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Enhanced Gene Expression in the Biosynthetic Pathway of 2-Acetyl-1-pyrroline in "Hom Bon" Fragrant Native Rice in Response to Varied Light Wavelength Conditions

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Abstract

The development of the Automated Agricultural Cabinet (AAC) prototype was driven by the goal of improving the aromatic qualities of locally grown rice. This compact prototype, designed with attention, employed Arduino programming to investigate genes related to the production of aromatic compounds in rice seedlings under different light conditions, including red, blue and white light. To ensure the reliability of experiments, a controlled environment was maintained at 25 °C and 75 % humidity, and rigorous testing confirmed precise light intensity measurements within the AAC. Results indicated specific light values: 9.25 ± 0.602 for red light, 11.47 ± 0.055 for blue light and 0.91 ± 0.027 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for white light. The study focused on up-regulated genes in 7-day-old 'Hom Bon' rice seedlings, revealing a significant increase in *P5CS* and *1Δ-pyrroline* gene expression. These genes are crucial in the biosynthesis pathway of 2-acetyl-1-pyrroline (2-AP), with *P5CS* acting as a precursor and *1Δ-pyrroline* as the final step. Interestingly, the study discovered that when 7-day-old 'Hom Bon' rice seedlings were exposed to both red and blue light, gene expression rose dramatically, contrasting when exposed to white light. This finding demonstrates that the wavelength of light exposure influences gene expression in the 2-acetyl-1-pyrroline (2-AP) biosynthetic pathway of 'Hom Bon' rice seedlings throughout the 7-day growth stage. This study demonstrates the feasibility of improving the smell of indigenous rice varieties by changing pre-planting light conditions, giving a promising avenue for increased commercial appeal. Furthermore, this project aims to improve the local community's financial well-being by supporting better farming methods.

Keywords: 2-AP, Automatic cabinet, Intensity, Model, RNA expression, Validation, Wavelength

Introduction

The issue of overpopulation has given rise to a multitude of challenges affecting both humanity and the environment. Among these challenges is the creation of unsuitable environments for plant growth, leading to pest infestations and subsequent food shortages [1]. In response, greenhouse cultivation has emerged as a viable solution, enabling the production of substantial crop yields while mitigating the risks of pest infestation and promoting continuous improvement. In the context of greenhouse agriculture, 4 key factors demand consideration: The choice of crop type, load-bearing capacity, light penetration and cost-effectiveness [2]. An effective approach to initiate greenhouse vegetable production involves utilizing the existing on-site soil, provided it is well-drained and conducive to plant growth. The precise regulation of greenhouse air temperature emerges as a critical factor influencing both vegetative and fruiting growth, demanding meticulous control and management [3]. In the realm of hydroponic greenhouse vegetable cultivation, crops are cultivated in substrates such as sand, gravel or artificial soil-less mixes contained within bags, tubes, tubs, tanks or troughs [4]. This approach facilitates the circulation of nutrient solutions essential for crop development, further enhancing production efficiency. Additionally, the introduction of supplemental carbon dioxide into the greenhouse environment has been demonstrated to yield significant increases in crop yields, particularly in the case of greenhouse tomatoes and other vegetable varieties [5]. The primary objective of plant cultivation, particularly when focusing on the production of secondary

metabolites, is driven by their considerable significance within the realms of pharmaceuticals, food production and agriculture. These secondary metabolites wield substantial economic importance [6,7]. Obtaining specific secondary metabolites in plants necessitates the modification of various plant development conditions. These metabolites play a pivotal role not only in plant growth and development but also in the domains of innate immunity and defense response signaling. Key factors influencing the pathways leading to the synthesis of secondary metabolites include variations in light wavelengths and light intensity [8]. The dynamic influence on morphogenesis, metabolism and photosynthetic capability is attributed to the wavelengths of red and blue light, as photosynthetic pigments primarily absorb light in the blue (400 - 500 nm) and red (600 -700 nm) spectra. The Light-emitting diode (LED) technology holds promise for creating customized light formulas to influence plant traits effectively, allowing for the manipulation of characteristics such as flowering induction, branching, compactness, root development and leaf expansion in order to achieve desired plant qualities [9-11]. Using LED lighting systems instead of traditional fluorescent lamps in plant *in vitro* cultures is suggested. The study found that both *Drosera binata* and *Drosera peltata* plants grown under LED lights showed similar growth rates and produced comparable amounts of biologically active compounds to those grown under fluorescent lamps [12].

Nakhon Si Thammarat is a province situated in the southern region of Thailand, renowned for its abundant natural resources, courtesy of its favorable climatic conditions conducive to the growth of diverse organisms [13]. Among the array of natural resources, rice stands out prominently, with the region boasting a distinct native rice variety known as 'Hom Bon' rice. This particular rice variety is noteworthy for its exceptional fragrance, characterized by an aroma reminiscent of potato, particularly when the rice is steamed [14]. The captivating aroma emanates from a fragrant compound known as 2-Acetyl-1-pyrroline (2-AP). 2-Acetyl-1-pyrroline (2-AP) is a chemical compound renowned for imparting the characteristic aromatic fragrance to select rice cultivars, most notably Basmati and Jasmine rice [14,15]. In the rice biosynthetic pathway leading to the formation of 2-AP involves a series of enzymatic processes. The amino acid precursors, ornithine and proline, serve as substrates in the synthesis of 2-AP. Specifically, the enzyme proline dehydrogenase (ProDH) catalyzes the conversion of proline into 1-pyrroline-5-carboxylate (*P5C*), involving an oxidation reaction. A pivotal juncture in this metabolic pathway is the conversion of P5C into 2-AP, a process facilitated by a specific enzyme known as 1-pyrroline-5-carboxylate reductase (P5CR). P5CR is responsible for the enzymatic degradation of P5C, culminating in the formation of 2-AP, the aromatic compound responsible for the distinctive olfactory profile of fragrant rice varieties [16-19]. This study is undertaken with the primary objective of establishing an advanced indoor cultivation system within the domain of smart agriculture. The primary goal is to increase the creation of secondary metabolites in rice by using painstakingly controlled parameters such as light intensity, long-lasting LED lighting and computer-controlled temperature and humidity conditions, with a particular emphasis on rice growth. Furthermore, this study aims to investigate the up-regulated gene expressions involved in the production pathway of 2-Acetyl-1-pyrroline (2-AP) in a 7-day-old seedling of the local rice known as "Hom Bon." The presence of 2-AP, a key aromatic compound, in fragrant rice cultivars not only endows them with distinctive and appealing characteristics, elevating their cultural and culinary significance worldwide but also presents an exemplary model for augmenting the synthesis of secondary metabolites in diverse plant species.

Materials and methods

An automated agricultural cabinet (AAC) designation

A cabinet has been designed in accordance with the apparatus design process, encompassing distinct input, output and processing components. The input module comprises a Light Sensor (BH1750FVI, ROHM semiconductor, Germany), a Temperature and Humidity Sensor (SHT20, Sensirion, Switzerland), a Distance sensor (SCSR04, SEIC stackpole Electronics) and a Water level sensor (HY-FRF04). For the processing component, the chosen model incorporates an Arduino board (UNO, Atmel, USA). This selection is attributed to its status as an open-source programming device, ideally suited for forward-thinking developers due to its extensive library support, catering to a multitude of applications. The output apparatus encompasses an LED RGB strip light (TRON11-3000k-24V, Lamp and Light, Thailand) characterized by a wavelength range spanning 300 - 800 nm, a 10×10 cm^2 square Fan (XF-12038, People, China) employed for humidity control and house ventilation, a mist spray head and a cooling fan (AN-PAC07LA ACONATIC 7000 BTU). Complementary components include a power supply unit (ALT-0902), a breadboard (ZSB120, Taiwan), a Channel relay (FL-3FF-S-2), a Water Pump (flt-A-2002G, FILETOP, China) and a solenoid valve (2w20020, CNHH, Northern Ireland).

An automated agricultural cabinet (AAC) validation

The validation of the AAC cabinet was conducted through inter-day and intraday assessments of temperature and humidity, comparing these parameters to both the ambient environmental conditions and standard measurement apparatus. Additionally, the validation encompassed the assessment of light intensity in the red, blue and white spectrums. Data for the validation process was collected using the Arduino processing control system and was systematically recorded and exported as an Excel file at 1-minute intervals over a 24 h period, spanning each day of evaluation. Data acquisition was executed at one-minute intervals utilizing the Arduino program interfaced with a computer. For intraday validation, data points were recorded hourly and followed by 1 h resting period, with each measurement recorded in triplicate. Inter-day validation was extended over a duration of three consecutive days, maintaining the same conditions as observed during the intraday assessments. To validate temperature, humidity and light intensity, comparisons were made against standard instruments, including a Lux meter (DT-1309 CEM Light Meter with RS-232 interface) for light intensity validation, and a thermometer (Hygrometer testo 605-H1, Testo AG, Germany) for temperature and relative humidity measurements.

Rice plantation

The rice strain employed in this study was a locally cultivated variety known as 'Khao Hom Bon' from Nakhon Si Thammarat province, Thailand, characterized by its distinctive potato-like aroma. This upland rice cultivar was sourced from the Department of Agriculture in Nakhon Si Thammarat province. The experimental, wherein paddy rice was planted in 100-well pots and nurtured within the automate agriculture cabinet, exposed to red, blue and white lighting conditions over a period of 7 days. The control system, managed through an Arduino processing system, alongside real-time monitoring of temperature and humidity levels, was seamlessly displayed on a computer screen. The lighting regimen within the growth chamber consisted of a 12 h photoperiod, with daily watering routines to sustain optimal plant growth and development.

RNA isolation, cDNA transcription and relative quantitative expression

Seedlings of the rice at 7 days was collected from an AAC and immediately dip to the liquid nitrogen. The RNA was extracted by ground the sample using the sterilized pestle and mortar and then isolated for the RNA according to the manufacture's instruction of Plant RNasey kit (Qiagen). Briefly, 100 mg of powdered tissue was combined with a lysis buffer mixture in a 2 mL microcentrifuge tube. The next stage involved centrifugation at 14,000 rpm, during which RNA preferentially adhered to extraction columns, while impurities were efficiently removed. The elution buffer provided in the kit was employed to elute pure RNA from the column. The concentration and purity of the extracted RNA were determined using an $OD_{260/280}$ spectrophotometer to ensure its high quality. For cDNA synthesis, 1 µg of total RNA was reverse transcribed into a 25 µL reaction using Moloney Murine Leukemia Virus Reverse Transcriptase reverse transcriptase (MMLV- reverse transcriptase) (USB) containing 1x MMLV reverse transcriptase buffer, 100 U of MMLV reverse transcriptase, 4 U of RNase Inhibitor, 0.4 µM of oligo dT18 primer, 4 µM of dNTP mixes and distilled H_2O. The reaction commenced with an initial incubation at 72 °C for 5 min, followed by cDNA extension at 42 °C for 1 h. Subsequently, the cDNA was assessed for relative quantitative expression within a 20 µL QuantiNova SYBR Green PCR reaction (Qiagen), comprising 2× QuantiNova SYBR Green PCR Master mix, 2 µL of QN ROX reference Dye, 0.4 µM of primer pairs, 20 µg of cDNA and RNase-Free water to reach the desired volume. The primer pairs were specifically designed for the up-regulated genes involved in the 2-AP biosynthetic pathway, encompassing *P5CS*, *TPI*, *GAPDH* and *Δ1-Pyrroline*, with the *18SrRNA* serving as the housekeeping gene for normalization. Detailed primer sequences can be found in **Table 1**. Gene expression analysis was conducted for relative quantitation using the QIAquant 96 5-plex real-time PCR machine (QIAgen). The real-time thermal profile consisted of an initial pre-heating step at 95 °C for 5 min, followed by an amplification phase comprising 40 cycles with the following conditions: Denaturation at 95 °C for 1 min, annealing at 60 °C for 30 s and extension at 72 °C for 30 s. Subsequently, an extension step at 72 °C for 2 min was performed. The analysis also included the evaluation of the melting curve and the determination of relative quantitative expression levels.

Table 1 Primer lists.

Primer name	Primer sequence (5′→3′)
The up-regulated genes	
P5CS-forward	CAACTGGATGTCTCGTCATCTC
P5CS-reverse	CCAACAGTCCTGCTAAACTGTC
TPI-forward	AGATCGTCACCGTCCTCAAC
TPI-reverse	TCCGCTCAGAGTGTCCCAGAATC
rice-GAPDH-forward	CATCACCACCGACTACATGAC
rice-GAPDH-reverse	ACCTTCTTGGCACCACCCTTC
Δ1-Pyrroline-forward	TGGAAAGGCTTGTGCCGCTGTTGG
Δ1-Pyrroline-reverse	ATGGAGCCTTTCTAGTGCTGATGG
The house keeping gene	
18SrRNA-forward	CTTGAGTGGAGGCACCGATG
18SrRNA-reverse	GAGCTACACCGGGTACGCAAAG

Statistical analysis

The data was summarized by calculating the mean and standard deviation, and subsequent statistical analysis was carried out using the 1-way Analysis of Variance (1-way ANOVA) test, followed by Tukey's multiple comparison post-hoc test. A significance level of *p-value* < 0.05 was adopted to determine statistical significance.

Results and discussion

Automated Agricultural Cabinet (AAC) prototype

Overall design

A specialized cabinet, constructed using robust materials and designed with the assistance of SketchUp: 3D Design Software (utilizing the free trial version), was developed to cater to the specific requirements of enhancing secondary metabolite production in plants. The cabinet was fabricated using temperature-regulating building materials denoted as "No. 8." The prototype occupied an area of 0.6 m^2 and featured an upper chamber with dimensions measuring 50 (width)×120 (depth)×60 (height) cm^3, as illustrated in **Figures 1(a)** and **1(b)**. The control system (**Figure 1(c)**), a pivotal component of the setup, consisted of a Greenhouse Control Box (referred to as "No. 6") and a Power Supply Box (designated as "No. 7"), strategically positioned at the rear of the chamber. To manage humidity levels effectively, a ventilation fan (comprising components "No. 1" and "No. 2") was integrated into the system through a side channel denoted as "No. 3" and "No. 9." The front panel of the cabinet was purposefully designed as a functional door, facilitating convenient access and creating an ergonomic workstation environment for users. The LED lighting system, a critical element of the cabinet's design, was situated atop the chamber. It featured a light range selector positioned above the plant specimens. Precise control over luminosity was achieved, targeting a lux reading of the white light (labeled as "No. 5"). This level of control was made possible through the implementation of an LED panel set marked as "No. 4." The LED panel set emitted light within the approximate wavelength range of 300 - 800 nm. To further enhance environmental control within the cabinet, an air conditioning unit (identified as "No. 10"), humidity circulation water pipes and temperature regulation mechanisms were seamlessly integrated into the lower section of the cabinet system.

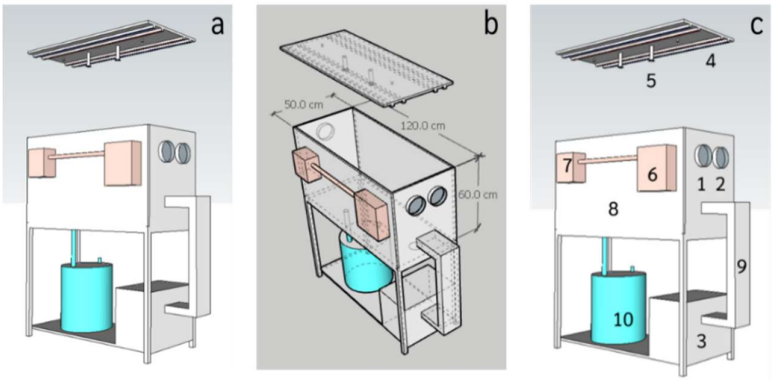

Figure 1 The overall design of the model with the automatic closing system, (a) overview of the model, (b) model size at the top view and (c) device location.

Through the design phase, a prototype of the AAC was constructed, as depicted in **Figure 2**, employing computerized control mechanisms driven by Arduino programming. An AAC is depicted in the following manner. **Figure 2(a)** illustrates a side view revealing the airflow; **Figure 2(b)** provides a side view encompassing the air circulation system, inclusive of the air conditioner; **Figure 2(c)** showcases the control system box connected to the computer through the utilization of the Arduino program; and **Figure 2(d)** offers an internal view of the chamber.

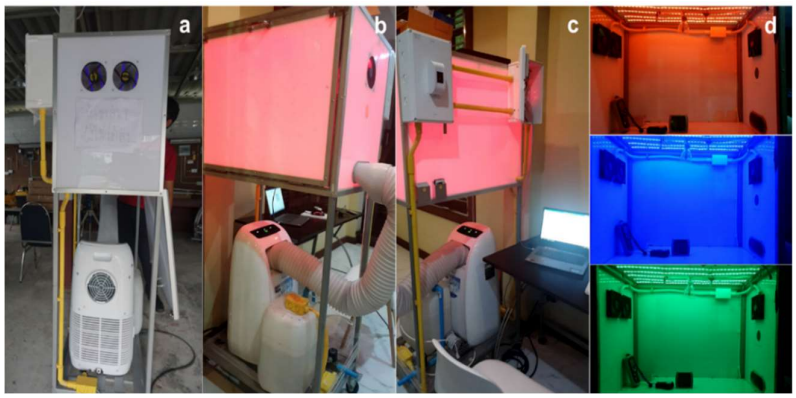

Figure 2 An autonomous agricultural cabinet (AAC) prototype, (a) show the side view with the 2 Fan, (b) the side view of the air circulation system, (c) the back view connected to the computer and (d) inside the chamber.

Air flow design

The airflow within the cabinet was facilitated by a 7,000 BTU air conditioning system positioned beneath the chamber, serving the dual purpose of temperature and humidity control. Air was channeled directly from the air conditioner to the chamber via the leaf of the chamber air conditioner. To regulate the airflow within the chamber, an air conditioning inlet (labeled as 'Air Flow,' No. 1 in **Figure 1(c)**) was incorporated, alongside an air conditioning outlet (No. 2, **Figure 1(c)**) for precise control of the internal airflow, thereby maintaining stable temperature and suitable humidity levels (No. 2, **Figure 1(c)**). To ensure the maintenance of an appropriate internal temperature range, refrigeration equipment was integrated (No. 3, **Figure 1(c)**). In instances where the humidity in the air needed adjustment, a mist nozzle (No. 5 in **Figure 1(c)**) was employed to appropriately increase humidity levels. A piping system (No. 9 in **Figure 1(c)**) was employed to regulate the temperature transfer from the cooler to the chamber. Additionally, a water tank (No. 10 in **Figure 1(c)**) was equipped with a hose to supply water to the fog nozzle.

Control system

The design of the control system is structured into 3 distinct segments, consisting of input, process and output, as illustrated in **Figure 3**. The input component of the model encompasses various elements.

Firstly, the LED RGB strip light emitting light within the wavelength range of 300 - 800 nm were employed to provide simulated lighting in lieu of natural sunlight. A fan was strategically integrated to both decrease humidity levels in the surrounding air and facilitate ventilation within the space. To augment atmospheric moisture, a mist nozzle was incorporated to increase humidity levels. The cooling component of the system was facilitated by a cooling fan (AN-PAC07LA ACONATIC 7000 BTU), serving to regulate the indoor temperature to a comfortable level. A water pump was employed to control humidity levels within the chamber by supplying water for use in conjunction with the fog nozzle. Furthermore, a solenoid valve was employed to manage the water level within the tank. To enable signal processing via the microcontroller, 3 distinct types of sensors were selected as input signals. The first, an Ambient Light Sensor (BH1750FVI), was employed to gauge the intensity of light emitted by the LED RGB strip light. The second, a Temperature and Humidity Sensor (SHT20), served to determine the temperature and humidity levels of the air, with a measurement range spanning from −40 to 125 °C for temperature and 0 - 100 % for relative humidity. The third sensor, a Water Level Sensor (HY-FRF04), was utilized to ascertain the water level within the storage tank, with a detection range spanning from 2 to 450 cm.

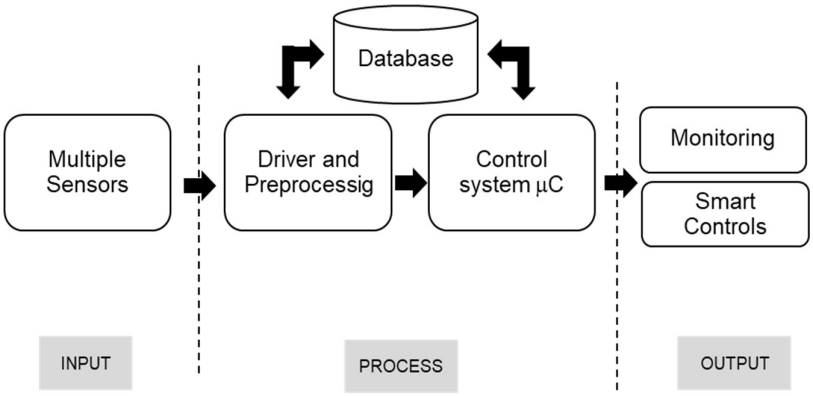

Figure 3 Control system of a automate cabinet.

The designed model utilizes an Arduino UNO board for processing, which is an open-source programmable device. Its adaptability is notably enhanced by an extensive array of libraries, making it particularly well-suited for innovative developers. Schematics serve as the foundational basis for designing the circuit connections, as depicted in **Figure 4**.

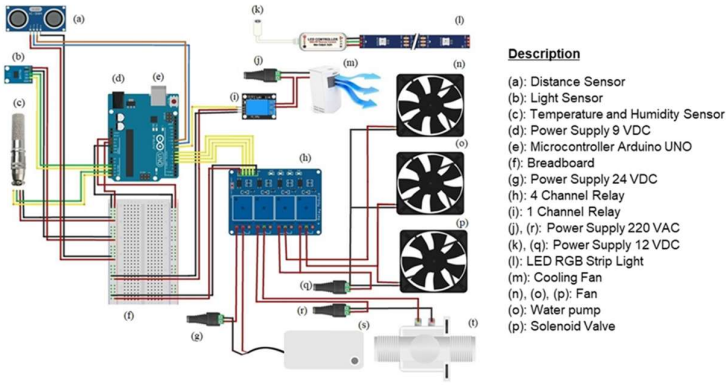

Figure 4 Control system diagram or wiring diagram.

The sensor transmits an input signal to the Arduino board, which subsequently processes the programmed sequence, as depicted in the flowchart illustrated in **Figure 5**, thereby regulating the output devices to establish and maintain the environmental conditions within the model house. The operational procedure commenced with the activation of the computer, where upon libraries and variables were configured to facilitate program execution. Subsequently, utilizing real-time data from the sensors, including temperature, humidity, light intensity and water tank water level distance, the LED lighting was

adjusted as deemed necessary. The control process initiated by activating Fan1 (Air Intake) and Fan2 (Air Out) to ensure a continuous flow of air within the enclosure. Further steps involved assessing the environmental conditions; if the temperature exceeded the predetermined threshold, the cooling fan was engaged to lower the temperature. Conversely, if the requirements were met, the cooling fan was deactivated. Similarly, humidity levels were closely monitored; when humidity exceeded the desired range, Fan3 was activated, and the misting pump was deactivated to reduce humidity. Conversely, if humidity levels were too low, Fan3 was deactivated, and the misting pump was activated. The process also included checking the water level in the tank; if the water level exceeded the intended value, the solenoid valve was opened to maintain the required water level. Conversely, if the condition was not met, the solenoid valve was closed. Real-time values of variables, such as temperature, humidity, light intensity and water tank water level distance, were continually displayed on the computer screen. The entire process operated in a loop to ensure the program's continuous execution (**Figure 5**).

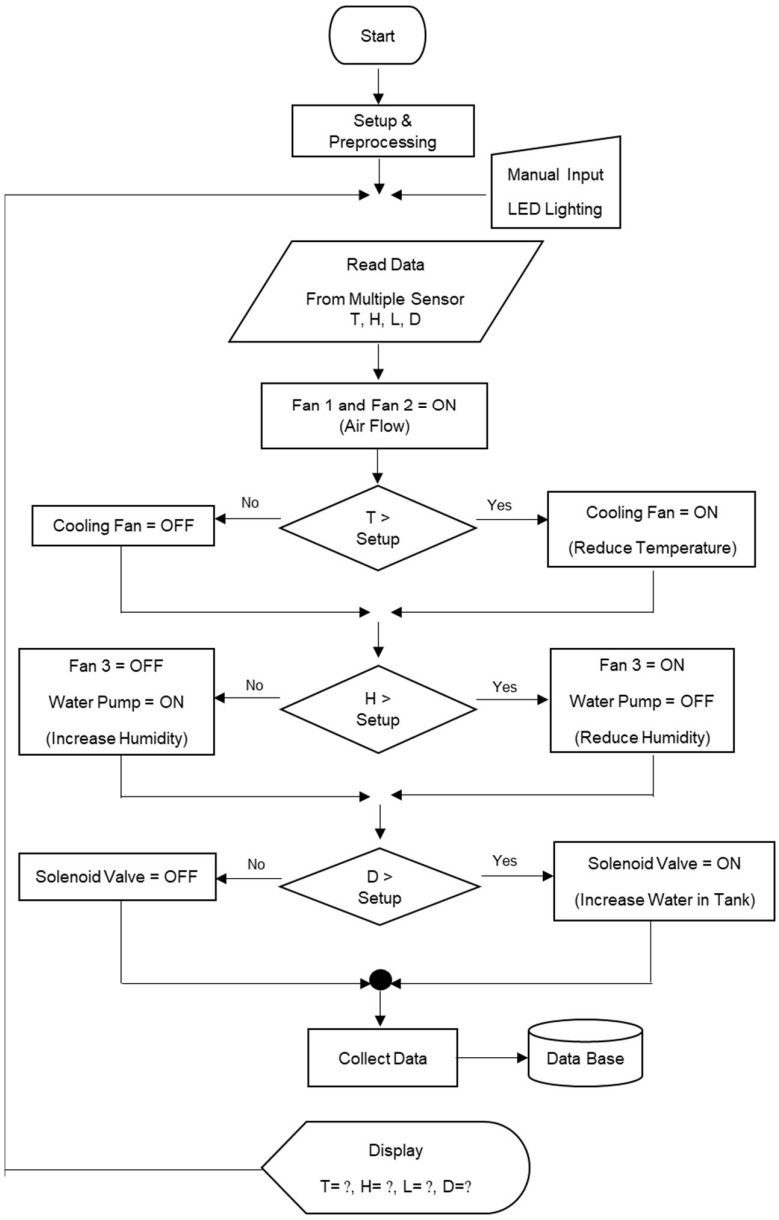

Figure 5 The control algorytm system flow chart; T = Temperature (°C), H = Humidity (% RH), L = Lighting (lux) and D = Distance (cm).

The light intensity validation with the actual temperature and relative humidity control

Validation of the AAC prototype involved rigorous assessment of temperature, humidity and light intensity, both inter-day and intra-day (triplicate measurements). Red and blue wavelengths were specifically chosen for validation, given their relevance to chloroplast absorption across various plant species. White light was utilized as a control variable. Regarding light intensity validation, the red wavelength intensity averaged 9.25 ± 0.602 μmol·m^{-2}·s^{-1}, while the blue light intensity measured an average of 11.47 ± 0.055 μmol·m^{-2}·s^{-1}. Notably, the white light intensity, measuring approximately 0.91 ± 0.027 μmol·m^{-2}·s^{-1} (**Figure 6(a)**). The validation process was conducted at a set temperature of 25 °C with a relative humidity of 75 %. The average temperature recorded inside the chamber was 25.14 ± 0.12 °C over the course of inter-day validation conducted over 3 days while the ambient temperature itself averaged 34.55 ± 0.06 °C (**Figure 6(b)**). Relative humidity levels within the cabinet were found to be approximately 75.43 ± 0.83 %, while ambient relative humidity averaged 55.64 ± 0.65 % during the cabinet validation process (**Figure 6(c)**). The advantage of using the ACC is the ease of operation, little downtime and dependability of PC systems equipped with Arduino UNO board processors in a variety of scenarios. Furthermore, ACC can be applied to a wide range of plant species with the goal of producing certain crops.

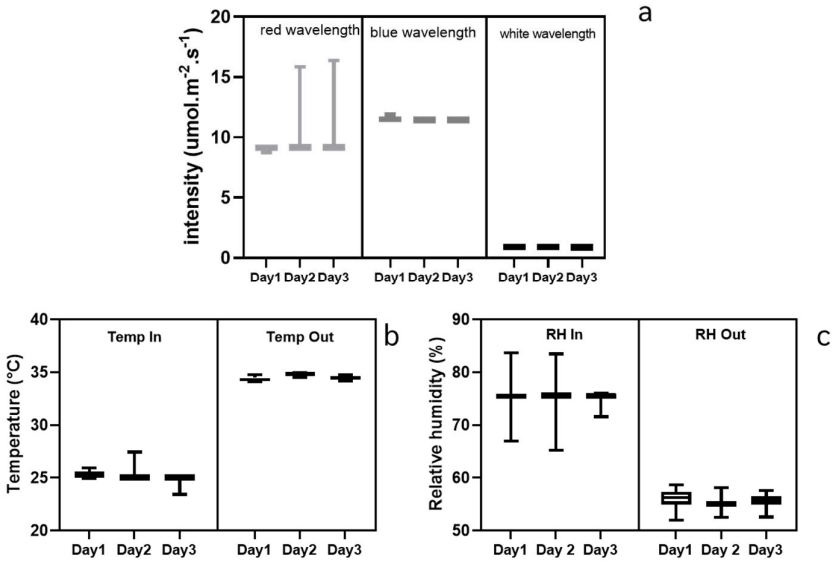

Figure 6 The validation of the red, blue and white wavelength at 25 °C and 75 % relative humidity, (a) the validation of the inter-day and intraday of the red wavelength, (b) the validation of the temperature inside the chamber and the ambient and (c) the validation of the relative humidity between the inner and outer the chamber.

The predecessor of the AAC demonstrated a consistent efficiency over a minimum period of 7 days, with no concerns regarding temperature, relative humidity (RH) or light intensity management. This apparatus, when activated, occupies a compact area of 0.6 m^2 and features wheels for enhanced mobility, ensuring ease of operation and compatibility with any computer running the Arduino program. Inside the chamber, an LED RGB strip light system is integrated, capable of modulating the illumination spectrum and adjustable to induce specific phytochemical responses in plants. During instrument validation, it was observed that light intensity readings remained consistent and accurate at a temperature of 25 °C and a relative humidity of 75 %, even when external environmental conditions differed. To induce the up-regulation of genes involved in the 2-AP biosynthesis pathway, we chose blue and red-light wavelengths over white light due to their crucial roles in supporting plant growth and development. These light properties are well-recognized for their capacity to regulate the accumulation of bioactive compounds in medicinal plants. This wavelength mechanism has the potential to enhance the production of specific bioactive compounds, such as flavonoids, terpenoids and alkaloids, unique to certain plant species, by acting as elicitors [7]. In our study, we observed an increase in gene expression along the 2-AP biosynthetic pathway in 7-day-old rice seedlings when cultivated under the red chamber, which had significantly higher light intensity compared to both the blue and white chambers. The quality of light has a significant impact on the growth and early development of both Arabidopsis and tomato plants. In Arabidopsis, exposure to

monochromatic red light led to enhanced shoot growth at the expense of root development and delayed flowering, while monochromatic blue light resulted in reduced shoot growth and early flowering. In tomato plants, monochromatic red light promoted increased shoot growth and development alongside a decrease in leaf surface area, whereas monochromatic blue light resulted in reduced shoot growth in vegetative plants but increased shoot growth in flowering plants [20]. The blue light exerts an up-regulating effect on various cytochrome P450 enzymes, including CYP85A1, consequently facilitating the biosynthesis of castasterone, a biologically active brassinosteroid in rice. In light of these discoveries, it is posited that the bending and unrolling of rice leaves, induced by blue light, can be attributed to the heightened production of endogenous castasterone [21]. An elevation in the ratio of blue to red light resulted in an augmentation of transcript levels for the gene encoding the gibberellic acid (GA) inactivation enzyme, specifically SlGA2ox7 [22]. In contrast, when the gene expression levels of key enzymes in pakchoi plants (*Brassica campestris* L. var. Suzhouqing) were compared under red, blue and white light conditions, it was evident that the expression levels were notably elevated under the influence of blue light [23]. Nonetheless, the absorption of this light quality relies on the presence of pigments within the plant [24].

In terms of light intensity, the AAC prototype provided a red wavelength intensity that was less than the blue wavelength, suggesting it could serve as an effective elicitor for plants to shift their pathway towards producing phytochemicals instead of following the primary pathway. This variance in light intensity may have consequential implications for the production of secondary metabolites, as evidenced by a substantial increase in total free amino acid content in response to heightened light intensity and a significant reduction observed in pink light conditions as compared to the standard white light intensity [25]. Light intensity influences secondary metabolite formation; light intensities of 12 and 6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ influence anthocyanin accumulation to variable degrees in *Ocimum basilicum* (Sweet Genovese) [9]. Light intensity may be altered by the buildup of carotenoid in *Brassia oleravea* at 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In the context of phytochemical groups, light intensity levels ranged from 40 to 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in *Cichorium intybus*, *Dracocephalum forrestii* and *Ocimum basilicum*, leading to increased polyphenol accumulation [26-28]. To enhance terpenoid content in *Dysphania ambrosioides*, *Perovskia atriplicifolia* and *Aquilaria agallocha*, the optimal light intensity fell within the range of 40 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ [29-31]. Meanwhile, for alkaloid production in *Camptotheca acuminata*, *Catharanthus roseus* and *Psychotria leiocarpa*, the appropriate light intensity ranged from 120 to 1,200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ [32-34]. In our research, the measured light intensities were 9.25 for red light, 11.47 for blue light and 0.91 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Both red and blue wavelengths exhibited the potential to act as elicitors in the phytochemical pathway of Hom Bon native rice. Nevertheless, the ACC prototype devised for inducing gene expression in this study has endured for a duration of 7 days, maintaining consistent levels of light intensity, temperature and humidity. However, for a more comprehensive understanding, extended investigations over a longer duration may be imperative. The 3 wavelengths were assessed for a singular cabinet size. To facilitate the mass production induction of the secondary metabolite, preparations should be made to examine the precision of the light elicitor.

The up-regulated gene expression in 2-acetyl-1-pyrroline biosynthetic pathway

The effects of the up-regulated genes involved the 2-cetyl-1-pyrroline biosynthetic pathway

In an autonomous agricultural cabinet where seedlings were cultivated, the relative fold expression of the up-regulated gene in the 2-AP biosynthetic pathway exhibited a significant increase, particularly in response to both red and blue wavelengths when compared to gene expression levels observed under white lighting conditions. The gene expression analysis encompassed examination of up-regulated genes within the 2-AP biosynthetic pathway, including *GP5S*, *Δ1-Pyrroline*, *TPI* and *rice-GAPDH*. Relative expression levels, in relation to the housekeeping gene *18SrRNA*, revealed that the white light condition exhibited the lowest relative expression when compared to the red and blue wavelengths (**Figure 7(a)**). The relative expression levels of up-regulated genes involved in the biosynthetic pathway of 2-AP under red and blue wavelengths were significantly different compared to those in the fluorescent chamber. According to the results of the 1-way analysis of variance (ANOVA) test, it was observed that the up-regulated genes exhibited statistically significant differences, with a p-value of 0.0032 [$F(D_{Fn},D_{Fd}) = F(4,10)$, $Df = 4$]. Subsequent analysis using the Tukey multiple comparison test revealed statistically significant differences between *P5CS* and *rice-GADPH* (p-value = 0.0462), *rice-GADPH* and *Δ1-Pyrroline* (p-value = 0.0056), as well as *18SrRNA* and *rice-GADPH* (p-value = 0.0080) (**Figure 7(b)**). These findings were derived under the assumption of a normal data distribution, as depicted in **Figures 7(c) - 7(e)**. The expression levels of up-regulated genes, namely *rice-GAPDH*, *P5CS*, *TPI* and *Δ1-Pyrroline*, exhibited statistically significant differences (p-value < 0.05) when compared to the housekeeping gene (**Figure 7(a)**) for red, blue and white wavelength. The expression of the *TPI* gene, which did not show significant differences across all light wavelengths. However, it is noteworthy that the *P5CS* and *TPI* genes did not display statistically significant

differences between the red and blue wavelengths. In contrast, both *rice-GAPDH* and *Δ1-Pyrroline* exhibited statistically significant differences (p-value < 0.05) in their expression levels.

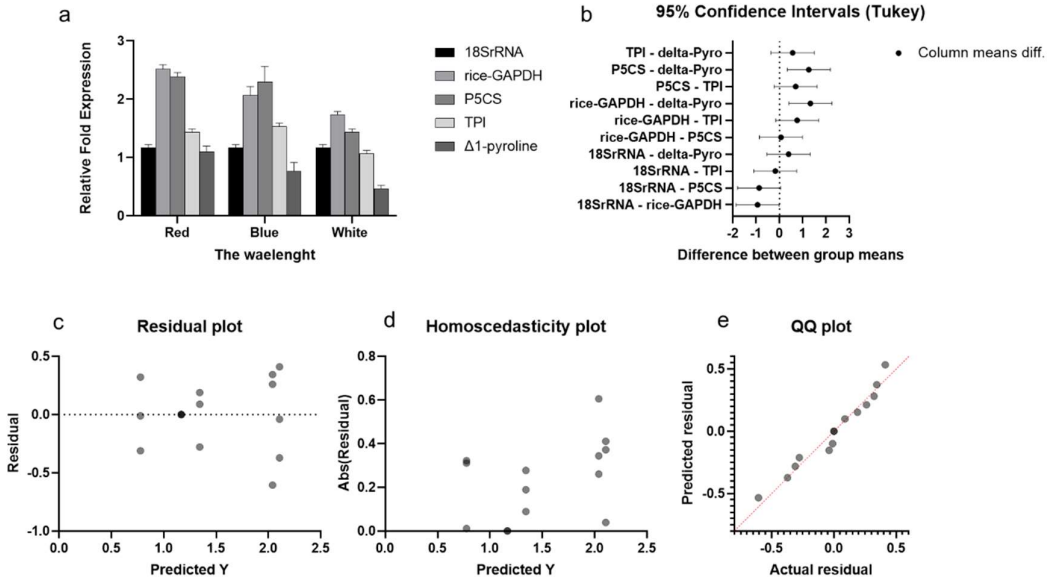

Figure 7 The relative expression of the up-regulated genes involved the 2-Acetyl-1-pyrroline biosynthetic pathway.

The investigation of the up-regulated gene expression in this study, red and blue light activated 2 related genes, resulting in the production of higher relative expression fold fragrance components, which might be a viable technique for enhancing natural fragrance for rice seedlings prior to planting. We did not add any additional parameters to AAC other than lighting intensity set in the room, however, aromatic substance production in rice was depended on other parameters, such as the 2-AP content, varied depending on the tillage conditions over both seasons. Furthermore, differential expression analysis under stress revealed divergent patterns, with 2 up-regulated genes, *P5CS* and *Δ1-pyrroline*, exhibiting differing responses [35]. Increased *GABA* and *Δ1-pyrroline* gene concentrations may aid in nitrogen usage, promoting the development of fragrant rice during the biosynthesis process [36,37]. Augmenting zinc (Zn) application in shaded conditions can intensify the biosynthesis of 2AP, photosynthesis (Pn), proline, pyrroline-5-carboxylate (P5C), gamma-aminobutyric acid (GABA), pyruvate dehydrogenase (PDH), pyrroline-5-carboxylate synthetase (P5CS), superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), while simultaneously reducing malondialdehyde (MDA) levels. However, it should be highlighted that under these conditions, the potential to increase agricultural productivity remains restricted [38]. As a consequence, the light tested in this study may play the important factor to activate an up-regulated gene in the 2-AP biosynthesis pathway in the "Hom Bon" native rice variety. The light quality treatments exhibited a notable impact by diminishing the 2AP content in mature grains [39] while numerous experiments have explored the impact of specific light ranges on the production of secondary metabolites, this study adopts a novel approach. It focuses on utilizing light to induce gene expressions associated with the biosynthesis of fragrance compounds, specifically within the 2-AP pathway, during the early stages of rice growth, specifically in the seedling phase, followed by natural sowing. This novel approach holds the potential to enhance the aromatic qualities of the rice, thereby imparting it with a more distinctive and unique scent. The presence of a distinctive and unique aroma in local rice varieties signifies their potential desirability among consumers. Such desirability has the capacity to elevate the intrinsic value of rice, generating economic worth and, significantly, augmenting the income of farmers engaged in the cultivation and development of these rice varieties.

The primary objective of this investigation was to design an Artificial Climate Chamber (ACC) with the capability to generate light tailored to plant-specific requirements and induce the expression of genes associated with 2-Acetyl-1-pyrroline (2AP) in rice seedlings. The up-regulation of a specific gene within this pathway was scrutinized utilizing the precursor, Δ1Pyrroline-5 carboxylate synthetase (P5CS) [40]. Remarkably, it was determined that 7-day-old seedling rice cultivated within the ACC exhibited the highest

gene expression levels when subjected to red wavelength illumination. In the conclusive phase before the synthesis of 2AP, the gene *Δ1-Pyrroline* demonstrated superior performance under red wavelength conditions compared to the control group [40]. However, despite these intriguing findings, the olfactory compound 2AP, residing in the seed or husk and potentially discernible when volatile during boiling, remained undetectable throughout the course of this investigation. Further exploration of this phenomenon may necessitate an extended cultivation period for the 7-day-old seedling rice until seed harvesting, in order to facilitate a more comprehensive investigation.

Conclusions

The portable and light-controlled nature of the Automated Agricultural Cabinet (AAC) provides a distinct advantage, enabling the initiation of gene expression associated with the 2-acetyl-1-pyrroline (2-AP) biosynthesis pathway in indigenous rice seedlings before planting. The application of red and blue wavelengths has demonstrated a significant accumulation of up-regulated gene expression, particularly in the precursor gene *P5CS* and the final step gene, *Δ1-Pyrroline*, before 2-AP during the seedling stage of 'Hom Bon' rice cultivation within the AAC. The significance of 2-AP in rice cultivation extends beyond its chemical composition, encompassing vital roles in enhancing aroma, flavor, cultural significance, culinary appeal, consumer preference, economic value and global agricultural trade. The presence of 2-AP in fragrant rice varieties contributes significantly to their uniqueness and popularity, making them invaluable and esteemed components of diverse global cuisines and cultural traditions.

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