ESTIMATING PHYTOSEQUESTRATION CAPACITY AND THE EFFECT OF ENVIRONMENTAL POLLUTION ON CARBON SEQUESTRATION OF SOME PLANT SPECIES AND ITS CHLOROPHYLL CORRELATION

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خلاصہ

کاربن ڈائی آکسائیڈ زمین کی فضامیں موجود ہے اور یہ گرین ہاؤس گیسوں میں ایک اہم شراکت دار کے طور پر کام کرتی ہے۔ کاربن ڈائی آکسائیڈ کی نمائش کسی شخص کی صحت پر تباہ کن اثرات مرتب کر سکتی ہے۔ گرین بائیو ٹیکنالوجی گرین ہاؤس گیسوں کے اخراج کو کم کرنے میں کردار ادا کر سکتی ہے، جس میں پودے فوٹو سنتھیس کے ذریعے کاربن کو الگ کر کے کاربن ڈوبنے کے طور پر اہم کردار ادا کرتے ہیں۔ کاربن سیکوسٹریشن سے مراد پودوں، مٹیوں، ارضیاتی شکلوں اور سمندروں میں کاربن کا طویل مدتی ذخیرہ ہے۔ موجودہ کام سبز بائیو ٹیکنالوجی کے مختلف طریقوں سے کاربن ڈائی آکسائیڈ کے اخراج کو تلاش کرنے پر مرکوز ہے اور یہ کہ ماحولیاتی آلودگی اس کے اخراج کو کیسے متاثر کرتی ہے۔ اس مطالعے کا مقصد یہ جانچنا ہے کہ ماحولیاتی آلودگی اور کاربن کے حصول کے درمیان تعلق کو سبز بایو ٹیکنالوجی کی تکنیک کے ذریعے کس طرح بہتر بنایا جا سکتا ہے جو پودوں کی مجموعی صحت کو متاثر کر رہی ہے۔ چھبیس پودوں کی انواع کے پتے جن میں Michelia، Anona Squamosa، Catharantha roseus، Elaeis Guineenis، Hibiscus rosa sinensis، اور Epipremnun pinnatum شامل ہیں، کو لے کر دو سیٹوں میں تقسیم کیا گیا۔ ایک سیٹ کو پانی سے دھویا گیا اور دوسرے کو اس پر گندگی کے ساتھ چھوڑ دیا گیا تاکہ یہ ظاہر کیا جا سکے کہ کاربن کے اخراج پر آلودگی کا کیا اثر ہے۔ پتیوں کو ایک شفاف فوٹو سنتھیٹک چیمبر میں رکھا گیا تھا جس میں CO_2 اور O_2، اور ہلکے بایو سینسرز تھے۔ اس کے بعد، کلوروفیل کی حراستی کا تعین سپیکٹروفوٹو میٹر کے ذریعے کیا گیا۔ پودوں کی چھبیس مختلف انواع سے، Hibiscus rosa sinensis نے 42 ppm کے ساتھ تمام پودوں میں CO_2 کی کم سے کم مقدار کو الگ کیا جبکہ Anona Squamosa 1025 ppm کے ساتھ CO_2 کی سب سے زیادہ مقدار کو ظاہر کرتا ہے۔ کاربن کے حصول اور کلوروفل کے مواد کے درمیان ایک تعلق ہے۔ وہ پودے جو کافی مقدار میں کاربن کو الگ کر دیتے ہیں ان میں بھی کلوروفل کی زیادہ مقدار ہوتی ہے جو فتو سنتھیس میں مدد کرتی ہے۔ مزید برآں، ہمیں پتہ چلا کہ مشیلیا کے غیر آلودہ پتوں نے ماحول سے 388 پی پی ایم CO_2 کو ہٹا دیا جبکہ آلودہ پتوں نے صرف 145 پی پی ایم CO_2 کو ہٹا دیا۔ موجودہ کام کاربن ڈائی آکسائیڈ کے اخراج کو کم کرنے اور فضا میں کاربن کو الگ کرنے کے لیے پودوں کے ممکنہ امیدواروں میں سے کچھ پر روشنی ڈالتا ہے اور یہ کہ ماحولیاتی آلودگی پودوں میں کاربن کے اخراج کو کیسے متاثر کرتی ہے۔

Abstract

Carbon dioxide is present in the Earth's atmosphere and it acts as a major contributor in greenhouse gases. Exposure to carbon dioxide can have devastating effects on a person’s health. Green Biotechnology may contribute to the reduction of greenhouse gas emissions, in which plants play an important role as carbon sinks by sequestrating carbon through photosynthesis. Carbon sequestration refers to the long-term storage of carbon in plants, soils, geologic formations, and the ocean. Current work is focused on exploring carbon dioxide uptake by different methods of green biotechnology and how environmental pollution affects that uptake. The study examined how the relationship between environmental pollution and carbon sequestration can be optimized by the technique of green biotechnology which affects the overall plant health. For this purpose, leaves from twenty-six plant species including *Michelia, Anona Squamosa*, *Catharantha roseus*, *Elaeis Guineenis*, *Hibiscus rosa sinensis,* and *Epipremnun pinnatum* were taken and divided into two sets. One set was washed with water and the other one was left with dirt on it to demonstrate the effect of pollution on carbon sequestration. Leaves were placed in a transparent photosynthetic chamber equipped with CO_2 and O_2, and light biosensors. Chlorophyll concentration was determined using a spectrophotometer. Out of twenty-six different species of plants, *Hibiscus rosa sinensis* sequestered the least amount of CO_2 among all the plants with 42 ppm while *Anona Squamosa* exhibited highest levels of CO_2 sequestration with 1025 ppm. There is a correlation between carbon sequestration and chlorophyll content. Plants that show a substantial amount of carbon sequestrated also have a greater amount of chlorophyll that help in photosynthesis. Furthermore, it was found that the unpolluted leaves of *Michelia* removed 388 ppm CO_2 from the environment while the polluted leaves removed only 145 ppm CO_2.

Keywords: Carbon capture; Environmental Pollution; Photosynthesis; Phyto-sequestration.

Introduction

The level of CO_2 in the atmosphere is increasing by some 2 ppm annually due to multiple natural sources which may include volcanic outgassing, the combustion of organic matter, and human activities such as the combustion of fossil fuel (Canadell *et al*., 2007). It is also produced by various microorganisms as a result of fermentation and cellular respiration. CO_2 is present in the Earth's atmosphere at a low concentration, and it acts as a major contributor to greenhouse gases (GHG). The rapid increase in the level of carbon dioxide is considered a primary contributor to global warming (Houghton *et al*., 2001). This study aims to provide scientific contributions to the estimation of phytosequestration, particularly by using locally available plant species as carbon-capturing agent by optimizing their environmental conditions.

One of the environmental factors that interact with elevated CO_2 is atmospheric ozone. A high atmospheric concentration of ozone produces detrimental effects on plant leaves, and it also decreases plant growth and photosynthesis. The site of action of ozone injury to plants is the internal tissues of leaves. Under elevated CO_2, stomatal openings get reduced and hence can reduce the exposure of sensitive tissues to ozone (Morgan *et al*., 2003; Feng *et al*., 2008). Moreover, rising levels of CO_2 are likely to have significant effects on plant growth, physiology, and chemistry (Ziska, 2008). Exposure to CO_2 can have devastating effects on a person's health as well. Headaches, dizziness, increased sweating, and elevated heart rate are to name a few (Satish *et al*., 2012). Carbon dioxide levels and potential health problems are indicated below:

Carbon dioxide levels and potential health problems

250-350ppm	Normal background concentration in outdoor ambient air.
350-1,000ppm	Concentrations typical of occupied indoor spaces with good air exchange.
1,000-2,000ppm	Complaints of drowsiness and poor air.
2,000-5,000 ppm	Headaches, sleepiness and stagnant, stale, stuffy air. Poor concentration, loss of attention, increased heart rate and slight nausea may also be present.
5,000	Workplace exposure limit (as 8-hour TWA) in most jurisdictions.
>40,000 ppm	Exposure may lead to serious oxygen deprivation resulting in permanent brain damage, coma, even death.

It is, therefore, imperative not only to lower the emissions but also to find means of sequestering atmospheric CO_2 over the distant future (Montzka *et al*., 2011). Due to rising concentrations of CO_2 in the atmosphere, impressive intrigue has been attracted to the possibility of expanding the pace of carbon sequestration through changes in land use and forestry. Since plants convert carbon dioxide to oxygen by the process of photosynthesis while performing carbon sequestration, also called phytosequestration (Watson *et al*., 2000), current work is focused to explore CO_2 uptake by different plant species and how environmental pollution affects that uptake. Plants sequester carbon by absorbing carbon dioxide from the atmosphere and converting it into biomass through photosynthesis (Chen, 2018). In plants, photosynthesis takes place in chloroplasts, which contain chlorophyll. Chlorophyll "a" absorbs light in the blue violet region and reflects green light. Whereas chlorophyll "b" absorbs red light and reflects green light (Emerson, 2021). This study contributes to identifying plant species that are good enough to sequester CO_2 from the atmosphere and will also help in reducing environmental pollution.

Materials and Methods

Estimating background CO_2 Level: Six different spots were chosen at Korangi creek, Karachi (latitude = 24.79, longitude = 67.11, GPS positioning sensor) and the level of CO_2 in the atmosphere was monitored by using CO_2 sensor (PS-2110, PASCO) to achieve the average CO_2 of that area.

Collection of Plants: Leaves from twenty-six economically important plant species were taken for carbon sequestration as mentioned in **Table 1**. The leaves were taken to the lab and divided into two sets. One set was washed with distilled water and the other one was left with dirt on it. All the leaves were then weighed, and their biomasses were recorded.

Measurements of Carbon Sequestration: The plant leaves were then placed in a transparent photosynthetic chamber which provided a closed space to measure the changes in levels of carbon dioxide in a chamber. The chamber was equipped with CO_2 and O_2, and light sensors (PASCO) as shown in **Figure 1**. The leaves of each plant species were placed in the chamber one by one, and the lid was closed. Then the level of CO_2 was observed and noted as mean $\pm$ standard deviation.

Effect of environmental pollution on Carbon sequestration: The purpose of this study was to compare the level of CO_2 sequestration in clean and dirty leaves and how pollution affects carbon sequestration in plants in the experiment.

Chlorophyll Determination: For the estimation of chlorophyll content, leaves were washed with distilled water, dried cut into small pieces, and major veins and tough fibrous tissue were discarded. With the help of mortals or pestles, a powder form of leaves was formed. Then stainless-steel strainer was used in order to discard the remaining large particles. Approximately, 100 mg of material was used for grinding and then was dissolved in 10ml of 80% acetone solution. The homogenate was filtered through filter paper and the filtrate was collected in the test tube. Ten-Fold dilutions were made by pouring 4.5ml of 80% acetone solution into the test tubes and 1.5ml of sample extract was then serially diluted. All the tubes were then centrifuged for 10 minutes at 500 rpm and then analyzed at 645nm and 663nm wavelengths for chlorophyll pigment a and B respectively. The 80% aqueous acetone is used as the blank to zero the instrument initially and after every wavelength resetting (N.A. Wagay, 2019). Equation 1 is used for calculating the total chlorophyll content.

Total Chlorophyll (mg/mL) = Chlorophyll a + Chlorophyll b (Equation 1)

Results and Discussion

Normal amount of indoor CO_2 considered healthy is 350-1000 ppm but, the readings we observed gave an average of 1242.6 ppm as depicted in Table 1. Exposure to higher levels of carbon dioxide can produce a variety of health effects. These may include headaches, dizziness, restlessness, a tingling or pins or needles feeling, difficulty breathing, sweating, tiredness, and increased heart rate. All of these conditions can cause injurious effects on a person's well-being including Sick Building Syndrome (Satish *et al*., 2012). One of the studies revealed that CO_2 concentrations surpassing 1000 ppm are related to reduce school participation. Teaching faculty also reported neuro-physiologic symptoms at CO_2 levels exceeding 1000 ppm (Gaihre, Semple, Miller, Fielding, & Turner, 2014), (Muscatiello *et al*., 2015). Moreover, elevated levels of atmospheric CO_2 may have detrimental effects on plant roots to soil interactions (De Deyn *et al*., 2008).

The principal procedure in green biotechnology for soil working is to sequester and transform atmospheric CO_2 in plant photosynthesis followed by its translocation below ground into plant roots (Hodge *et al*., 2009). Since plants play an important role in an environment as carbon sinks by sequestrating carbon, leaves from four different plant species were taken for carbon sequestration. We observed that plant species removed different levels of CO_2 from the environment. Out of six different species of plants, *Hibiscus rosa sinensis* sequestered the least amount of CO_2 among all the plants with 42 ppm while *Anona Squamosa* exhibits the highest levels of CO_2 sequestration with 1025 ppm as shown in Table 2. Figures 2 and 3 exhibit CO_2 concentration in the leaf chamber containing leaves of *Hibiscus rosa sinensis* and *Epipremnun pinnatum*, respectively. One of the research studies showed that elevated levels of CO_2 alleviate plant leaf's stomatal conductance of water by an average of 22 % which would decrease overall plant water use and ultimately produce a negative impact on soil moisture content (Ainsworth & Rogers, 2007). The high carbon level has left plants with a different chemical composition (Taub, 2010).

Next, we found out that the unpolluted leaves of *Michelia* removed 388 ppm CO_2 from the environment while the polluted leaves removed only 145 ppm CO_2 as exhibited in Table 3. The amount of CO_2 removed by the unpolluted leaves was almost double compared to the unpolluted leaves. A previous study indicates that air pollution due to vehicles reduces the concentration of photosynthetic pigments in the trees exposed to roadside pollution (Joshi & Swami, 2009). Our work is in concordance with past investigations and affirms the thought that the dirt and tar collected on the polluted leaves severely reduced the plant’s ability to remove atmospheric carbon dioxide.

Furthermore, the total chlorophyll content of the plant was measured by adding the values of chlorophyll pigment ‘a’ and chlorophyll pigment ‘b’. Table 4 and Figure 4 depict the chlorophyll content of five significant plant species. The graph shows that all the plants that absorb a considerable amount of carbon dioxide also contain an adequate amount of chlorophyll content. There is a correlation between carbon sequestration and chlorophyll content as shown in Figure 5. Plants that show a substantial amount of carbon sequestrated also have a greater amount of chlorophyll that help in photosynthesis. As a result, plants will perform photosynthesis rapidly and for more time due to which plants will also be able to absorb carbon dioxide more and will be able to remove pollutants from the environment.

Table 1. Estimating background level of CO_2

Location	Time period	First Reading	Second Reading	Third Reading	Mean
Indoor	5 minutes	1262ppm	1236ppm	1230ppm	1242.6ppm
Outdoor	5 minutes	1085ppm	617ppm	401ppm	701ppm

Table 2. Estimating Phytosequestration of some plant species

S#	Plant Species	Biomass (gms)	Light Intensity level (lux)	O_2 Level (ppm)	Mean CO_2 sequestered (ppm)
1	Champa (Michelia)	6.51	13088.02	203812	510
2	Sharifa (Anona Squamosa)	5.56	17995.61	196771	1025
3	Sadabahar (Catharantha Roseus)	5.51	13184.02	194949	892
4	Malaysian Palm (Elaeis Guineenis)	5.03	13468.27	190101	281.5
5	China rose (Hibiscus rosa sinensis)	5.0	19720.56	186519	42
6	Epipremnun pinnatum	5.0	20687.26	203812	57
7	Ficus elastica 10.7016	10.70	19004.56	186519	512
8	Dieffenbachia compacta	16.70	14054.26	189681	290
9	Euphorbia milli	7.08	18152.03	191437	282
10	Polyscias scutellaria	11.92	13458.27	190038	559.3
11	Polyscias fructicosa	2.52	16216.06	194949	314
12	Dracena reflexa	8.77	13084.02	193193	393.3
13	Dracena marginata coloroma	4.34	19719.56	192842	294.6
14	Syngonium podophyllum	5.94	17086.15	193896	533.3
15	Syngonium neglectum	5.31	17086.15	193896	244
16	Epipremnum aureum	23.17	17803.34	204924	525.3
17	Epipremnum aureum jade	23.31	17736.05	200847	395
18	Codiaeum variegatum var. pictum	6.61	20302.73	190101	222.3
19	Codiaeum variegatum	3.99	17995.61	197142	223.6
20	Cleorodendrum quadriloculase	6.81	13669.74	203812	317.3
21	Ipomea Batatas	4.10	16133.88	203812	275
22	Cassia abbreviata	3.57	17114.41	187136	272.3
23	Dieffenbachia ameona	11.14	20687.26	201218	189.3
24	Gossypium herbaceum	5.14	13477.10	185289	165.6
25	Plumerica pudica	8.99	20168.15	196771	327.3
26	Acalypha hispida alba	6.08	20229.03	198624	496

Table 3. Effect of air pollution on Carbon sequestration with leaves of *Michelia*

Leaf type	Initial CO_2 level	Final CO_2 Level	CO_2 sequestered
Polluted	1168ppm	1023ppm	145ppm
Un Polluted	388ppm	0ppm	388ppm

Table 4. Estimation of chlorophyll content of Plant species

S#	Plant species	Chl-a (645nm)	Chl-b (663nm)	Chl= a+b
1	Ficus elastica	0.438	0.583	1.021
2	Polyscias scutellaria	0.434	0.460	0.794
3	Syngonium podophyllum	0.394	0.446	0.841
4	Epipremnum aureum	0.397	0.541	0.938
5	Acalypha hispida alba	0.449	0.574	1.023

Fig. 1. Leaf Chamber containing CO_2 and O_2, and Light sensors

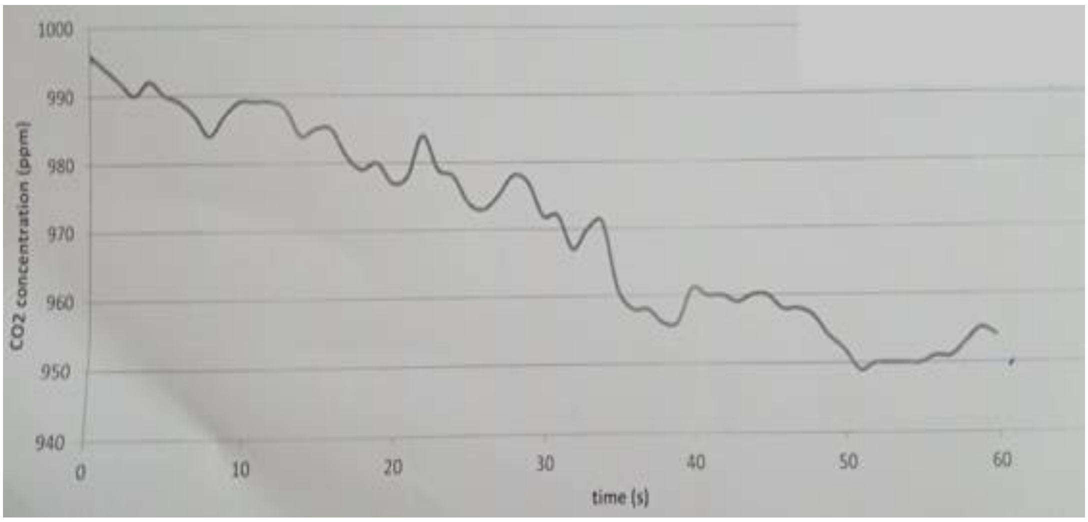

Fig. 2. CO_2 Phytosequestration by leaves of *Hibiscus rosa sinensis*

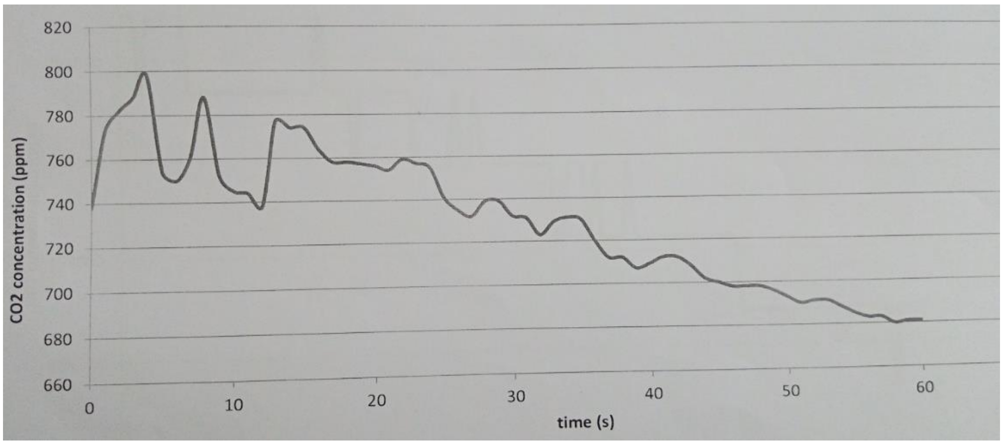

Fig 3. CO_2 Phytosequestration by leaves of *Epipremnun pinnatum*

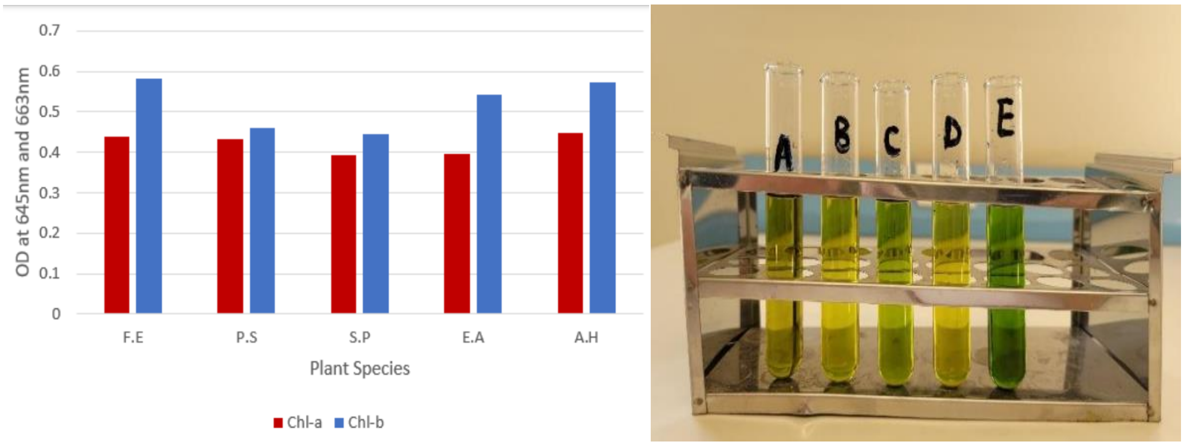

Fig 4. Estimation of chlorophyll content of Plant species
(F.E = Ficus elastica, P.S = Polyscias Scutellaria, S.P= Syngonium podophyllum, E.A= Epipremnum aureum, and A.H = Acalypha hispida alba).

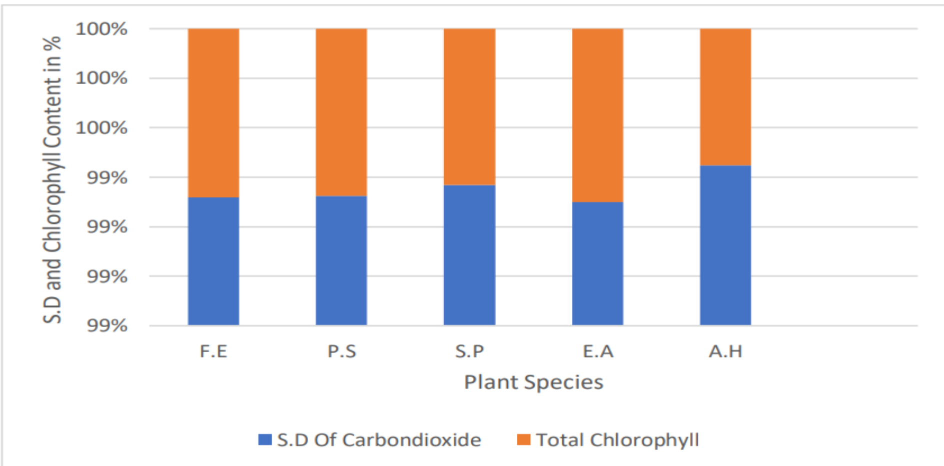

Fig. 5. The correlation between carbon sequestration and the total chlorophyll content among different plant species
(F.E = Ficus elastica, P.S = Polyscias Scutellaria, S.P= Syngonium podophyllum, E.A= Epipremnum aureum, and A.H = Acalypha hispida alba).

Conclusion

The current study highlights some of the potential plant candidates for mitigating carbon dioxide emission and sequestering carbon from the atmosphere using green biotechnology and how environmental pollution affects carbon sequestration in plants. However, green biotech plants can, and already do, contribute positively in reducing CO_2 emissions and anticipating the impact of climate change. Further research activities including plant genetic engineering is required to enhance carbon sequestration in above and below-ground plant biomass. The environmental pollution level of CO_2 is increasing day by day since 3,220,000 motor vehicles register per year in Pakistan. The plants presented in the current study can be used as ornamental plants and they can reduce the level of CO_2 as per the study.

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