

## Physiological, Morphological and Biochemical Responses of Strawberry (*Fragaria × ananassa* D.) Against the Environmental Conditions of Agra

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### ABSTRACT

The changing global food scene requires moving various foods worldwide, stressing resources and causing environmental issues. In this context, strawberries have become popular in India, leading to significant imports to satisfy demand. Yet, it's crucial to start growing exotic fruits locally to ease resource strain. Adapting crops to new environmental conditions is crucial, especially in semi-arid climates with hot, dry summers and mild winters. This study investigates the physiological, morphological and biochemical aspects of growing strawberries in Agra's climate, using a holistic organic farming approach. Employing Infra-red Gas Analysis, the research observes an average photosynthetic assimilation rate of  $11.5433 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Light curve analysis indicates an optimal Photosynthetic Photon Flux Density near  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . An average  $138.4 \mu\text{mol mol}^{-1}$  disparity between  $C_a$  and  $C_i$  is noted. Plant height exhibits

consistent growth, starting at 7.74 cm on day 0 and reaching 23.56 cm on day 60. The growth trajectory accelerates initially, signalling maturation later on. Leaf area expands from  $17.03 \text{ cm}^2$  on day 0 to  $88.93 \text{ cm}^2$  on day 55, crucial for photosynthesis and nutrient assimilation with 13.8 compound leaves, each consisting of three leaflets. Plant height correlates positively with leaf area, petiole length, diameter. The fruit contains 59.90 mg/100 g of vitamin C. The study found an average NDVI value of 73.11 and an average soil-plant analysis development value of 0.58. This research offers insights into improving strawberry cultivation in shifting climates and semi-arid conditions, aiding sustainable local production and decreasing reliance on imports.

**Keywords** Strawberry, IRGA, Physiological response, NDVI, SPAD.

### INTRODUCTION

Globalization fosters interconnections and cultural exchange among nations, introducing diverse cuisines and altering dietary patterns. Urbanization amplifies this shift by offering urban dwellers varied food options due to expanded culinary availability (Connell *et al.* 2020, Putra *et al.* 2020, Colozza and Avendano 2020). Sustainability gains priority as people embrace local, organic, and eco-friendly diets like plant-based alternatives (Susilo *et al.* 2023, Schiano and Drake *et al.* 2021). Heightened health consciousness, evolving

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preferences, accessibility, and cultural influences surge strawberry demand in India (Shetty *et al.* 2021). However, import-related emissions, waste, and distance can harm the environment. Growing organic farming awareness elevates the appeal of organic fruits and veggies, with the global market exceeding \$100 billion by 2019 (Ma *et al.* 2021, Esteves *et al.* 2021). This increase originates from worries about pesticides and the biodiversity-focused methods of organic farming that inherently improve the well-being of plants.

Understanding the intricate physiological and morphological adaptations of crops in response to climate change assumes paramount significance, particularly in the context of identifying well-suited exotic crops capable of thriving within local ecosystems and attaining substantial economic viability. With the ever-shifting climate patterns impacting conventional agricultural zones, the imperative to cultivate robust plant varieties that can adeptly endure evolving environmental dynamics escalates.

The present investigation is meticulously directed towards an in-depth exploration of the physiological and morphological reactions exhibited by strawberries. This encompasses a comprehensive analysis encompassing metrics such as stem length, stem width, leaf count, and the inherent attributes of fruits, gauged with precision via Vernier calliper measurements. The physiological dataset is meticulously acquired via infrared gas analyzer measurements that encompass parameters including photosynthetic assimilation (A), stomatal conductance, water use efficiency (WUE), and transpiration rate (E). Moreover, a meticulous biochemical scrutiny of the fruits is undertaken to discern their intrinsic characteristics. The assessments included measuring fruit brix, determining chlorophyll content, acquiring SPAD readings, and calculating NDVI. These analyses offered insights into fruit ripeness, plant health, chlorophyll concentration, and vegetative growth. Together, they provided a holistic understanding of the subject. At the core of this study lies the principal goal of meticulously documenting the morphological, biochemical, and physiological retorts of strawberries, thereby culminating in the formulation of tailored cultivation methodologies adeptly suited for the unique semi-arid

climatic conditions. This endeavor holds particular significance as strawberries represent a potentially pioneering crop within the regions of Agra and other global locales. Furthermore, the research seeks to unravel the intricate physiological disparities between strawberries cultivated in their native habitats and those subjected to newer environments, thus paving the way for prospective commercial applications.

## MATERIALS AND METHODS

### Cultivation site & climate

The cultivation was done in 2019-20, 2020-21 at Biodiversity Park, (27°14'06" N, 78°01'28" E), located 169 meters above sea level. It is an experimental site of the Department of Botany, Dayalbagh Educational Institute, Dayalbagh, Agra. The average annual rainfall is 548 mm in Agra (Guhathakurta *et al.* 2020). According to world meteorological organization during summer days maximum temperature daily is found to be between 28°C to 40°C and winter minimum temperature daily means 7°C to 22°C. A typical monsoon season rainfall ranges from 600 mm to 650 mm from June to September (WMO 2022).

### Soil analysis

Multiple soil samples were taken from the cultivation site to make composite soil samples. The soil sample was collected from a depth of 12-16 cm in zigzag fashion. The composite sample is immediately brought to the laboratory and in open air dried. After drying the sample gravels, large biomass debris is removed and gently grinded by mortar and pestle. The soil sample was sieved by 2.0 mm sieve, and further analysis was done by the following methods. Table 1 summarizes the results of physical and parameter measurements.

### Cultivation

In the first week of November, approximately 6 tonnes of manure were evenly distributed across 0.3 acres of cultivated land using a tractor-driven cultivator, which tilled the soil to a depth of 6 inches. Raised beds were then created using a bed shaper, dividing the cultivation land into three parts. Mother plants

**Table 1.** Physico-chemical analysis: Parameters and methods.

Properties	Method	Unit	Reference
Texture	Soil textural triangle (FAO)	-	FAO (2018)
Bulk density	Bulk density (FAO)	g/cm <sup>3</sup>	FAO, (2020)
pH	Soil-H <sub>2</sub> O system (FAO)	-	FAO, (2021a)
EC	Conductivity meter (FAO)	μS/cm	FAO, (2021d)
Organic carbon	Oxidation method	%	Walkey and Black (1934)
Total nitrogen	Kjeldahl method	%	FAO (2021b)
Phosphorus	Mehlich I method	kg/ha	FAO (2021c)
Potassium	Flame photometric method	kg/ha	Chavan and Pansare (2018)

of *Fragaria* × *ananassa*, locally popular as Chandler strawberries, were procured from the market in the Indian state of Himachal Pradesh. Plantation was carried out at a depth of 2-3 inches in the second week of November, with the raised bed distance and height shown in Fig. 1. After plantation, the plots were irrigated using surface irrigation. After 30 days of plantation, a foliar spray of Jivamrut (a traditional biofertilizer) at a concentration of 3% was applied to increase the biological efficiency of the plants (Patel *et al.* 2021). A seaweed-based product named Sagarika, manufactured by Indian Farmers Fertilizer Cooperative Limited (IFFCO), was sprayed at a rate of 3 ml per liter of water to enhance tillering and

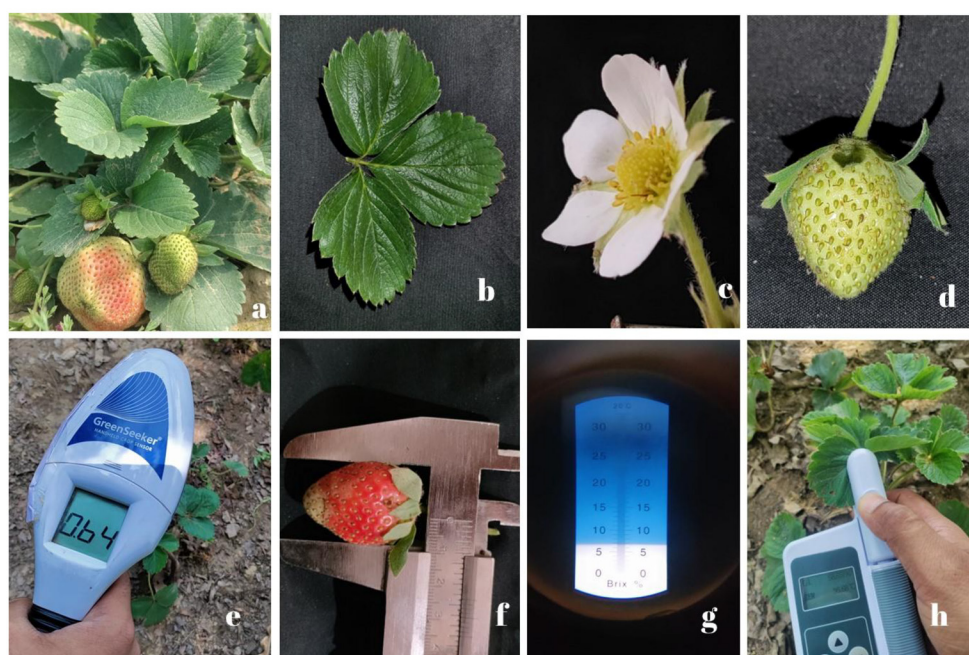
plant growth (Kumar *et al.* 2021) after 23 days of planting the mother plants. The same product and quantity were also sprayed during flowering of the plants. Sewage-treated water was used as a source for irrigation, and surface irrigation was carried out. Irrigation was done every 10–12 days to account for the sandy soil. In total, 8 irrigations were carried out until the final harvest of the crop.

### Morphology

Strawberry plants were selected with similar appearance to ensure consistency. A longitudinal study was conducted to monitor morphology of the plant over a



**Fig. 1.** This figure illustrates field preparation of strawberry cultivation: (a) A view of the cultivation field, (b) Raised bed setup, (c) Optimal distance between rows, height of bunds and (d) Strawberry plants placed at a planting depth of 2-3 inches.



**Fig. 2.** Exploring strawberry growth: from plant to fruit a. Mature strawberry plant with fruit, b. Compound leaf with three leaflets c. Intricate flower with reproductive organs, d. Young strawberry fruit, e. Monitoring NDVI with green sensor, f. Measuring fruit diameter with caliper, g. Calculating fruit sweetness with refractometers, h. Assessing chlorophyll content with SPAD meter.

period of 60 days data at the interval of 5 days. Plant height measured from the base to the highest point of foliage. Petiole measured from the main stem to the leaf blade. The petiole diameter is measured using vernier calliper (Tang *et al.* 2019). The leaf area is measure by using leaf area meter (LI-3000C LI-COR Inc, Lincoln, NE, USA) (Li *et al.* 2021). The average values of each day provided along with the standard deviation to represent validity within the data, phenological observations, which involve observing events such as bud break, leaf emergence, flowering, fruiting, and senescence, are also conducted through field observations and also shown in Fig. 2.

### Physiology

The Infra-red Gas Analyzer (IRGA) LI 6800 portable photosynthesis system, developed by LI-COR, Inc-NE, USA, is used to study physiological responses, including the assimilation rate ( $A$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), Intercellular  $\text{CO}_2$  ( $g$ ,  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) and Transpiration rate ( $E$   $\text{mol m}^{-2} \text{s}^{-1}$ ). These parameters were measured at an ambient  $\text{CO}_2$  concentration of approximately

$400 \mu\text{mol}$  and total amount of light energy received by plant ( $Q_{in}$ ) of  $1600 \mu\text{mol m}^{-2} \text{s}^{-1}$ , with respect to the soil and climate conditions of the Agra region. Additionally, consecutively two light response curves were also studied from  $Q_{in}$  zero to  $3000 \mu\text{mol m}^{-2} \text{s}^{-1}$  to determine the optimum light intensity for plant growth and development.

### Biochemical analysis

Total chlorophyll content of leaves is measured using SPAD meter 502. The pH of the fruit juice is measured by using Systronic T-361. The RHB32ATC refractometer, manufactured by Yhequipment Co, Ltd, is utilized for studying the Brix % of strawberry pulp. This refractometer is capable of measuring Brix % within the range of 0 to 32% and offers a measurement accuracy of 0.2%. It is specifically designed to operate effectively within a temperature range of 10 to 30 degrees Celsius. The ascorbic acid test was performed using Dichlorophenolindophenol (DCPIP) titration to estimate the concentration of ascorbic acid (Tinceva 2019).



## RESULTS

### Cultivation site and soil

In Agra, the months of November through February are ideal for strawberry production due to the variation of temperatures. Luckily, it rarely gets colder than 4°C at night in this area. Because frosting is uncommon in the area, the strawberries don't need to be covered or protected with mulch. The light intensity reaches over 10,000 lux on bright, sunny days, which is perfect for strawberry development. However, due to cloudy and foggy weather, visibility is frequently restricted to about 5000 lux throughout the winter.

The soil composition in the cultivation area consists of 89.3% sand, 9.41% silt, and 1.29% organic matter, clay, and other unidentified particles texture and other parameters in detailed mentioned in Table 2. Cultivation field, the pH level is between 7.93 and 8.10. The bulk density of the soil, at 1.53

g/cm<sup>3</sup>, is suitable for cultivation. The low electrical conductivity of the soil, measuring 86.32 µS/cm, is due to the low levels of macronutrients present. The soil has low levels of nitrogen (0.17%), phosphorus (12.75 kg/ha<sup>-1</sup>), and potassium (132 kg/ha<sup>-1</sup>).

### Morphological responses

The morphological characteristics of strawberry plants were assessed over a period of 60 days, including plant height, petiole length, petiole diameter, and leaf area. The plant height measured from root tip to the top, exhibited a steady increase throughout the experimental period, starting from 7.14±0.54 cm on day 0 and reaching 23.56±1.30 cm on day 60. The growth rate accelerated during the initial phase and gradually slowed down, indicating the plant's maturation, detailed data is given in Table 3.

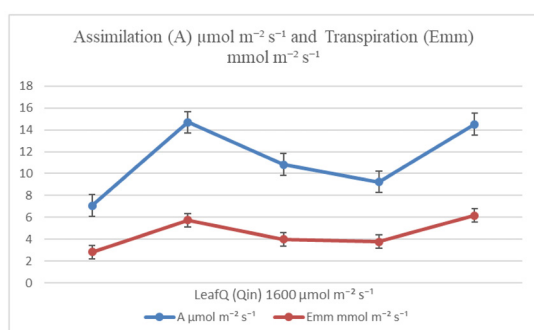
Leaf area showed a consistent increase, starting from 17.03 cm<sup>2</sup> on day 0 and reaching 88.93 cm<sup>2</sup> on day 55. The expanding leaf area reflects the growth and development of the plant's foliage, which is vital for photosynthesis and nutrient assimilation. The correlation analysis showed that plant height was highly and significantly positively correlated with both leaf area ( $r = 0.99$ ,  $p < 0.01$ ) and petiole length ( $r = 0.97$ ,  $p < 0.01$ ) at a significance level of 0.01. Similar to plant height, petiole length also increased progressively over time. The initial petiole length was 4.21 cm on day 0 and reached 16.14 cm on day 60. This indicates elongation and development of

**Table 2.** Results of analysis of soil.

Properties	Result	Unit
Texture	Loamy sand	-
Bulk density	1.53	g/cm <sup>3</sup>
pH	7.54	-
EC	86.32	µS/cm
Organic carbon	0.42	%
Total nitrogen	0.17	%
Phosphorus	12.75	kg/ha <sup>-1</sup>
Potassium	132.5	kg/ha <sup>-1</sup>

**Table 3.** Growth, physiological and biochemical parameters of strawberry plants over time.

Day	Plant height (cm)	Petiole length (cm)	Petiole diameter (mm)	Leaf area (cm <sup>2</sup> )	SPAD	NDVI
0	7.74±0.54	4.21±0.61	1.1±0.08	17.03±1.15	52.89±1.36	0.42±0.01
5	8.27±0.34	4.94±0.76	1.1±0.07	18.60±1.02	52.44±1.4	0.43±0.01
10	9.04±0.36	5.7±0.96	1.2±0.06	22.04±2.76	56.86±0.78	0.45±0.02
15	10.54±1.5	6.32±1.14	1.2±0.02	22.53±1.82	61.91±0.88	0.47±0.01
20	13.27±1.9	7.64±1.10	1.2±0.07	23.35±2.31	66.77±0.87	0.5±0.01
25	16.02±1.51	8.12±0.91	1.3±0.02	32.97±1.79	68.93±1.61	0.51±0.02
30	17.70±1.5	9.16±0.78	1.4±0.05	45.30±2.36	79.79±0.98	0.6±0.01
35	19.34±1.49	9.97±1.21	1.4±0.04	57.14±3.75	86.11±0.96	0.66±0.01
40	19.77±1.50	10.37±0.82	1.5±0.07	63.76±3.94	87.84±0.96	0.71±0.01
45	21±1.31	12.47±1.40	1.6±0.04	74.46±2.42	88.81±0.72	0.72±0.01
50	21.71±2.14	14.89±1.10	1.6±0.06	81.22±4.70	88.2±0.57	0.76±0.02
55	22.14±1.71	15.36±1.11	1.6±0.04	88.93±2.41	88.37±0.97	0.8±0.01
60	23.56±1.30	16.14±0.96	1.6±0.07	90.37±2.76	85.08±2.33	0.8±0.01

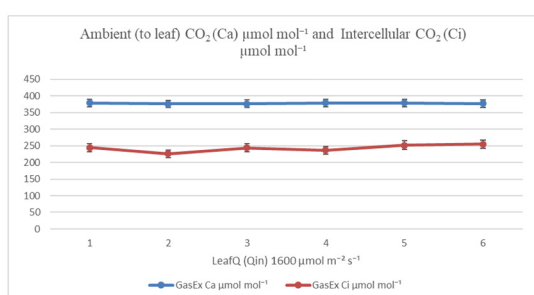


**Fig. 3.** Correlation between assimilation rate (A) and transpiration Rate (E). This graph showcases the relationship between assimilation rate (A) and transpiration rate (E), both measured in  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

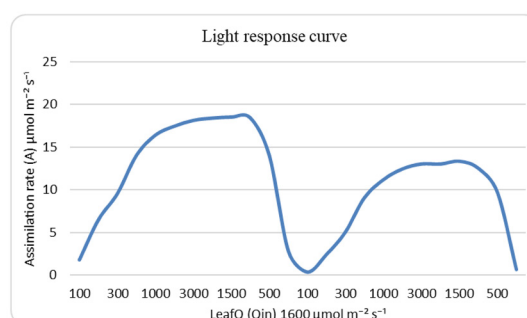
the petioles, supporting the growth and expansion of the strawberry plant. The petiole diameter remained relatively stable, ranging from 1.1 mm to 1.6 mm throughout the experiment. This suggests that the thickness of the petioles did not undergo significant changes during the observed period.

### Physiological responses

The gaseous exchange measurements were conducted under specific conditions in the Agra region, with an ambient  $\text{CO}_2$  concentration of approximately  $400 \mu\text{mol}$  and a total amount of light energy received by the plant (Qin) of  $1600 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The assimilation rate (A), intercellular  $\text{CO}_2$  concentration (Ci), and transpiration rate (E) were recorded (Fig. 3). The A represents the rate at which plants convert  $\text{CO}_2$  into organic compounds through photosynthesis.



**Fig. 4.** This figure presents an investigation into the correlation between  $\text{CO}_2$  concentration on the leaf surface/leaf chamber (Ca) measured in  $\mu\text{mol mol}^{-1}$  and intercellular  $\text{CO}_2$  concentration (Ci) also in  $\mu\text{mol mol}^{-1}$ .



**Fig. 5.** Response curves revealing assimilation rate and photoinhibition. The curves offer a comprehensive perspective on the interplay between assimilation rate and light intensity, aiding in the evaluation of photoinhibition as depicted by the second curve. On the x-axis, assimilation rate is plotted ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), while the y-axis represents light intensity ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), unraveling the intricate relationship between these critical factors.

The difference between the  $\text{CO}_2$  on leaf surface/leaf chamber (Ca) and intercellular  $\text{CO}_2$  (Ci) shows the photosynthetic ability of the plant. The Ca  $377.65 \pm 1.4 \mu\text{mol mol}^{-1}$  and Ci  $242.83 \pm 10.63 \mu\text{mol mol}^{-1}$ .

The light response curve was constructed by measuring the photosynthetic rate (A) at different light intensities (Qin). The data collected is shown in Fig. 4. The light response curve provides insights into the plant's photosynthetic performance at different light intensities. As the light intensity (Qin) increases, we observed a corresponding increase in the photosynthetic rate (A) of the plant (Fig. 5).

At low light intensities (e.g.,  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), the photosynthetic rate is relatively low, with values around  $1.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ . As the light intensity gradually increases to 200 and  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ , the photosynthetic rate also rises, reaching  $6.51 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $9.59 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively. However, beyond a certain threshold (around  $300\text{--}500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), further increases in light intensity have a diminishing effect on the photosynthetic rate.

### Biochemical analysis

SPAD readings collected over 0 to 60 days, with a 5-day interval, offer insights into chlorophyll content dynamics and plant health. Values showed steady

increase, from an initial  $52.89 \pm 1.36$  at day 0 to  $85.08 \pm 2.33$  by day 60. NDVI was monitored over a span of 60 days at 5-day intervals to understand the temporal dynamics of plant health.

The recorded NDVI values displayed a discernible trend over the observation period. Initially, on day 0, the NDVI value was measured at  $0.42 \pm 0.01$ , signifying the baseline vegetation health. As time progressed, the NDVI values exhibited a gradual increase. By day 60, the NDVI had reached  $0.8 \pm 0.01$ , indicating enhanced plant vitality (Jiyang *et al.* 2021).

Strawberry pH analysis revealed a mean of  $3.35 \pm 0.12$ , indicating high acidity. Brix measures soluble solids like sugars in liquids, gauging sweetness. Strawberries have  $8.11 \pm 0.79$  brix, denoting 8 g sucrose per 100 g liquid. This suggests moderate sweetness and aligns with the tangy flavor of strawberries, contributing to their unique taste profile. Ascorbic acid is estimated  $59.90 \pm 3.37$  mg/100 g. A concentration of 59.90 mg of ascorbic acid (vitamin C) per 100 g.

## DISCUSSION

In a general context, strawberries demonstrate optimal growth at an average temperature of  $15^\circ\text{C}$ , displaying a preference for cool to mild conditions (Hancock *et al.* 1991). Temperature of Agra below  $4^\circ\text{C}$  is uncommon, so frosting is rare which is good for growth of strawberry. The region maintains an average light intensity of approximately 5000 lux, effectively mitigating potential damage to the photosynthetic system attributed to excessive light energy absorption by Photosystem II (PSII) (Zhang *et al.* 2019).

Successful strawberry cultivation necessitates well-drained soil to prevent waterlogging, a condition conducive to root rot and fruit rot (Azam *et al.* 2019). The high sand content in the soil is favorable for strawberry plant growth. An ideal soil pH for cultivation ranges from 5.7 to 6.5, with recommendations for mitigating increased pH through the application of aluminum sulfate or elemental sulfur (Tabak *et al.* 2020). Experimental evidence indicates the adaptability of strawberry plants to slightly alkaline soils ranging from 7.93 to 8.10 pH, addressed through the

addition of organic manure considering alkaline soil and low nutrient content, featuring an organic carbon level of 0.42%.

Implementation of raised beds aims to improve soil drainage, enhance aeration, control weeds and pests, and prevent fruit contact with water, thus reducing spoilage and fungal infections. Application of Jivamrit, a microorganism-rich biofertilizer, enhances soil health, nutrient availability, and provides environmental benefits. Additionally, Sagarika, a seaweed-based product, is employed for tillering and overall plant growth. Irrigation, utilizing sewage-treated water, occurs every 10-12 days due to sandy soil characteristics, totaling eight irrigations until the final harvest.

Phenological observations reveal nuanced plant growth patterns, with new leaves emerging 2 days post-plantation, bud break varying after 42 days, and optimal budding and flowering occurring from November to February. Fruit harvesting spans from the second week of November to the end of March. Decline and senescence are observed during April to June heat, with waterlogging in July and August posing challenges, although some plants survive all seasons.

Photosynthetic rate data indicates a positive correlation ( $r=0.94$ ,  $p < 0.05$ ) between assimilation (A) and transpiration (E) shown in Fig. 3. The average difference between intercellular ( $C_i$ ) and atmospheric ( $C_a$ )  $\text{CO}_2$  concentrations is  $138.4 \mu\text{mol mol}^{-1}$ , with notable changes equivalent to assimilation rates of  $11.54 \pm 3.04 \mu\text{mol m}^{-2} \text{s}^{-1}$  shown in Fig. 4. A plateau in photosynthetic rate is observed at light intensities of 1000 to 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , with a subsequent gradual decline depicted in Fig. 5.

High-intensity light is associated with photoinhibition, damaging the photosynthetic machinery, particularly PSII. Reactive oxygen species (ROS) generated under high-intensity light induce oxidative stress, evident in decreased assimilation. Light intensities exceeding 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  are deemed unsuitable for strawberries.

Soil-Plant Analysis Development (SPAD) chlo-

rophyll meter and green seeker data indicate positive correlations between rising values and enhanced plant health, photosynthetic activity, and overall vigour during growth stages. Strawberry pH analysis reveals high acidity, Brix measurements suggest moderate sweetness, and vitamin C levels are notably high, contributing significantly to daily requirements (Nielsen *et al.* 2021).

## CONCLUSION

In conclusion, the investigation into the morphological and physiological responses of strawberries has revealed their potential not only for successful cultivation but also for transforming into a lucrative high-value crop within the climate and soil conditions of Agra. This study underscores the viability of strawberries as a high-value alternative crop in regions sharing similar climatic and soil attributes worldwide. Furthermore, the study demonstrates the remarkable resilience of the plants in adverse native conditions and soil compositions, underscoring their impressive tolerance capabilities. The conducted biochemical analysis has confirmed the suitability of the strawberries for consumption, adding to their appeal as a viable agricultural option. Therefore, it is strongly recommended that farmers and agricultural entrepreneurs explore strawberry cultivation as a profitable alternative.

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