSO, and MVO effectively determine the best global solution for nonlinear problems such as the maximization of chlorophyll-*a* concentrations as a function of carbon dioxide, light intensity, air temperature, and humidity (Table 2). Prior studies have reported that for soilless lettuce cultivation, the acceptable threshold for carbon dioxide concentration, light intensity, temperature, and humidity is 803.019 to 1300 ppm [17], [21], ≥ 175.23 μmol m^{-2} s^{-1} [15], [17], [23], 18 to 23 °C [14], [17], [18], and 60% to 75% [14], respectively. Based on the converged best solutions of GA, the temperature is above the ideal range, and the humidity is slightly short (Table 2). The PSO converged with inadequate carbon dioxide concentration despite that 400 to 1000 ppm is the actual concentration in a standard enclosed room where plants can grow (Table 3). Hence, a distinct combination of pre-harvest growth factors is necessary for a high photosynthetic rate through the evidence of accumulation of chlorophyll-*a* concentration of lettuce leaf in mesophyll tissues. The MVO-optimized pre-harvest growth factors of 880.744 ppm of CO_2, 543.147 μmol m^{-2} s^{-1} of the visible white light spectrum, 22.238 °C air temperature, and 67.742% humidity are all within the ranges of reported appropriate levels. Prior studies have noted the importance of the combined influence of growth factors on leaf canopy area [1], [2], [17], [21] and root emergence and area [6], but none was found to optimize these environmental stressors based on photosynthetic pigment. [1], [2] used ANFIS and GA in optimizing carbon dioxide, light intensity, and temperature. Hence, no prior studies were found to optimize humidity with carbon dioxide. The current study found out that based on the chlorophyll-*a* concentration of aquaponic loose-leaf lettuce, the ideal environment condition that will induce the best photosynthetic reaction is through the generated solutions of the multiverse optimization algorithm.

The chlorophyll *a* and *b* concentrations, vitamin C, and fresh and dry weights of lettuce systems cultivated in unoptimized, and MVO-optimized conditions were quantified (Table 3) to verify the reliability of the generated best combination of pre-harvest growth rate factors and its impact on other lettuce yield signatures (Fig. 8, Table 3). The chlorophyll-*a* of head development and harvest lettuce leaves cultivated in MVO-optimized environment condition is 105.386% and 138.489% more concentrated, respectively, than its corresponding growth stage cultivates in partially diffused unoptimized environmental conditions (Fig. 8). The fresh weight of the optimized head development and harvest stage lettuce shoot system is 266.188% and 225.68% heavier than the lettuces in unoptimized conditions. The fresh weight of the optimized head development and harvest stage lettuce root system is 298.201% and 465.187% heavier than its unoptimized counterpart (Fig. 8). This means that MVO-optimized pre-harvest growth factors are effective and valid. In return, lettuce yields are also optimized, as initially manifested by their weights. It is also interesting that the dry shoot weight of optimized head development lettuce is 396.62% heavier than the unoptimized one and is 115.61% heavier than the optimized harvest lettuce. It means that much biomass is formed on the shoot system of optimized head development lettuce than the harvested lettuce. Within a week of harvesting it, some part of its shoot architecture becomes more soluble to water which allows it to be consumed.

On the contrary, the dry weight of the optimized harvest shoot system is 412.023% heavier than its unoptimized counterpart and 94.992% heavier than the optimized head development. It means that this specific transitioning period (WAS 5 to 6) delivers more of the energy during dark respiration to different plant organelles for growth expansion, especially to leaves, resulting in higher biomass concentration on the shoot system. The leaf weight ratio (LWR) is highest in optimized WAS 5 lettuce (0.944 g g^{-1}) followed by the optimized WAS 6 (0.934 g g^{-1}), and unoptimized WAS 6 (0.92 g g^{-1}) and WAS 5 (0.920 g g^{-1}). Since it is the ratio of dry leaf weight to whole plant weight, as the value gets closer to 1, root system biomass is sufficiently small, and the leaf canopy is essentially broad and thick. The root to shoot ratio (RSR) is highest on unoptimized WAS 5 (0.080 g g^{-1}) followed by optimized WAS 5 (0.079 g g^{-1}) and WAS 6 (0.066 g g^{-1}), and unoptimized WAS 5 (0.056 g g^{-1}) lettuces. As it is the ratio of dry shoot weight to shoot biomass when RSR approaches 0, the shoot system biomass is getting larger and larger despite the small root network. In this study, both LWR and RSR are considered here as secondary determinants of environmental stimuli. Hence, the abundance of water concentration was observed in optimized lettuces.

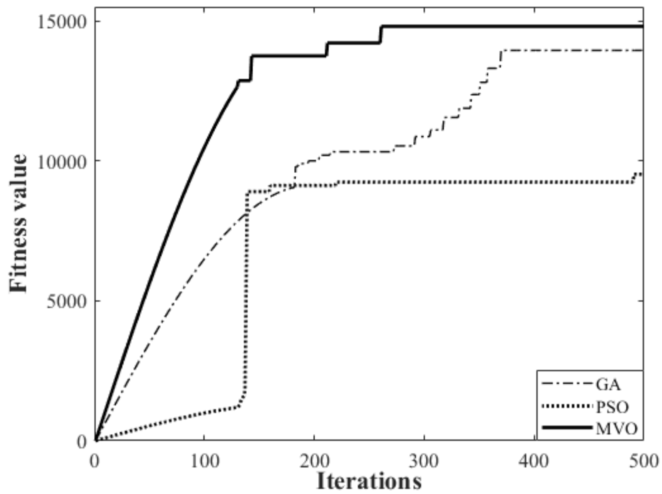

Fig. 7 Convergence curve of genetic algorithm, particle swarm optimization, and multiverse optimization for 500 iterations in determining the best combination of pre-harvest growth factors to maximize the chlorophyll-*a* concentration of aquaponic lettuce leaves.

TABLE II
GENERATED OPTIMAL PRE-HARVEST GROWTH STRESSORS BASED ON CHLOROPHYLL CONDITION USING BIOINSPIRED ALGORITHM

Model	CO_2 (ppm)	Light Intensity (µmol m^{-2} s^{-1})	Temperature (°C)	Humidity (%)
GA	864.13	633.209	26.788	57.745
PSO	769.163	841.155	18.744	33.411
MVO	880.744	543.147	22.238	69.742

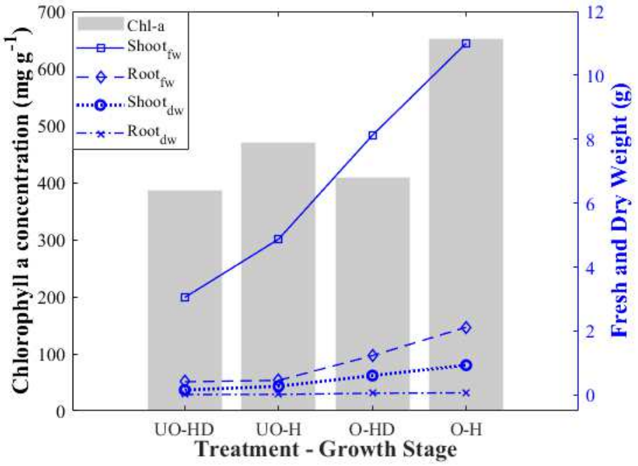

Fig. 8 Improvement of chlorophyll-*a* concentration, and fresh and dry weights of shoot and root systems of aquaponic lettuce. UO is unoptimized, O is optimized, HD is head development, and H is harvest.

This study showed that not only water and soil nutrients could sufficiently affect the growth of lettuce. Rather, it is a combined influence together with carbon dioxide, light intensity, air temperature, and humidity (Fig. 8). With that, this study established a standard for optimized aquaponic lettuce fresh weight, chlorophylls *a* and *b*, and vitamin C concentrations for 6-week life cycle cultivation (Table 6). This is based on the optimized condition generated by the multiverse optimization algorithm with 880.744 ppm of CO_2, 543.147 µmol m^{-2} s^{-1} of the visible white light spectrum, 22.238 °C air temperature, and 67.742% humidity (Table 2). This standard table can be used as the basis for controlling a nutrient management system adaptively. Other nutrient systems are solely based on pH, electrical conductivity, ammonia, and nitrate levels on the water system without considering the essential yield parameters, such as indicated in Table 3 [5, 6, 11].

TABLE III
ESTABLISHED OPTIMIZED AQUAPONIC LETTUCE FRESH WEIGHT, CHLOROPHYLL-*A*, CHLOROPHYLL-*B*, AND VITAMIN C LEVELS FOR 6-WEEK LIFE CYCLE CULTIVATION

Week	Fresh weight (g)	Chl-*a* (mg g^{-1})	Chl-*b* (mg g^{-1})	Vitamin C (mg g^{-1})
1	0.98	131.258	34.137	0.113
2	1.454	179.658	53.698	0.184
3	3.366	260.874	78.766	0.239
4	7.339	331.147	118.784	0.488
5	9.35	408.325	146.985	0.551
6	13.114	651.144	169.348	0.693

As a significant microscopy result of optimized environment condition, the xylem vessels of transected stems, stomata presence, and leaf intracellular chloroplast formation of unoptimized and optimized lettuces were visualized and characterized (Figs. 9 and 10). The optimized lettuces exhibited an average of 141 xylems in a transected stem tissue, while unoptimized lettuce has 43.5 only.

However, the average diameter of each xylem vessel in optimized lettuces is slightly greater than the unoptimized one (36.5 µm is to 35 µm) (Fig. 10). The current study found that xylem strongly relates to photosynthesis and chlorophyll-*a* concentration (R^2=0.927). Since xylems are physiologically responsible for transporting water from root to leaves necessary for photosynthesis and relative to crop growth, a high number is expected to occur in a more conducive environment. The occurrence that even plants are anatomically optimized using the MVO-generated combination of pre-harvest growth factors is evident in Fig. 9a. It agrees with the report of [6], [21]. Also, the stomatal leaf density is observed to be more prominent in optimized lettuce (127.389 stomata mm^{-2}) than unoptimized (63.694 stomata mm^{-2}) (Fig. 9b). The higher the stomatal density, the more active the plant is in leaf gas exchange, inducing photosynthesis [21]. It is similar to the intracellular chloroplasts, where the chlorophyll molecules reside, and the actual photosynthesis occurs. For both environmental conditions, chloroplasts move along and reside right on the outermost region of each cell wall. The only major difference is that the MVO-optimized environment induced the leaf mesophyll tissue to have abundant chloroplasts than the unoptimized setting (Fig. 9c). It is caused by weaker light intensity and high air temperature (Figs. 4 and 5). This significantly resulted in advanced photosynthesis and better growth in the optimized lettuces.

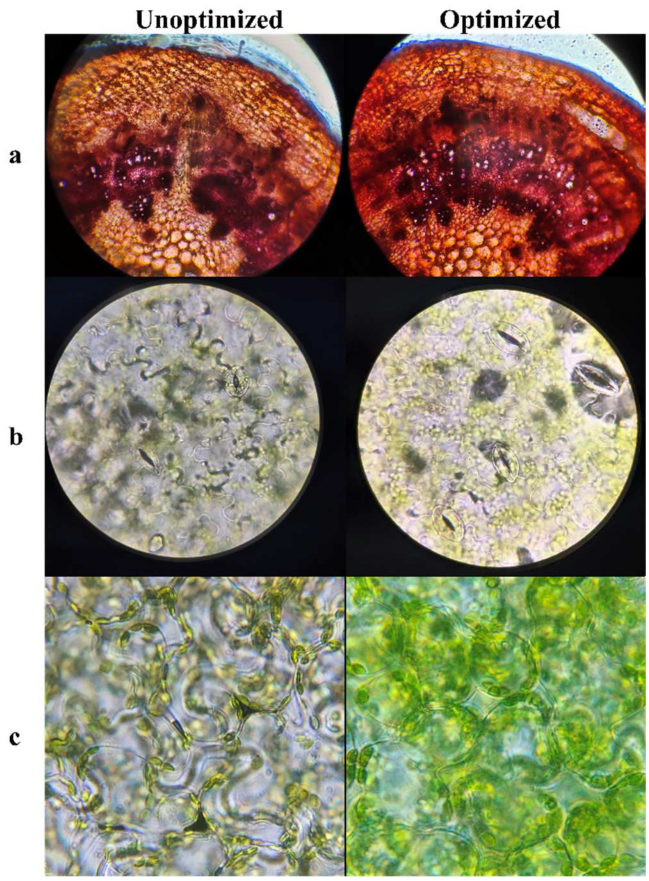

Fig. 9 Differential influence of unoptimized and MVO-optimized environmental conditions on (a) safranin-stained transected stem anatomy, (b) leaf stomata, and (c) chloroplast formation of aquaponic lettuce. Mature 6 WAS stem and leaf tissues were used.

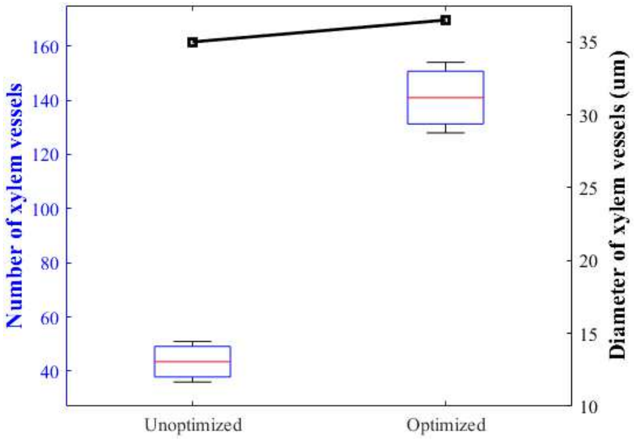

Fig. 10 Number and diameter of xylem vessels found in transections of aquaponic green loose-leaf lettuce cultivated in unoptimized partially diffused and MVO-optimized environment.

Overall, the results of this study showed that hybrid genetic programming and multiverse optimization-generated combinations of abiotic pre-harvest growth factors (Fig. 3, Table 2). This study has been proven valid, reliable, and effective in cultivating green loose-leaf lettuce in aquaponic nutrient film technique as observed on the significant increase in chlorophyll-*a* concentration, fresh and dry weights of root and shoot sections, xylem vessel count and diameter, stomatal density, and chloroplast formation as measures of better photosynthetic yield (Figs. 8-10).

IV. Conclusion

This study showed that hybrid multigene genetic programming and multiverse optimization algorithm effectively determines the ideal combination of photosynthetic pre-harvest growth parameters for aquaponic lettuce. Carbon dioxide was 880.744 ppm, light intensity was 543.147 µmol m^{-2} s^{-1}, the air temperature was 22.238 °C, and humidity was 67.742% could maximize the chlorophyll-*a* concentration as a strong determinant for better photosynthetic reaction in artificial photosynthetic light. Carbon dioxide and humidity have a strong positive impact on chlorophyll-*a* concentration. Heat radiation from a light source revealed that Vis/IR of 7.817 impedes photosynthesis. Genetic algorithm and particle swarm optimization exhibited off-bound solutions for best pre-harvest growth factors. Light spectrum characterization through leaf disk floatation technique deduced white light as the most inducive in photosynthesis based on the gross photosynthetic rate for light reaction and dark respiration of lettuce. The xylem vessel count, stomatal density, and intracellular chloroplast formation of lettuces cultivated in optimized conditions were quantified to have substantially higher than the unoptimized lettuces. The established standard fresh weight, chlorophylls *a* and *b*, and vitamin C concentrations for a 6-week aquaponic lettuce life cycle can help automate the nutrient management system in large-scale farms. Also, as future work, the nutrient use efficiency of lettuce based on this optimized environment condition can be quantified further to understand the impact of these stressors on plant growth.

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