EVALUATION OF CO_2 ENRICHMENT ON GROWTH, DEVELOPMENT AND SOFT ROT TOLERANCE IN GINGER (*Zingiber officinale* Rosc.)

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(2016-21-023)

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF AGRICULTURE

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By

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THESIS

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2021

DECLARATION

I, hereby declare that this thesis entitled **"Evaluation of CO_2 enrichment on growth, development and soft rot tolerance in ginger (*Zingiber officinale* Rosc.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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Certified that this thesis entitled **"Evaluation of CO_2 enrichment on growth, development and soft rot tolerance in ginger (*Zingiber officinale* Rosc.)"** is a record of research work done independently by Ms. Manasa R. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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LIST OF ABBREVIATIONS

CO_2	Carbon-dioxide
ppm	Parts per million
%	Per cent
^0C	Degrees Celsius
cm	Centimetre
g	Gram
$\mu mol\ mol^{-1}$	Micro mole per molecule
FW	Fresh weight
mg	Milligram
$mmol\ mol^{-1}$	Milli mole per molecule
FACE	Free air CO_2 enrichment
h	Hours
ROS	Reactive Oxygen Species
µS	Microsiemens
PAL	Phenylalanine ammonia-lyase activity
PR	Pathogenesis-related
CAT	Catalase
µg	Micro gram
ml	Milli litre
Fv/Fm	Variable to Maximum fluorescence
NPQ	Non-photochemical quenching
kDa	Kilo Dalton
CAD	Cinnamyl alcohol dehydrogenase
CHS	Chalcone synthase
OTC	Open Top Chamber
CRD	Completely Randomised Design
IPCC	Intergovernmental Panel on Climate Change
SLA	Specific leaf area
m	Metre

v/v	Volume per volume
s	second
BSA	Bovine serum albumin
FW	Fresh Weight
DW	Dry Weight
nm	nanometre
min	minute
rpm	Rotations per minute
v/w	Volume per weight
Fo	Basal fluorescence
Fm	Maximal fluorescence
Fv	Variable flueroscence
PAR	Photosynthetically Active Radiation
SDS	Sodium dodecyl sulfate
V	Volts
SEm	Standard Error (Mean)
CD	Critical Difference
NBT	Nitro blue tetrazolium chloride
APX	Ascorbate Peroxide
DAB	3,3′-Diaminobenzidine

Introduction

1. INTRODUCTION

Global atmospheric CO_2 concentration has increased at an alarmingly rapid rate exceeding 407 ppm (Dlugokencky *et al*., 2019), nearly a 30 per cent increase, since 1950 and is projected to increase twofold by the end of this century. The resulting increase in CO_2 levels will lead to both positive and negative effects on major agricultural crops used to feed the global population, which many of them are yet to be known.

A few of the positive impacts include stimulation of plant biomass production, changes in the photosynthate allocation pattern, phytochemical profiles and yield. Although elevated CO_2 concentration considerably affects the yield and quality of crops, the physiological, biochemical and molecular parameters conferring to these variations among cultivars have not been fully understood.

When grown at enhanced levels of CO_2, there is consistent intraspecific differences among crop cultivars in growth and yield, few exhibiting a strong stimulation of yield, others little or no stimulation (Bishop *et al*., 2015). Understanding the basis for these variations may be crucial in identifying those cultivars that can convert additional CO_2 into greater yield. Such investigation will fundamentally benefit future breeding strategies to enhance the crop adaptability to elevated CO_2 environments.

While much is known regarding the effects of elevated CO_2 on crop plants, horticultural species in general and ginger in particular have received much less attention. Ginger (*Zingiber officinale* Rosc.) of the family Zingiberaceae is an important spice and cash crop, commercially cultivated in South and Southeast Asia, tropical parts of Africa, Latin America, and the Caribbean (Zest, 2018). As a spice, ginger is an integral part of all distinguished cultures of the world for its unique aroma and sweet-spicy flavor.

In the history of traditional medicinal applications, ginger has earned a place among the most healing herbs around the world (Das *et al*., 2020). India, renouned as 'the spice bowl of the world,' enjoys a unique position in the production and export of ginger. From time immemorial it has been cultivated in Kerala both as a fresh vegetable and as dry spice.

Although the increasing demand of ginger for its multitude of use as food, spice, medicine, beverages, flavor and pharmaceuticals has created a demand for its regular cultivation, the annual production of ginger is crucially limited by phytopathogens such as fungi, viruses, bacteria, and nematodes (Le *et al*., 2014). As the largest group of plant fungal pathogens, the oomycete genus y*Pythium* causes severe crop losses worldwide. *Pythium* species alone are accountable for losses of multibillion-dollar worldwide (West *et al*., 2003). Presently, most of the available ginger cultivars are susceptible to plant diseases (Nair and Thomas, 2007b) such as soft rot caused by *Pythium* spp. (Le *et al*., 2014). Kavitha and Thomas (2008) have identified durable resistance in a wild relative of cultivated ginger in *Zingiber zerumbet* (L.) Smith, against *Pythium* spp.

Shin and Yun (2010) reported that increase in temperature by 5°C and CO_2 of 700 ppm, increased bacterial spot and wilt on pepper by 25% and 24%, respectively. In contrast, the oomycete-plant interactions of soybean and *Peronospora manshurica*, the CO_2 concentration of 550 ppm decreased the severity of the disease by more than 50% (Eastburn *et al*., 2010). The varying findings on the effects of CO_2 concentrations on plant-pathogen interactions are not adequate to draw any unifying principles. Therefore, they emphasize the importance of examining each pathosystem associated with important agricultural crops to predict disease susceptibility under future climatic conditions.

Hence, extensive studies are required for understanding the crop response to an invading pathogen involving complex physiological, biochemical and molecular processes that lead to development of disease (Jones and Dangl, 2006). Studies on possible effects of elevated CO_2 on ginger and the major disease, soft rot will be useful to comprehend adaptive behavior and to devise production technologies for the future. It is very crucial for the scientific community to be equipped with suitable research findings to cope up with the effects of enhanced levels of atmospheric CO_2.

The present study entitled “Evaluation of CO_2 enrichment on growth, development and soft rot tolerance in ginger (*Zingiber officinale* Rosc.)” envisages to address various perceptions and priorities concerning the above mentioned issues. For elevating the comparative effect of elevated CO_2 on development, yield, quality and

tolerance to soft rot in cultivated ginger and its tolerant counterpart (*Z. zerumbet*) the following objectives have been formulated.

1. Assessing the impact of elevated CO_2 on growth, development and quality of ginger.

2. Assessing the impact of elevated CO_2 on the response of ginger to *Pythium*. *aphanidermatum.*

3. Molecular studies on the impact of elevated CO_2 on the response of ginger to *P. aphanidermatum.*

Review of literature

2. REVIEW OF LITERATURE

Climate change

The change in climate has been majorly attributed to the rise in atmospheric CO_2 concentration and a corresponding increase in temperature (Vanaja *et al*., 2015). It is apparent that the continuous increase in atmospheric CO_2 influences the growth, development and physiology of crops. This rise in atomopheric CO_2 has drawn the scientist's interest towards the response of crop plants to CO_2 enrichment.

However, one imperative topic that has not been focused majorly is the effect of increased levels of atmospheric CO_2 on the growth of medicinal and spice plants in general; and ginger in particular and their production of secondary metabolites of therapeutic value. As CO_2 concentrations are predicted to raise, there would be greater concern on plant responses to atmospheric CO_2 in the future.

Elevated CO_2

Currently, the level of atmospheric CO_2 exceeds 407 ppm (Dlugokencky *et al.*, 2019) which is almost an increase of 30% since the 1950s and is expected to double by the end of this century (IPCC, 2014). The increase in CO_2 concentration is a major concern to plants and animals including humans because in the atmosphere it is a cause for warming of global temperatures, acidification the oceans and may ultimately deplete aquatic oxygen, leading to hypoxia (Harter, 2020). Some crops grown in CO_2 concentrations of 546 PPM to 584 PPM have less protein, iron, and zinc per calorie compared to crops grown at CO_2 concentrations of 363 PPM to 386 PPM (Krawisz, 2020).

Carbon dioxide plays a crucial role in plants. It is involved in the process of photosynthesis through which complex carbohydrates, sugars and carbon skeletons are produced for most of the organic compounds in plants. Along with the direct impact on the global temperature, elevated CO_2 has affected almost all crucial biological processes, including respiration, antioxidant systems, photosynthesis, and as well as other key secondary metabolisms in plants (Penuelas *et al*., 2013; Singh and Agrawal, 2015). In

some studies it is reported that elevated CO_2 concentrations increased photosynthesis in plants, which consequently enhanced the growth, biomass, and yield (Kooi *et al.,* 2016).

Elevated CO_2 also could have negative effects influencing the quality of crops leading to a decline in macro and micro elements as well as vitamins (Hogy and Fangmeier, 2009; Myers *et al.,* 2014) and proteins (Broberg *et al.,* 2017). In the light of these findings, it has become even more important to understand plant responses to elevated CO_2.

Ginger-The most valued spice

The plant *Zingiber officinale* was named by an English botanist William Roscoe in 1807. The genus name is derived from the Greek word 'zingiberis', which was inturn derived from the Sanskrit word 'shringavera', which means 'shaped like a deer's antlers'. The species name 'officinale' is attributed to the medicinal properties of the rhizomes (Elzebroek and Wind, 2008).

Ginger (*Zingiber officinale* Rosc.), is a perennial herb and belongs to the family Zingiberaceae which includes about 47 genera and 1400 species (Hogarth, 2000). It is widely believed to have originated in Asia or native to India. The economic part of the ginger plant is the rhizome which is utilised either freshly for culinary purposes or as a processed product which can be used as salted, dried, powdered, paste, or extracted as ginger oleoresin or oil (Kizhakkayil and Sasikumar, 2011). Besides as spices and condiments, ginger plays an important role in Ayurvedic or other ancient traditional medicinal practices which were used to treat nausea, headaches, arthritis, colds, rheumatism, inflammation and muscular discomfort (Baliga *et al*., 2011; Dehghani *et al.*, 2011).

Especially, the ginger extracts including gingerols, shogaols and zingerone possess medicinal properties such as anti-inflammatory (Baliga *et al.,* 2011), anti-oxidant and anti-emetic (Thomson *et al.,* 2014), anti-migraine (Semwal *et al.,* 2015), anti-tumor (Zhang *et al.,* 2017), immuno-modulatory and cardiovascular protection (Jafarnejad *et al*., 2017).

The approach of CO_2 enrichment

One of the most recent techniques that have been recently used extensively around the world to improve the qualitative and quantitative yield of plants is CO_2 enrichment (Wang *et al.*, 2003).

Moore *et al.* (1999) emphasised that elevated CO_2 enhanced the photosynthetic rate of plants which is attributed to due to enhanced Rubisco enzyme activity. He has also revealed that the leaf starch content increased by 160% and soluble carbohydrate content by 52% on an average. By taking into account the structural and physiological changes, and their interactions at whole plant level resulting from growth under elevated CO_2 will give a more comprehensive idea of how plants respond to increasing CO_2 can be deciphered (Murthy and Dougherty, 1997).

2.1 Effect of CO_2 enrichment on growth parameters

2.1.1 Plant height

High CO_2 levels result in an increase in plant height, larger canopy, dry biomass and crop yields (Kant *et al*., 2012). Saravanan and Karthi (2014) reported that maximum fresh weight and plant height were observed under higher concentration of elevated CO_2 levels (900 ppm and 600 ppm). At 550 ppm of elevated CO_2, the height of rice plants was 81.7 cm, while in ambient CO_2 (395 ppm) it was 76.9 cm with recommended dose of N fertilizer (Raj *et al.*, 2019).

Gladiolus plants when grown at varying elevated CO_2 concentrations of 900 ppm, 700 ppm and 400 ppm (control) showed that the maximum plant height was at 700 ppm. (Kadam *et al*. 2012). Mamatha *et al.* (2014) reported that tomato plants grown at 550 ppm of elevated CO_2 exhibited significantly higher plant height of 115.0 cm, than those grown at 700 ppm (81.8 cm) and the control (92.3 cm) which was 40.7% and 24.6% higher respectively. This implies that plant height increases up to an optimum CO_2 concentration beyond which the effect on plant height is not significant. However, this optimum CO_2 concentration varies between the crop species.

2.1.2 Number of leaves

Saravanan and Karthi (2014) reported that when *Catharanthus roseus* was grown at ambient and elevated CO_2 concentrations (600 ppm and 900 ppm), the maximum number of leaves was observed in 900 ppm (114) followed by 600 ppm (101) and control (62). Experimental plants of cowpea recorded higher number of leaves under elevated CO_2 of 500 ppm (78.00) which was significantly higher compared to open control (70.75) (Srikanth *et al.,* 2019).

The elevated CO_2 had a significant positive influence on leaves per plant in tomato which showed higher number of leaves of 88.3 at 700 ppm CO_2 as compared to the plants which were grown under ambient CO_2 (67.3) leaves (Mamatha *et al.,* 2014). Minu *et al.* (2015) found similar results in black pepper (*Piper nigrum* L.) plants under 500 ppm.

Plant development, is due to meristem initiation, which includes cell division, regulated cell expansion and differentiation (Taylor, 1997). These processes are coordinated, by genetically programmed and timed ontogenetic events (KoÈrner, 1991). They are also integrated with environmental cues to influence the shape, number and rate of organs formed by plants (Kerstetter and Hake, 1997).

2.1.3 Specific leaf area

Specific leaf area is defined as the area of a fresh leaf, divided by its dry mass; and is a function of leaf dry-matter content. Elevated CO_2 has been reported to alter the leaf size and anatomy. Higher levels of CO_2 increased the leaf thickness, single-leaf area and total leaf area per plant in many plant species (Pritchard *et al.*, 1999). Gladiolus plants exhibited significantly higher leaf area when grown at 700 and 900 ppm of CO_2 compared to the plants grown at 400 ppm (control) (Kadam *et al*., 2012). Reduction in specific leaf area was found in elevated CO_2 of 600 ppm (294.10 cm^2 g^{-1}) compared to open control (319.73 cm^2 g^{-1}) in tomato plants (Chatti and Manju, 2018). *Azolla filiculoides* grown under elevated CO_2 of 600 ppm experienced a 21% reduction in specific leaf area compared to the plants grown under CO_2 of 400 ppm (ambient) (Gufu *et al.,* 2019).

2.1.4 Biomass

Dilustro *et al*. (2002) reported that fine roots were strongly stimulated by elevated atmospheric CO_2. Increase in dry mass at elevated CO_2 conditions results from increased photosynthetic rate. Higher photosynthetic rate at elevated CO_2 conditions in mungbean contributed to higher biomass production (Chowdhury *et al*., 2005). Ghasemzadeh and Jaafar (2011) reported that when two *Zingiber officinale* varieties (Halia bentong and Halia bara) were exposed to varying concentrations of CO_2 (400 and 800 ppm), 800 ppm CO_2 resulted in enhancement of dry weight of leaves, stems and rhizomes by 76.3% in Halia Bara and 47.6% in Halia Bentong. At elevated CO_2 of 550 ppm there was an increase in shoot biomass in field pea (36%) and wheat (55%) (Kimball, 2016). Thinh *et al*. (2017) reported that under elevated CO_2, in Chinese yam the leaf area, leaf dry weight, vine length, and total plant dry weight were significantly greater than in atmospheric CO_2.

2.1.5 Stem diameter

Tyree and Alexander (1993) hypothesized that enhanced photosynthesis and increased availability of carbohydrates resulted in increased size and number of xylem elements (tracheids and vessel members) within the plants grown under elevated CO_2. Medeiros and Ward (2013) observed that, plants have larger xylem conduits and reduced ratio of conduit wall thickness to diameter when grown at elevated CO_2 compared to those grown at ambient CO_2. Foxtail millet grown at elevated CO_2 of 600 μmol mol^{-1} stem diameter was increased by 16% (Li *et al.*, 2019).

2.1.6 Tiller number

Mamatha *et al.* (2014) reported that tomato plants grown under elevated CO_2 significantly increased the number of leaves and branches per plant. Plants grown at 700 ppm of CO_2 showed higher number of leaves (88.3) and branches (17.3) compared to the control (380 ppm CO_2) 67.3 and 9.5 respectively. Jin *et al.* (2017) reported that elevated CO_2 (580 ppm) almost doubled the number of branches in two soyabean varieties Dongsheng 7 and Suinong 14. Foxtail millet crop grown at elevated CO_2 (600 μmol mol^{-1}) produced 27% more tillers compared to the control (Li *et al.*, 2019).

2.2 Effect of CO_2 enrichment on yield parameters

Using meta-analysis, Wang *et al*. (2013) estimated the overall CO_2 impacts on wheat crop physiology and yield, showing an average yield stimulation of 24%. Jin *et al.* (2017) reported that elevated CO_2 (580 ppm) increased yields of two soyabean varieties, Suinong 14 and Dongsheng 7 by 13% and 35% respectively. Total pod weight in cowpea increased under elevated CO_2 (500 ppm) with highest total pod weight (102.59 g $plant^{-1}$) compared to ambient CO_2 (81.68 g $plant^{-1}$) (Srikanth *et al.,* 2019). Elevated CO_2 of 550 ppm increased grain yield by 25.5% over ambient of 395 ppm of CO_2 with recommended dose of N (Raj *et al.,* 2019). Elevated CO_2 of ~200 μmol mol^{-1} above ambient enhanced rice yields by 32.8% 13.5%, 22.6% and for hybrid, japonica and indica cultivars (Lv *et al.,* 2020).

2.3 Effect of CO_2 enrichment on Physiological and Biochemical parameters

2.3.1 Chlorophyll

Mung bean plants grown under OTC 570 ppm conditions maintained a higher chlorophyll content compared to those grown under field conditions of 370 ppm and chlorophyll began to decrease from the flowering stage (after 30 days of emergence) (Haque *et al.,* 2005). Total chlorophyll (1.7 mg g^{-1} FW) and carotenoid contents(1.3 mg g^{-1} FW) of *Catharanthus roseus* L. plants were significantly higher under elevated CO_2(560±25 ppm) compared to ambient CO_2 of 375±30 ppm (Singh and Agrawal, 2015). Elevated CO_2 level significantly increased total chlorophyll content (3.1 mg g^{-1}) in the leaves of cowpea as compared to ambient CO_2 (2.6 mg g^{-1}) (Dey *et al.,* 2017). Similar results were evident in cucumber seedlings where chlorophyll concentrations were 1.95 mg g^{-1} FW and 1.64 mg g^{-1} FW at elevated CO_2 and ambient CO_2 respectively (Song *et al.,* 2020).

2.3.2 Stomatal conductance and transpiration

In ginger varieties, upon CO_2 enrichment of 800 μmol mol^{-1} reduction in stomatal conductance was evident by 30.76% in Halia Bentong and 24.52% in Halia Bara (Ghasemzadeh and Jaafar, 2011). For twice the CO_2 level, stomatal conductance reduced

by 30–40% (Liu *et al.,* 2008). In most plant species under elevated CO_2 decline in the level of, Rubisco and reduction in stomatal conductance was evident (Ainsworth and Rogers, 2007; Leakey *et al.,* 2009). Yusuke *et al*. (2007) reported that plants grown in elevated CO_2 concentrations exhibited low stomatal conductance and high Water Use Effeciency (WUE). The decreased magnitude of stomatal conductance by CO_2 enrichment greatly depends on environmental variables and species (Haworth *et al.*, 2013; Ward *et al.*, 2013).

2.3.3 Photosynthesis

Ghasemzadeh and Jaafar (2011) reported that photosynthetic rate increased in ginger varieties, Halia Bara to 46% and Halia Bentong to 65% under elevated CO_2 concentration. Higher rates of net photosynthesis due to greater activity of Rubisco enzyme, up on exposure of plants to elevated CO_2 usually leads to modification in the plant growth and partitioning of secondary metabolites (Mark, 2000). Elevated CO_2 of 548 ppm enhanced the net photosynthesis to 13% as well as the biomass to 23% in one year old potted paper birch (*Betula papyrifera*) (Mattson *et al*., 2005).

Katny *et al.* (2005) have conducted a study on potato and have reported that elevated CO_2 enhanced the photosynthesis by 10-40%. It was reported that elevated CO_2 increases the electron flow to RuBisCO carboxylation by decreasing the allocation of electron transport to photorespiration (Robredo *et al*., 2010). Tomato (cv. Hezuo 903) plants grown under elevated CO_2 of 800 μmol mol^{-1} and ambient 380 μmol mol^{-1} exhibited maximum carboxylation rate and maximum Rubisco regeneration which resulted in enhancement in the net photosynthetic rate (Pan *et al.*, 2018).

However, study by Ludewig and Sonnewald (2000) hypothesised that photosynthetic acclimation is resulted due to accumulation of sugars, they found in *Nicotiana tabacum* that high CO_2 caused accelerated leaf senescence, due to down-regulation of leaf photosynthetic related genes

2.3.4 Protein content

Taub and Wang (2008) showed that at an elevated CO_2 concentration of 540-958 μmol mol^{-1}, protein content was decreased by 10% to 15% in rice, wheat and barley

grains. Hogy and Fangmeier (2009) reported that high CO_2 concentration resulted in low tuber protein concentration in potato by 14%. Ghasemzadeh *et al.* (2014) reported that in ginger varieties at 800 μmol mol^{-1} of CO_2 the total protein content in the rhizomes decreased by 46.2% in Halia Bara and 29.9% in Halia Bentong compared to 400 μmol mol^{-1} of CO_2.

There was a decline in N allocation into leaf blades under elevated CO_2, which consequently reduced Rubisco and other protein synthesis in rice (Seneweera *et al*., 2011). However Jin *et al.* (2017) reported that in two soyabean varieties, Suinong 14 or Dongsheng 7 elevated CO_2 of 580 ppm did not affect the protein concentration which maintained an average of 387 mg g^{-1}. This indicates that grain quality was maintained as the N supply was adequate. Myers *et al.* (2014) also indicated that soybean protein concentration was not significantly varying by the elevated CO_2 effect.

2.3.5 Sugars and starch

Ghasemzadeh and Jaafar (2011) reported that two ginger varieties when exposed to 800 ppm of CO_2 concentration resulted in maximum carbohydrate content in Halia Bentong (38.22 mg g^{-1} dry weight) and Halia Bara (38.43 mg g^{-1} dry weight); and maximum starch content in Halia Bara (553.3 mg g^{-1} dry weight) and Halia Bentong (583.5 mg g^{-1} dry weight) compared to 400 ppm CO_2. Increasing of CO_2 level from 400 μmol mol^{-1} to elevated of 800 μmol mol^{-1} resulted in increased content of glucose, sucrose, and fructose in the young rhizome of Halia Bentong (81.03%, 33.13% and 147.67% respectively) and Halia Bara (94.62%, 51.44% and 132.63% respectively) (Ghasemzadeh *et al.,* 2014).

Upon CO_2 enrichment, due to the lower utilization of carbohydrates than their production, they accumulate in the plant tissues (Moore *et al*, 1998). Total sugars in tomato varieties increased by 14.16% for Eureka Premature, 20.85% for Eureka and 16.12% for Astra under elevated CO_2 of 1000 μmol mol^{-1} (Khan *et al.,* 2013).

Under elevated CO_2 the increase in total soluble sugar was highest in leafy vegetables (36.2%) compared to all other classes of vegetables. The increments of total soluble sugar were less in fruits (8.5%) and root vegetables (16.3%) (Jin *et al*., 2009).

This proves that the synthesized carbohydrates in leaves cannot be fully translocated to roots and fruits, this ability varies with the species. The total soluble sugar was increased by 20% in turnip and 13% in radish under 1000 mmol mol^{-1} CO_2 compared to control (400 mmol mol^{-1}) (Azam *et al.*, 2013). However, the total soluble sugar in stem vegetables is not affected under elevated CO_2. This is due the impairment of carbon transformation into soluble sugar accumulation as the soluble sugar is converted to lignin under elevated CO_2 (Liu *et al.,* 2018).

2.3.6 Effect on mineral nutrition

Upon CO_2 enrichment, in wheat grains the concentrations of nutrients such as Mn, Zn, Ca and Fe reduced in a number of studies (Fernando *et al*., 2012; Hogy *et al*., 2013). Myers *et al.* (2014) found that under elevated CO_2 Fe content reduced by 5.2%. Upon analysis of seven leafy vegetables, essential minerals such as Ca, Mg, P, Fe Mn, and Zn exhibited 20% reduction. It is postulated that dilution of elemental content is due to increase in the carbohydrates under elevated CO_2 and it is termed as "dilution effect" (Loladze, 2014).

A meta-analysis conducted by Dong *et al.* (2018) showed that elevated CO_2 decreased the concentrations of Zn, Mg and Fe by 9.4%, 9.2% and 16.0% respectively, while the concentrations of S, P, K, Mn and Cu were maintained in the vegetables as a whole. In a 7-year FACE study, when averaged across various crop species, seasons and soil type elevated CO_2 was found to reduce the concentrations of Zn, P and N by 10%, 5% and 6% respectively (Jin *et al*., 2019).

Elevated CO_2 significantly down-regulated 798 genes and up-regulated the expression of 529 genes by 1.5-fold under FACE conditions. Fourteen genes encoded transporters, 10 of these genes, including heavy metal transporters and K, were down-regulated. (Ujiie *et al.,* 2019).

2.4 Effect of CO_2 enrichment on quality parameters

Concomitant to increase in biomass production, a frequently observed phenomenon of, plants grown under enriched CO_2 exhibit significant changes in their

chemical composition (Kimball *et al.,* 2002). The studies on the impact of CO_2 concentration on the nutritional quality of crops are inadequate (Moretti *et al.,* 2010).

2.4.1 Fiber

Elevated CO_2 (1000 μmol mol^{-1}) in tomato depicted accumulation of fiber and soluble sugar in different varieties; for Eureka the increase was 4.40% and for Astra 5.25% (Khan *et al.,* 2013). In rice, the increase in soluble dietary fiber (82.10%) and dietary fiber (2.64%) was evident when grown under CO_2 enrichment (550 mol mol^{-1}) (Goufo *et al.,* 2014). The effect of elevated CO_2 on crude fiber found to be non significant among the three maize genotypes (Shankar *et al.,* 2015). However, superior vegetative growth and low fiber content are the breeding priorities for improvement of ginger (Ganapathy *et al.,* 2016).

2.4.2 Gingerols

Carbon-nutrient balance theory states that under elevated concentrations of CO_2 the carbon to nitrogen ratio increases leading to the majority of carbohydrates being allocated to the plant's secondary metabolism, resulting in higher amounts of carbon based secondary metabolites (Tisserat and Vaughn, 2001). Ginger is profoundly known for its medicinal value, which is attributed to several bioactive compounds such as gingerols, zingiberene and shogaols (Butt and Sultan, 2011). The pungent taste of fresh ginger rhizome is due to the presence of the gingerols in general and 6-Gingerol in particular.

2.4.3 Phenols

Total non-structural carbohydrates often increase upon CO_2 enrichment in plants and stimulates secondary metabolism (Booker 2000). The carbon partitioning to acid-detergent lignin, condensed tannins and phenolics in leaves enhanced at elevated CO_2 of 548 ppm. In a FACE experiment on 1-year-old potted paper birch, *B. papyrifera*, in the stem tissues, lignin and tannins increased but phenolics did not whereas tannins and phenolics increased, but lignin did not in root tissues (Mattson *et al.,* 2005).

Ghasemzadeh and Jaafar (2011) reported that elevated CO_2 increased phenolic content in rhizomes of ginger varieties, Halia Bentong and Halia Bara. Under elevated CO_2 of 800 µmol mol^{-1} among the phenolic acid compounds cinnamic acid, salicylic acid and vanillic acid were detected where as tannic acid was not. This implies that each of the phenolic components is synthesized at different CO_2 concentrations.

2.4.4 Oils

Oil formation is favored under elevated CO_2 which enhances photosynthesis, leading to reallocation of more sucrose and raffinose to grains of soyabean (Hymowitz *et al*., 1972). Studies on the effect of elevated CO_2 on oil percentage in seeds of soybean (Li *et al*., 2019; Köhler *et al*., 2019), sunflower (Pal *et al*., 2014), indicated that elevated CO_2 enhanced oil content in the seeds. The concentration of oil in soybean grains was 14% higher under elevated CO_2 when compared to the control (Qiao *et al*., 2019). Similar findings have been reported in soybean by Hao *et al.* (2014) who reported that high CO_2 stimulated and increased the concentration of palmitic acid and linoleic acid which enhanced the total oil production.

2.4.5 Ascorbic acid

Under elevated CO_2, the increase in total antioxidant capacity (72.5%) as well as ascorbic acid (15.3%) was evident in leafy vegetables compared to other vegetables. The enhanced synthesis and accumulation of antioxidants under higher concentration of CO_2 is due to the greater levels of soluble sugars which are the precursors (Jaafar *et al*., 2012; Becker and Klaring, 2016).

Previous research had shown that exposure to elevated CO_2 can enhance the production of ascorbic acid in tomato (Islam *et al*., 1996), *Capsicum annum* (Aloni and Karni, 2002), and potato (Vorne *et al*., 2002). In contrast, elevated CO_2 had no significant effect on the accumulation of pigments like carotenoids, total chlorophyll, anthocyanins and lycopene; and even reduced the concentration of carotenoids, ascorbic acid in root vegetables, chlorophyll a, and total antioxidant capacity in fruit vegetables by 8.1%, 14.8%, 14.1%, and 14.4% respectively (Dong *et al*., 2018).

2.5 Climate change effects on plant diseases

Many environmental conditions including light, temperature, wind speed, water availability, soil fertility, CO_2, atmospheric ozone and methane concentrations affect the development of plant disease. Water availability, CO_2 concentration and temperature are most likely predicted to change and can have positive, neutral or negative effects on disease development, as each disease may have different response to the variations occuring. Climate change alters pest and pathogen resistance by altering the nutritional status, morphology, physiology and/or host plant defence responses (Trebicki *et al*., 2017).

Pathogens and plants do not interact in isolation. The 'disease triangle' concept in plant pathology emphasizes the interaction of both plants and pathogens with the environment (Velasquez *et al*., 2018). The environment can have an influence on disease development and spread through its influence on survival, vigour, rate of multiplication, sporulation, direction, distance of dispersal of inoculums, rate of spore germination and penetration of plant pathogens (Kang *et al*., 2009).

The availability of information on the potential impacts of climate change on plant diseases is limited. More work is needed in this direction as pathogens reduce plant productivity in agricultural, forest and natural ecosystems worldwide (Pimentel *et al*., 2000). To elucidate the role of future climatic conditions on plant disease development, understanding such relationships is crucial in predicting the plant and ecosystem health and for managing plants in a range of eco-systems in the future.

2.6 Influence of elevated CO_2 on plant pathogens

Little attention has been received for experimental research on the effects of rising atmospheric concentrations of CO_2 on plant pathogen interactions. However, several conflicting results have been published. The three key components virulent pathogen, susceptible host, and favourable elevated CO_2 environments in plant disease are known to accelerate disease symptoms (Mc Elrone *et al*., 2001) or result in a new disease emergence (Anderson *et al*., 2004).

The growth of pathogens can be directly affected by elevated levels of CO_2. The susceptibility of wheat varieties increased under elevated CO_2 for the fungal pathogen *Fusarium graminearum*, and also enhanced the virulence of the fungal isolate resulting in higher disease incidence (Vary *et al.*, 2015).

Shin and Yun (2010) reported that the climate change treatment, of 5°C and 700 ppm of CO_2, increased the incidence of bacterial spot and wilt on pepper by 25% and 24% respectively. In contrast, 550 ppm of CO_2 decreased the severity of the disease by more than 50% in oomycete plant interactions of soybean and *Peronospora manshurica* (Eastburn *et al*., 2010).

Elevated CO_2 of 700 μM mol^{-1} increased the tolerance to infection by *Phytophthora parasitica* in tomato plants. Under higher CO_2, greater vegetative growth in aerial parts and in the root systems might have compensated the loss of growth caused by the root pathogen (Jwa and Walling, 2001). High CO_2 has impacts on the genes involved in redox regulation, hormone-dependent processes, pathogenesis-related responses and secondary metabolism (Niu *et al*., 2016). These contrasting reports on the effects of elevated CO_2 concentrations on plant pathogen interactions are not adequate to draw any unifying principles, therefore, there is a need to expand research in this area.

2.7 Effect of pathogens on ginger

Although there is a rise in demand of ginger for food and medicine, the annual production of ginger is limited by the plant pathogens such as fungi, bacteria, viruses and nematodes (Le *et al*., 2014). With complete sterility, ginger propagated vegetatively hinders the development of resistance to diseases by crossbreeding. Low genetic diversity and high pathogen susceptibility are resulted from obligate asexual reproduction compared to sexual reproduction. (Thomas *et al*., 2016). Therefore at present, most of the available ginger cultivars are susceptible to several plant diseases (Nair and Thomas 2007b) such as soft rot caused by *Pythium* spp. (Le *et al*., 2014), bacterial wilt caused by *Ralstonia solanacearum* (Prasath *et al*., 2014) and yellows disease caused by *Fusarium* spp. (Meenu and Kaushal, 2017).

2.8 Incidence and impact of *Pythium* on ginger

The genus *Pythium* belongs to the class oomycetes and includes more than 120 species (Dick, 1990). Le *et al.* (2014) reported that ginger is vulnerable to at least 24 different plant pathogens, including fungi, bacteria, viruses and nematodes. Among these, *Pythium* spp. are of primary concern, as various species of the pathogen can cause rotting and loss of yield in ginger at any stage of growth, including post-harvest storage. The pathogens responsible for soft rot can cause severe losses during postharvest storage by infecting rhizomes from latent infections.

Most of the *Pythium* spp. thrives in the field when the soil temperature is as high as 26-30 ° C and the soil moisture is near or saturated (Stirling *et al*., 2009). The storage losses due to *Pythium* spp. can be very high, ranging from 24 to 50% and occasionally exceeding 90% have been reported in India (Nepali *et al*., 2000). The disease usually occurs during the months of June to September in India, coinciding with the southwest monsoon.

Symptoms of soft rot first appear on the aerial parts at the intersection of the rhizome and stems or "collar region" as brown water soaked lesions. As the lesions enlarge and coalesce, the stem rots and eventually collapses (Dohroo, 2005). The initial symptoms appear as yellowing of the tips of older leaves with the chlorosis gradually progressing along the margin downwards to the rest of the leaf blade and, finally to the sheath.

As older leaves exhibits advanced symptoms of wilting and necrosis, younger leaves also begin to develop a similar symptom progression until the whole plant dies (ISPS, 2005). Since there is less structural integrity between the stem and rhizomes the diseased stems can be easily dislodged. The ginger plants may survive if the infection is not severe, but they will remain weak and stunted. Rhizomes from diseased plants appear water soaked, brown, soft and rotten, and will gradually decay (Dohroo, 2005).

2.8.1 Disease incidence and severity

Normal soils may have as much as 6-18% CO_2 depending on microbial and root respiration, pH and organic matter decomposition. Even a 10 to 20 fold increase in

concentration of atmospheric CO_2 can be tolerated by most of the soil inhabiting fungi. Some soil borne plant pathogens, such as species of *Pythium*, *Phytophthora*, *Sclerotium* and *Aphanomyces* pathotypes of *Fusarium oxysporum* are well adapted and even multiply better at low oxygen levels and high CO_2 (Manning and Tiedemann 1995).

2.8.2 Percentage Disease Index

Disease index is the percentage of host tissues or organ covered by lesion or symptom or damaged by the disease. It indicates the extent of damage caused by the disease. Susceptibility of 6 cultivars of ginger Pulpally, Kunduli, Himachal, Maran, Varada, Suprabha against *P. aphanidermatum* was examined. All test cultivars except Kunduli were moderately susceptible to the rhizome rot pathogen. Among the six cultivars, Kunduli appeared to be the least susceptible while Varada was the most susceptible (38% loss in fresh wt of rhizome), followed by Suprabha (32%) and Maran (18.56 %) (Karmakar *et al*., 2003).

Three months old 56 accessions of *Z. zerumbet* were tested against *P. aphanidermatum*. Plants were observed frequently and the host pathogen interaction phenotype were scored for a duration of 30 days using 0-9 scale: 0 no symptoms, 1 up to 25% tiller death, 3 up to 50% tiller death, 5 up to 51-75% tiller death, 7 more than 75% death of tiller after 25 days of post inoculation and 9 more than 75% tiller death within 25 days post inoculation. Accessions with a DI value more than 20% were classified as susceptible and those with a value < 20% were considered as resistant. The mean DI values for 56 accessions varied from 0 to 72.22% (Kavitha and Thomas, 2007). Rocket plants (*Eruca vesicaria*) were grown in the phytotrons under different temperature and CO_2 combinations and disease incidence was found to be higher at 400-450 ppm of CO_2 by 22.90% and at 800-850 ppm of CO_2 by 43.40%, when the temperatures were 26–30°C and 26–30°C respectively and 0% for control plants (Chitarra *et al*., 2015).

2.8.3 Number of days taken for symptom development

Sharma *et al.* (2015) reported that when pigeonpea was grown at ambient (380 ppm), 550 ppm and 700 ppm CO_2 and seven day-old seedlings were inoculated with *P. cajani* zoospore, the blight symptoms appeared after 30 h of inoculation under ambient

condition, after 36 h under elevated CO_2 levels of 550 ppm and 40 h under 700 ppm. But it was observed that disease progressed faster under elevated CO_2 compared to ambient.

A reduction in powdery mildew caused by *Erysiphe graminis* on wheat and barley was observed with enhanced CO_2 levels of 700 µmol mol^{-1} (Thompson and Drake, 1994). On the other side, higher threats of late blight in potato (*Phytophthora infestans*) and blast (*Pyricularia oryzae*) and sheath blight (*Rhizoctonia solani*) of rice have increased day to day due to elevated CO_2 concentration and higher temperature (Gautam *et al*., 2013).

2.8.4 Post harvest incidence

The pathogens responsible for soft rot can attack at any stage of growth and even during postharvest storage it can cause severe losses. Most *Pythium* spp. prefer and even flourish in the field with high soil temperature (26-30°C) and high water content in soils (Stirling *et al*., 2009).

In recent studies on host-pathogen interactions in selected fungal pathosystems, two important trends have emerged related to the effects of elevated CO_2. First, initial pathogen establishment may be delayed because of changes in pathogen aggressiveness and/or host susceptibility. Second, pathogens were more fecund under elevated CO_2 (Coakley *et al.,* 1999). The combination of increased fecundity and conducive microclimate within enlarged canopies may provide more opportunities for severe infection. Increased CO_2 could influence plant disease resistance by altering the nutritional value of plant tissues, by modifying pathways involved in plant resistance, or by modulating pathogen vigor and virulence (Melloy *et al.,* 2014; Zhang *et al.,* 2015).

2.9 Effect of pathogen inoculation on physiological and biochemical paramaters

2.9.1 Plant pigments

Scarpari *et al.* (2005) reported that in *Theobroma cacao* plants infected by the pathogen *Crinipellis pernicious*, found to have reduced chlorophyll a and b contents. *Colletotrichum lindemuthianum* on *Phaseolus vulgaris* showed significant decrease in photosynthetic pigments, photosynthesis rate and consequent yield reduction (Lobato *et*

al., 2009). Studies indicated that there was significant loss in pigments when it was infected by pathogens (Berova *et al.,* 2007). When *Phaseolus vulgaris* plants (cv. Mexico 222) were inoculated with *C. lindemuthianum* (race 2047), there was decrease in chlorophyll content by 6.4%, 20.6% and 21.3% and carotenoid levels by 8.9%, 28.3% and 35% on the 4^{th}, 8^{th} and 12^{th} day of infection respectively (Lobato *et al*., 2010).

A study was undertaken to assess the impact of downy mildew caused by *Peronospora plantaginis* on chlorophyll pigments in *Plantago ovata* was found that Chl a was highest (0.80 mg g^{-1} FW) and Chl b (0.15 mg g^{-1} FW) in healthy leaves. A reduction of 30% and 60% in slightly and severely chlorotic leaves respectively was observed in comparition to healthy leaves. A similar trend was also observed for Chl b content in leaves. Healthy leaves had maximum Chl b and reduction of 25% in slightly chlorotic (0.11 mg g^{-1} FW) and 40% in severely diseased leaves (0.08 mg g^{-1} FW) were estimated (Mandal *et al.*, 2009)

Non enzymatic antioxidative carotenoids, such as beta carotene and xanthophylls, could reduce ROS and stabilized photosynthetic complexes under oxidative stress (Munné and Peñuelas, 2003).

2.9.2 Photosynthetic rate

A study was undertaken to assess the impact of downy mildew caused by *Peronospora plantaginis* on *Plantago ovate*. Significant differences in photosynthetic rate were observed among healthy (21 µmol CO_2 m^{-2} s^{-1}), slightly chlorotic (15 µmol CO_2 m^{-2} s^{-1}) and severely chlorotic leaves (7 µmol CO_2 m^{-2} s^{-1}) (Mandal *et al.*, 2009). Lobato *et al.* (2010) reported that the photosynthetic rate was reduced by *C. lindemuthianum* pathogen on *Phaseolus vulgaris* (cv. Mexico 222), by 22%, 49.9% and 77.3% on the 4^{th}, 8^{th} and 12^{th} days after inoculation respectively.

2.9.3 Membrane integrity

There was a change in membrane permeability in the early disease process when wheat seedlings were exposed to brown rust (Singh, 2006). Cell wall acts as a plant defense in basal resistance as a protective barrier against pathogen entry (Wang *et al.,*

2015). Vandana *et al.* (2019) reported that when resistant black pepper line (04-P24) and highly susceptible line (Sreekara) were studied for root resistance to *Phytophthora capsici*, a significant increase in the conductivity of water from 3rd day onwards in the susceptible line and 4th day onwards in the resistant line was evident. The greatest increase was observed on the 8th day after inoculation in both the lines of Sreekara inoculated (680 µs), Sreekara unoculated (80 µs), 04-P24 uninoculated (75 µs) and 04-P24 inoculated (300 µs).

2.10 Effect of pathogen inoculation on oxidative stress

2.10.1 Malondialdehyde (MDA)

Malondialdehyde which is used as an indicator of lipid peroxidation is one of the final products of polyunsaturated fatty acids in the cells. The MDA content was assessed in sunflower susceptible and resistant lines against white mold caused by *Sclerotinia sclerotiorum* (Davar *et al*., 2013). Garcia-Limones *et al.* (2002) reported that chickpea infected with *Fusarium oxysporum* had increased MDA content in both susceptible and resistant cultivars. The MDA levels in resistant line with the fungal inoculation and its control were 2 µg g^{-1} FW, 1.2 µg g^{-1} FW, and susceptible with the inoculation and its control were 2 µg g^{-1} FW and 0.9 µg g^{-1} FW respectively. Significant increase in MDA content was evident in the inoculated plants when compared to the control (Davar *et al*., 2013).

2.10.2 Enzymatic activity

Reactive oxygen species (ROS), including, hydroxyl radical, superoxide, singlet oxygen and hydrogen peroxide can disturb normal plant metabolism through oxidative damage to nucleic acids, proteins, photosynthetic enzymes, lipids and pigments (Ozkur *et al.*, 2009).

To overcome oxidative stress, plants have developed enzymatic and non-enzymatic antioxidant defense mechanisms to scavenge ROS (Smirnoff, 1993). The most important antioxidant enzymes are peroxidase, catalase, superoxide dismutases, phenylalanine ammonia lyase and glucanase. Phenylalanine ammonia-lyase (PAL) and

Tyrosine ammonia-lyase (TAL) are key regulatory enzymes involved in the phenyl propanoid pathway (Vogt, 2010).

The increased phenolics are correlated with abundance of *PAL* transcripts, which in turn corresponds to disease resistance (Ju *et al.,* 1995). Ghosh (2015) reported that when ginger rhizomes of the cultivar Suprabha was inoculated with *P. aphanidermatum*, PAL activity increased up to 14 days (13 µmol cinnamic acid mg^{-1} protein min^{-1}) and then declined in leaves of inoculated plants (9 µmol cinnamic acid mg^{-1} protein min^{-1}). There was no much difference in the PAL activity (2 µmol cinnamic acid mg^{-1} protein min^{-1}) in the control plants throughout the experimental period of 28 days.

Pathogenesis-related Proteins (PR) are synthesised in plants in response to several pathogens. β-1,3-glucanases (Class II PR proteins) are abundant proteins that catalyze the hydrolysis of β-1,3-glucans which is a major component of the cell wall of most fungi (Ceasar and Ignacimuthu, 2012). Over-expression of tobacco β-1,3-glucanase in transgenic groundnut has enhanced resistance against two major fungal pathogens, *Aspergillus flavus* and *Cercospora arachidicola* (Sundaresha *et al.*, 2010).

Catalase (CAT) is an oxygen-scavenging enzyme that removes toxic substrates *viz.,* H_2O_2 during development, which are otherwise lethal. Peluffo *et al*. (2010) reported a significant increase in the CAT activity by 3-fold in a resistant line of sunflower that was inoculated with *S. sclerotiorum*. *Sclerotinia* infection in sunflower plants highly induced CAT in resistant line (Davar *et al*., 2013). Nasir *et al.* (2017) reported that maximum CAT activity was observed in healthy plants of Kabuli genotype Noor-2009 while minimum in Desi genotype Bittle-98. CAT activity significantly decreased in leaves of phytoplasma-infected plants of Noor-2009.

When screening of tomato cultivars for bacterial spot, it revealed that upon pathogen inoculation, a drastic increase in Ascorbate peroxidase (APX) activity was evident in the seedlings of highly resistant cultivars compared to the susceptible ones (Chandrashekar and Umesha, 2012). Davar *et al*. (2013) observed that the APX activity in the resistant line increased when *S. sclerotiorum* was inoculated to sunflower at 12, 24

and 48 h after inoculation. However, at 48 h after inoculation, the APX activity was highest (3 folds).

Induction of defense-related enzyme activity of peroxidase, polyphenol oxidase, β-1,3 glucanase and chitinase was observed in banana (Grand Naine variety) plants on interaction with dead or live pathogen, *Fusarium oxysporum* f. sp. *cubense*, the causative agent of Panama disease (Thakker *et al.*, 2013).

2.10.3 Ascorbic acid

Several antioxidants, such as ascorbate, carotenoids, proline and glutathione provide non-enzymatic protection against ROS. Ascorbic acid is small, water soluble, reductone sugar acid with antioxidant properties and acts as a primary substrate in the cyclic pathway for enzymatic detoxification of a number of reactive oxygen species. It affects cell signaling during pathogenesis induced resistance (Khan *et al.*, 2016).

Results from studies by Dias *et al.* (2011) confirmed that ascorbic acid is the main precursor of oxalic acid in susceptible and resistant cocao infected by the hemibiotrophic fungus *Moniliophthora perniciosa.* Oxalic acid help in synthesis of H_2O_2 in plant pathogen interaction which play a role in inhibition of growth of biotrophic pathogens and could help in prevention of the infection/colonization process of necrotrophic pathogens in plants.

2.10.4 Phenols

Among the several defense mechanisms present in plants, the low molecular weight phenolics act as an inbuilt constitutive chemical barrier for plant diseases (Hammerschmidt 2005; Mary 2006). Systemic induction and accumulation of plant polyphenolics were evident in response to several diseases (Petkovsek *et al.*, 2008). When black pepper line (04-P24) showing resistance to *Phytophthora capsici* was studied in comparison with a highly susceptible line (Sreekara), the total phenolics peaked at 8^{th} day after inoculation in 04-P24. The total phenol content was 31 µg ml^{-1} (Sreekara inoculated), 10 µg ml^{-1} (Sreekara unoculated), 7 µg ml^{-1} (04-P24 uninoculated) and 11 µg ml^{-1} (04-P24 inoculated) (Vandana *et al.*, 2019).

2.10.5 Chlorophyll fluorescence signals

Biotic stresses (fungal, viral, bacterial or pathogen infection) have been shown to negatively affect the photosynthetic electron transport rate and downstream metabolic reactions (Rolfe and Scholes, 2010). Chlorophyll fluorescence imaging is an important tool to understand the activity of photosynthesis and also for phenotyping of the plants (Bueno *et al.,* 2019).

Muniz *et al.* (2014) reported that when cashew seedlings inoculated with two different isolates of *Lasiodiplodia theobromae* exhibited significantly lower FV/FM values respecting to the controls, as well as decays in ΦPSII and increases in NPQ values before any visual symptoms appeared. In the *Bipolaris sorokiniana* infected leaves of susceptible wheat plants, there was a progressive decrease of photosynthesis (measured as FV/FM and ΦPSII) which was correlated to the expansion of lesions, as well as to a progressive loss of chlorophyll (Rios *et al.,* 2017).

A study was undertaken to assess the impact of downy mildew caused by *Peronospora plantaginis* on *Plantago ovate.* Maximum fluorescence showed gradual decrease with the increase in disease pressure. Maximum quantum yield of PSII (Fv/Fm) was highest in healthy leaves (0.82) and infection by downy mildew pathogen significantly reduced it in both slightly (0.63) and severely chlorotic leaves (0.47). Significant reduction in PSII activity (ϕPSII) was observed in diseased leaves as compared to their healthy counterparts. The ϕ PSII was maximum in healthy leaves (0.32) and a reduction of 21.87% was recorded in slightly chlorotic leaves. However, the severely diseased leaves had very low ϕ PSII (0.09) (Mandal *et al*., 2009).

2.11 Molecular studies on the impact of elevated CO_2 on disease development

2.11.1 Protein profile

The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique is a powerful tool for estimating the molecular weights of proteins. Sinha and Prasad (1983) reported that amino acids and proteins are indicators of host-pathogen

interaction metabolites and they influence the course of disease development or of resistance in plants.

Four week old ginger cultivar Suprabha was inoculated with *P. aphanidermatum* (Edson) Fitz and comparison of protein patterns in leaves of inoculated and uninoculated ginger plants revealed that number of defence related proteins was 3 in the inoculated plants and 0 under uninoculated plants. Molecular weights of defence related proteins were 67, 56 and 14 kDa (Karmakar *et al.,* 2003).

2.11.2 Expression pattern in *PAL, CAD, CHS*

PCR-based assays are rapid techniques with high specificity and sensitivity, has been used for gene expression analysis. The Real-time PCR is based on the labelling of primers, probes, or amplicon with fluorogenic molecules and allows detection of the target fragment to be monitored while the amplification is in progress.

Transcriptome profiling of a resistant turmeric genotype (Cl210-27) after 72 h of inoculation with *P. aphanidermatum*, the transcripts associated with the phenylpropanoid metabolic pathway, including trans-cinnamate 4-monooxygenase (8.29 fold), caffeic acid 3-O-methyltransferase (7.41 fold) and cinnamyl alcohol dehydrogenase (6.77 fold) exhibited induced expression (Chand *et al.,* 2016). Guo *et al.* (2016) reported that from 8 to 48 h post-infection with Tomato yellow leaf curl virus artificially, elevated CO_2 increased the expression of genes encoding *PAL5* and *PR1*a involved in the SA signaling pathway in tomato variety Moneymaker. *PHENYLALANINE AMMONIA LYASE*, the core gene involved in the phenylpropanoid biosynthetic pathway was induced in *Z. zerumbet* following pathogen inoculation. Transcripts of the genes involved in the monolignol biosynthetic pathway including *4-COUMARATE-CoA LIGASE 2*, *CINNAMOYL-CoA REDUCTASE* , *CAFFEIC ACID/5-HYDROXYFERULIC ACID O-METHYLTRANSFERASE* 1 and *CINNAMYL ALCOHOL DEHYDROGENASE 2* were strongly elevated in *Z. zerumbet* upon pathogen inoculation (Geetha *et al.,* 2019).

Material and methods

3. MATERIALS AND METHODS

The experiment was undertaken with the main objective of assessing the impact of elevated CO_2 on rhizome development, yield, quality and tolerance to soft rot in ginger (*Zingiber officinale* Rosc.) through morphological, physiological, biochemical and molecular analysis. Pot experiments were under taken in the Open Top Chamber (OTC) facility present at the Department of Plant Physiology, College of Agriculture, Vellayani, Thiruvananthapuram.

3.1 EXPERIMENTAL DETAILS

3.1.1 Location

The pot experiments were conducted in OTC facitity at College of Agriculture Vellayani, located at 8°5' N latitude and 76°9' E longitude and an altitude of 29 m above mean sea level.

3.1.2 Planting material

Ginger rhizomes of varieties Aswathy, Athira and cv. Maran were procured from Regional Agricultural Research Station, Ambalavail, Wayanad, Kerala. Wild ginger (*Zingiber zerumbet*) was collected from *Thenmala* area near Punalur town, Kollam district in *Kerala.*

3.1.3 Layout of the Experiment

The experiment was conducted in CRD with two treatments and four replications.

3.1.4 Technique for CO_2 enrichment

Open Top Chambers (OTC) facility is the square type chamber designed to maintain elevated and near natural CO_2 conditions for research environments. The basic structure of OTC is built of metal frame and installed in the experimental field. It is covered with UV poly sheet of 200 micron. The chamber is 3 m X 3 m X 3 m dimension, 45° slope and 1 m^2 opening at the top, CO_2 was released into the chamber from a CO_2 cylinder in a controlled manner for the entire active experimental period during the day

time. Measurements of the microclimatic parameters (temperature and humidity) were done inside and outside the OTC with the help of sensors on a real time basis. On an average basis, mean temperature of 36.2 °C and relative humidity of 87.60% were recorded inside chamber during the experimental period.

The 500 ppm of elevated CO_2 concentration was opted based on the report from IPCC (2007) which stated that atmospheric concentrations of carbon dioxide have been steadily rising with an average annual increase rate of 2 ppm and continues to rise 500-1000 ppm by the year 2100.

3.2 EXPERIMENT I

Assessing the impact of elevated CO_2 on growth, development and quality of ginger

3.2.1 Treatments:

(1) Conditions:

T_1- OTC with elevated CO_2 (500ppm)

T_2- open control

(2) Varieties:

1. Aswathy

2. Athira

3. Maran

3.2.2 Methodology

Treated rhizomes (0.3% mancozeb for 30 min) of all the three varieties selected based popularity and high yielding ones were grown in protrays and two month old plants were transplanted to pots. The potting mixture of sand soil and farmyard manure were mixed in 2:1:1 ratio was utilised. Nutrients were supplied as per POP recommendation. One set of 3 month old plants were transferred to OTC and another set was retained at the ambient CO_2 (Plate 1).

3.2.3 Observations recorded

Growth, biochemical and physiological parameters were recorded at 4^{th}, 5^{th}, 7^{th} and 8^{th} month after planting (MAP) (Plate 2). Observations on yield and yield parameters, quality parameters and tissue nutrient status were made at 8 MAP.

3.2.4 Morphological parameters

3.2.4.1 Growth parameters

3.2.4.1.1 Plant height

The plant height was measured from the base of the plant (soil level) to the tip of the top leaf of the main shoot (pseudostem) and expressed in cm.

3.2.4.1.2 Number of leaves

The number of fully opened leaves of each tiller from sample plants was counted.

3.2.4.1.3 Specific leaf area **(SLA)**

Leaf area was measured from the fully expanded third leaf of the main pseudostem. Leaves were dried for 2 days at 80°C and dry weight was recorded. SLA was calculated using the formula:

$$\text{SLA } (cm^2\ g^{-1}) = \frac{\text{Leaf area}}{\text{Dry weight}}$$

3.2.4.1.4 Root weight

The fresh root weight from control and treated plants were recorded after separating roots from rhizomes by destructive sampling and are expressed in g $plant^{-1}$.

3.2.4.1.5 Shoot weight

The shoot fresh weight after separating the rhizome from control and treated plants were recorded by destructive sampling and are expressed in g $plant^{-1}$.

3.2.4.1.6 Stem diameter

The stem diameter was measured from the tallest pseudostem, 5 cm away from the base of the plant and expressed in cm.

3.2.4.1.7 Number of tillers

The number of aerial shoots arising around a single plant was counted.

3.2.4.2 Yield parameters

3.2.4.2.1 Total fresh weight of rhizome

The rhizomes after harvest (8 MAP) were washed to get rid of soil particles. After removing the roots, the rhizomes were weighed and expressed in g plant^{-1}.

3.2.4.2.2 Total dry weight of rhizome

The rhizomes after harvest (8 MAP) were washed to get rid of soil particles and after removing roots were allowed to dry under sun for one week. It was then kept in hot air oven at 80-100°C till constant weight was obtained and expressed in g plant^{-1}.

3.2.4.2.3 Number of fingers per rhizomes

The number of fingers per rhizome was determined as the number of rhizome fingers produced from mother rhizome

.3.2.4.2.4 Rhizome spread

The maximum width of the rhizomes was measured and expressed in cm.

3.2.4.2.5 Rhizome thickness

Vernier calliper was utilized to measure the rhizome thickness and expressed in cm.

3.2.5 Physiological and Biochemical parameters

3.2.5.1 Total chlorophyll

Leaves collected from both control and OTC grown plants were washed in distilled water and used for the estimation of chlorophyll. The leaf bits of 100 mg were

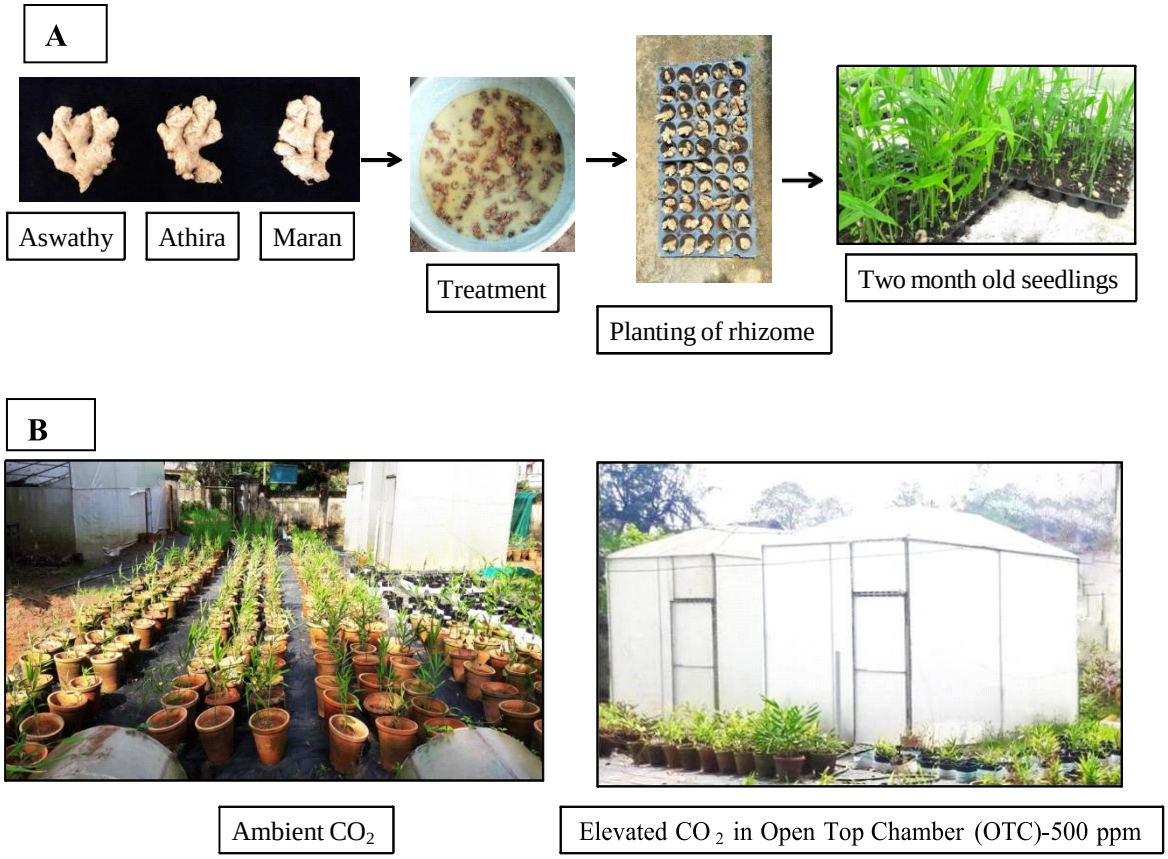
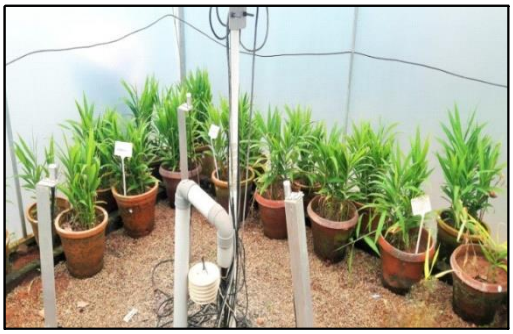


Plate 1. A) Methodology followed for raising seedlings
B) Seedlings transplanted to pots and placed at ambient and elevated CO_2

4MAP



6MAP

8MAP

Plate 2. Growth phases of ginger plants under elevated CO_2

incubated in 80 % Acetone: DMSO (Dimethyl sulfoxide (1:1 v/v) mixture in the dark for overnight. The chlorophyll extract was drained into measuring cylinder and the final volume was made up to 10 ml. Using UV-visible spectrophotometer the absorbance was recorded at 663 and 645 nm. Total chlorophyll content was estimated as described by Arnon (1949) and was calculated by using the formula and expressed as mg g^{-1} of FW.

Total chlorophyll = [{20.2 (A645) + 8.01 (A663)} X volume/ weight] X 1000

3.2.5.2 Stomatal conductance

Stomatal conductance was measured between 8:30 am and 11 am using a portable photosynthetic system (CIRAS-3, PP systems U.S.A) and was expressed in mmoles m^{-2} s^{-1}.

3.2.5.3 Photosynthetic rate

Photosynthetic rate was measured between 8:30 am and 11 am using portable photosynthetic system (CIRAS-3, PP systems U.S.A) and was expressed in µmoles CO_2 m^{-2} s^{-1}.

3.2.5.4 Transpiration rate

Transpiration rate was measured between 8:30 am and 11 am using portable photosynthetic system (CIRAS-3, PP systems U.S.A) and was expressed in mmoles H_2O m^{-2} s^{-1}.

3.2.5.5 Total protein

Total protein content was estimated as described by He (2011) using bovine serum albumin as the standard. Ginger sample of 0.5 g was homogenised in 2.5 ml of ice cold extraction phosphate buffer (pH- 7.8) containing 1 mM EDTA, 2% (w/w) and isolation was done at 4^{o}C on ice. The samples were centrifuged at 15000g for 20 min at 4^{o}C. To the 100 µl of the supernatant 3 ml of Bradford reagent was added and mixed well. The absorbance of the solution was recorded within 30 min using the BSA standard in the range of (10-100 mg) at 595 nm using spectrophotometer. The total protein content was expressed as mg g^{-1} DW.

3.2.5.6 Total sugars

Total sugars were estimated utilising the procedure described by Sadasivam and Manickam (2008). Each rhizome samples of 100 mg were taken in test tubes and were hydrolysed by boiling in a water bath for 3 h with 5 ml of 2.5N HCl. The samples were allowed to cool down to room temperature and were neutralized by adding sodium carbonate until effervescence ceases. These solutions were made up to 100 ml. After centrifuging, the supernatant was collected and 1ml of solution was taken for analysis. To this anthrone reagent of 4 ml was added. This mixture was heated for 8 min (water bath) and cooled rapidly. Absorbance values were measured at 630 nm using spectrophotometer. Final values were obtained from a standard graph prepared from working standard glucose solution and expressed in mg g^{-1} DW.

3.2.5.7 Tissue nutrient status

The rhizomes samples collected from both ambient and elevated CO_2 grown plants were initially sundried and then oven dried until it reached to a constant weight. The dried rhizomes were ground and sieved finally. Nitrogen was estimated by micro kjeldahl method (Jackson, 1973). P, K, Fe, Cu, Zn were analysed by diacid extracts. This was prepared by digesting 1 g of the sample in 10 ml of 9:4 concentrated nitric acid and perchloric acid mixture. Aliquots of the digests were taken for the analysis. 'P' was determined by vanadomolybdate yellow colour method. The yellow colour was read using spectrophotometer at a wave length of 470 nm. K was estimated using flame photometer. Fe, Zn and Cu were estimated using atomic absorption spectrophotometer and the content of each nutrient was calculated and expressed in ppm. The NPK content were expressed in mg g^{-1} DW.

3.2.6 Quality parameters

3.2.6.1 Fiber content

For crude fiber estimation, 2 g of rhizome was weighed in a beaker and 50 ml of 10% v/v nitric acid was added. This mixture was heated to boil with constant stirring and strained through fine cloth on a Buchner funnel. Residue was washed with hot water and

transferred to a beaker from cloth. To this 50 ml of 2.5% v/v NaOH was added, heated and maintained at boiling point for 30 min with constant stirring. The residue was strained and washed with hot water as mentioned earlier. For quantitative determinations, the residue was transferred to a cleaned and dried crucible. The residue was weighed and expressed as mg g^{-1} (Chulet *et al*., 2010).

3.2.6.2 6-Gingerol estimation

Gingerol content in the rhizomes was estimated at the Sophisticated Analytical Instrument Facility available at Indian Institute of Horticultural Research, Bengaluru. Liquid chromatography–mass spectrometry (LC-MS) analysis was done for estimation of gingerol. The extracted oleoresin (50 µl diluted to 1ml in methanol) 5 µl was injected to LC-MS and final values were obtained from standard graph prepared from working standard of [6]-Gingerol and is expressed in mg g^{-1} FW.

3.2.6.3 Phenol content

The phenol content was estimated according to the protocol described by Bray and Thorpe (1954). One g of the rhizome sample was homogenized in 10 ml (80%) of ethanol. After centrifugating the homogenate at 10,000 rpm for 20 min, the supernatant was collected and the residue was reextracted with 5 times the volume of 80% ethanol and centrifugation was repeated. The supernatant was evaporated in a boiling water bath until the phenolic residues were left over. This residue was dissolved in 5 ml of distilled water. An aliquot of 0.2 ml was pipetted out and made up to 3 ml with distilled water. Folin- Ciocalteau reagent (0.5 ml) and 2 ml of 20% sodium carbonate solution were added to each tube after 3 min. This was mixed thoroughly and kept in boiling water for 1 min. The reaction mixture was cooled and absorbance was measured at 650 nm against a reagent blank. Standard curve was prepared using different concentrations of catechol. Phenol content was expressed as mg g^{-1} DW.

3.2.6.4 Total oleoresin

Total oleoresin was extracted using Soxhlet apparatus (Braga *et al.,* 1998). Twenty gram of dry ginger sample was wrapped to form a thimble. Thimble was placed

inside Soxhlet extractor. In a round bottom flask 300 ml of acetone was heated to boiling. Vapours raised due to boiling reached the distillation arm and fell into sample. As a result ginger rhizome immersed in the acetone extracted the oleoresins from cells. When the Soxhlet chamber was nearly filled, the chamber got emptied automatically by siphon side arm by solvent running back to the distillation flask. This process was repeated for 3 h until all the oleoresin was extracted. Round bottom flask now contained both solvent and extract. The solvent was removed before it siphoned back to flask and the extract was collected. Total oleoresin content in the sample was expressed in percentage.

Percentage of oleoresin (V/W) = (Volume of extract (ml)/ Weight of sample (g)) x 100

3.2.6.5 Ascorbic acid

The ascorbic acid in the dry rhizome was volumetrically measured as per the method of Harris and Ray (1935). A mixture of 10 ml of 4% oxalic acid and 5 ml of working standard solution was titrated against dye (V_1 ml). The end point of the reaction was appearance of pink colour. The dye consumed for obtaining the end point is equivalent to the amount of ascorbic acid. Sample (1 g of rhizome) was taken and extracted with 4% oxalic acid. The extract was made up to 100 ml and centrifuged. Five ml of supernatant was pipette out from that and added 10 ml of 4% oxalic acid. This solution was titrated against dye (V_2 ml) until the appearance of pink colour. The dye used was Dichlorophenol 2 indophenol (DCPIP) solution.

$$\text{Amount of ascorbic acid mg/100g sample} = \frac{0.5\text{mg}}{V_1\ \text{mL}} \times \frac{V_2}{5\ \text{mL}} \times \frac{100\ \text{mL}}{\text{Weight of the sample}} \times 100$$

3.2.6.6 Starch content

The estimation was done by anthrone method by Sadashivam and Manickam (2008). Five hundred mg of rhizome was homogenized in hot 80% ethanol to remove sugars. The homogenate was centrifuged. After centrifugation, the retained residue was washed repeatedly with 80% ethanol till the washing did not give colour with anthrone reagent. This residue was dried in water bath, and to this 6.5 ml perchloric acid and 5 ml distilled water was added and then centrifuged at 0° C for 20 min. The supernatant was saved for estimation. Again this extraction was repeated with perchloric acid. The

supernatant was made up to 100 ml and from this 0.1 ml was taken and made up to 1ml with distilled water. To this 4 ml of anthrone reagent was added and these tubes were heated for 8 min and cooled rapidly. The intensity of colour change was measured at 630 nm. The standard curve was used to obtain glucose content. This glucose value was multiplied by a factor of 0.9 to calculate the starch content in sample which is expressed in per cent.

3.2.6.7 Essential oils

Essential oil content of ginger rhizome was extracted by hydro-distillation method using Clevenger apparatus (Pruthi, 1993). The essential oil constituents have much lower boiling point than that of water; hence they volatilize and get distilled before water vapour. Dry 20 g ginger powder was mixed in deionised water (300 ml) and was poured into a 1 litre round bottom flask and allowed to distill for a period of 3 h. After distillation, water and oil got separated due to difference in specific gravity. Total essential oil in the sample was expressed in percentage.

Percentage of essential oils = (Volume of oil (ml) / Weight of sample (g)) X100

3.3 EXPERIMENT II

Assessing the impact of elevated CO_2 on the response of ginger to *P. aphani dermatum*

3.3.1 Treatments:

(1) Conditions

T_1 - OTC with elevated CO_2 (500 ppm).

T_2 - Open control

(1) Varieties:

1. Athira
2. Aswathy
3. Maran
4. *Zingiber zerumbet*

Replications- 3

Number of pots/replication-2

Design- CRD

3.3.2 Methodology

Plants were raised as in the case of Experiment I and were maintained under open field conditions. After 3½ months, one set of plants were transferred to OTC and allowed to acclimatize for a period of 15 days and another set was maintained under open field condition. Pathogen inoculation was given to four month old plants with uniform growth habits through soil inoculation.

Standardization of medium for multiplication was carried out in order to inoculate *Pythium aphanidermatum* to soil with modifications (Nikam *et al*., 2011). Autoclaved river bed sand, powdered maize, powdered corn, FYM were mixed thoroughly in the ratio (sand: maize (9:1), sand: maize (19:1), sand: FYM: soil (1:1:1), sand: corn (9:1) and sand: corn (19:1) with double distilled water and about 20 per cent of each was added to pre autoclaved tissue cultured bottles. Then the media was autoclaved for 40 min and autoclaved again after 24 h. Five mm discs of three day old subcultured *P. aphanidermatum* (procured from Department of Plant Pathology, Kerala Agricultural University, Thrissur) maintained at room temperature (25 °C) in potato dextrose agar (PDA) medium was inoculated in to the media. Allowed to colonize for 10 days and bottles were shaken every other day to ensure even distribution throughout the media (Plate 3). For the study sand: maize meal medium of 9:1 was utilised since the pathogen could efficiently multiply and abundant growth of mycelia was evident and subsequently mixed with the top soil in each pot (80 g pot^{-1}).

3.3.3 Disease incidence and severity

3.3.3.1 Percentage Disease Incidence (PDI)

The number of plants infected to the number of plant assessed was recorded after one week of inoculation. PDI was calculated using the formula and expressed in percentage.

Sand: maize meal medium (9:1)

Sand: maize meal medium (19:1)

Sand: corn meal medium (9:1)

Sand: corn meal medium (19:1)

Sand: fym: soil medium (1:1:1)

Plate 3. Standardisation of meal medium for multiplication of *P. aphanidermatum*

$$PDI = \frac{\text{No. of infected plants}}{\text{Total no. of plant assessed}} \times 100$$

3.3.3.2 Percentage Disease Index

The per cent disease index (PDI) was calculated after 20 days of pathogen inoculation using formula:

Disease index = [(sum of scores)*100/ (N * maximum score)]

N being the number of plants inoculated per variety

Rhizome rot was assessed on 0-4 scale as 0 =no infection; 1= 1-25% area infected; 2= 26-50% area infected and rotted; 3= 51-75% area rotted; 4 =76-100% area rotted due to infection (Shanmugam *et al*., 2013).

3.3.3.3 Number of days taken for symptom development

Plants were observed regularly from the day of inoculation and the day of first symptom appearance *i.e.*, yellowing of the tip of the lower leaves was recorded.

3.3.3.4 Post harvest incidence / severity of the disease

From the day of harvest, the rhizomes were spread under shade and were observed for the incidence of disease for a period of two months.

3.3.4 Growth parameters

3.3.4.1 Number of leaves

Number of leaves was recorded as in case of experiment 1 after one week of inoculation.

3.3.4.2 Plant height

The height of the plant was measured same as in case of experiment 1 after one week of inoculation.

3.3.4.3. Number of tillers

Number of tillers was recorded as in case of experiment I after one week of inoculation.

3.3.4.5 Total fresh weight of rhizome

The fresh rhizome weight was recorded as in case of experiment I on the 20^{th} day after inoculation.

3.3.4.6 Total dry weight of rhizome

The rhizomes after harvest were washed and then kept in hot air oven at 70-80°C till constant weight was obtained.

3.3.5 Physiological and biochemical observations

3.3.5.1 Chlorophyll a, b, total chlorophyll and carotenoids

Leaf bits collected from both control and treated plants after one week of inoculation were washed in distilled water and used for the estimation of chlorophyll and carotenoid pigments. Pigments were extracted from leaf bits by using acetone (80 %): DMSO (Dimethyl sulfoxide) (1:1 v/v) mixture. The leaf bits were incubated in acetone: DMSO solution in the dark for overnight. The coloured solution was decanted into measuring cylinder and made up to 10 ml. The absorbance was recorded at 663, 645, 510 and 480 nm using UV-visible spectrophotometer. Total chlorophyll was estimated as described by Arnon (1949). Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were calculated by using the following formulae and expressed as mg g^{-1} FW.

Chlorophyll a = [{12.7 (A663) – 2.69 (A645)} X volume/ weight] X 1000

Chlorophyll b = [{22.9 (A645) – 4.68 (A663)} X volume/ weight] X 1000

Total chlorophyll = [{20.2 (A645) + 8.01 (A663)} X volume/ weight] X 1000

Carotenoid content= [{7.6 (A480) – 1.49 (A510)} X volume/ weight] X 1000

3.3.5.2 Xanthophyll

Xanthophyll was estimated in control and treated plants after one week of inoculation as per Lawrence (1990). One hundred mg of leaf was weighed in to a 100 ml volumetric flask. Pipetted 2 ml of 40 per cent methanolic KOH into flask and stirred for one min. Refluxed the flask in a water bath and cooled the samples. Kept the sample for one h then pipetted 30 ml hexane into flask. Shaken for one min and made up the volume with 10 per cent sodium sulphate solution and shaken vigorously for one min. After one h, the upper phase was collected in a 50 ml volumetric flask. Pipetted 3 ml of upper phase into 100 ml volumetric flask and made up the volume with hexane and mixed well. The absorbance was measured at 474 nm.

Total xanthophylls content in the sample was calculated by using the formula

$$\text{Total xanthophylls (mg/g)} = \frac{A_{474} * D}{W * 236}$$

Where,

A_{474} = Absorbance at 474 nm

W= Weight of the sample in g

D= Final dilution = $\frac{50*100}{3}$

236= Trans-lutein specific absorptivity for 1g/ litre

3.3.5.3 Photosynthetic rate

Photosynthetic rate was measured similarly as in case of experiment I from the diseased and control plants after one week of inoculation and was expressed in µmoles CO_2 m^{-2} s^{-1}.

3.3.5.4 Membrane integrity

Membrane integrity was calculated in terms of percentage leakage (Chatti and Manju, 2017). Fully expanded leaves from both control and inoculated plants were

excised with their petioles intact in water and allowed to regain turgidity by incubating in distilled water for 45 min. Turgid weight was taken and leaves were allowed to wilt for three h. After 40 to 60% loss of the fresh weight, leaf punches of 1 cm diameter were taken and washed for 1-2 min to leach out their solutes from the cut ends and blotted on clean filter paper. Ten leaf punches were incubated in test tubes containing 20 ml distilled water for 3 h. Leakage of the solutions in their bathing medium was estimated by recording its absorbance at 273 nm (initial leakage of solutes). Test tubes were incubated in hot water bath (100^{o} c) for 15 min. Absorbance of bathing medium was again read out at 273 nm to indicate final absorbance.

% leakage = Initial absorbance of bathing medium / Final absorbance of bathing medium x 100

3.3.6 Oxidative stress

3.3.6.1 Malondialdehyde

Melondialdehyde (MDA) is a decomposition product of lipid peroxidation and can be used as index for lipid peroxidation. The MDA content was determined after the one week of pathogen inoculation by the reaction of thiobarbituric acid (TBA), as described by Yagi (1998). Leaf tissue (0.5 g) was collected and homogenized in 2 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 5 min. One ml of solution containing (4% (w/v) TCA+0.5% TBA (w/v)) was added to 0.5 ml of supernatant. The mixture was heated at 95^{o}C for 1 h, then cooled down to room temperature and centrifuged at 10,000 rpm for 5 min. Absorbance of the clear solution was measured at 532 nm and corrected for non specific turbidity by subtracting the absorbance at 600 nm.

3.3.6.2 Catalase activity

CAT Activity was measured on 2^{nd} day after symptom development according the method described by (Aebi *et al*., 1984). Enzyme extract was prepared by freezing 0.3 g of leaf sample in liquid nitrogen to prevent proteolytic activity followed by grinding with 2 ml extraction buffer 100 mM phosphate buffer (pH 7.5) containing 0.5 mM EDTA.

Extracts were centrifuged at 4^{o}C for 20 min at 15,000 g and the supernatant was used for enzyme assay.

The 3.0 ml reaction mixture consisted of 1.5 ml of potassium phosphate buffer (100mM buffer, pH 7.0), 0.5 ml of 75 mM H_2O_2, enzyme 50 µl, and 950 µl double distilled water. Addition of H_2O_2 started the reaction. The rate of decrease in absorbance at 240 nm was recorded for 1 min using UV visible spectrophotometer and absorption coefficient of 39.4 mM^{-1} was used to calculate the enzyme activity. The amount of CAT that can decompose 1 µmol H_2O_2 min^{-1} was defined as one unit.

3.3.6.3 Ascorbate peroxidase activity

APX was assayed on the 2nd day of symptom development by recording the decrease in absorbance due to ascorbic acid at 290 nm assuming an absorption coefficient of 2.8 Mm^{-1}(Nakano and Asada, 1981). Enzyme was extracted using the same procedure as followed for catalase. For assay, the 3 ml reaction mixture contained 1.5 ml of potassium phosphate buffer (100 mM) pH 7.0, ascorbic acid 0.5 ml of 3.0 mM, EDTA 0.1 ml (3.0 mM), 0.1 ml enzyme, and 900 µl double distilled water. The reaction was started with the addition of 0.2 ml of 3.0 mM of hydrogen peroxide. Decrease in absorbance was recorded for a period of 30 sec using UV visible spectrophotometer. The amount of APX that can oxidise 1µmol of ascorbic acid min^{-1} was defined as one unit.

3.3.6.4 Phenylalanine ammonia-lyase activity

Samples for the estimation of PAL activity were homogenized in sodium borate buffer (pH 8.8) using a cold mortar and pestle (0.3 g FW/2 ml of buffer) and homogenates were immediately centrifuged (12,000 ×g, 15 min, 4 ◦C). The reaction mixtures consisting of 500 µl buffer and 350 µl of homogenates were pre-incubated at 40°C for 5 min and the reaction was started by addition of 300 µl (50 mM) L-phenylalanine. After 1 h of incubation at 40°C, the reaction was stopped by the addition of 50 µl 5 N HCl. Parallel controls without L-phenylalanine addition were analyzed to determine plant endogenous transcinnamic acid (CA) content. The amount of CA was calculated from the calibration curve prepared with different CA concentrations. Proteins were quantified as in case of experiment 1. The amount of CA was monitored at 275 nm

(Kovaˇcik and Klejdus, 2012). The enzyme activity was expressed as nmoles of trans-cinnamic acid formed in mg protein^{-1} min^{-1}.

3.3.6.5 Ascorbic acid content

Ascorbic acid content was estimated in the diseased and healthy leaves after one week of inoculation similarly as followed in case of experiment I and expressed in terms of mg 100 g^{-1} FW.

3.3.6.6 Phenols

The phenol content was estimated following the procedure as in case of experiment I from the leaves of diseased and healthy plants after one week of inoculation symptom development and expressed in terms of mg g^{-1} DW.

3.3.6.7 Glucanase activity

The activity of glucanase in the leaf tissue after 2nd day of symptom development was measured according to the procedure of Cheng and Kuc (1995). Enzyme was extracted using 0.3 g sample in 2 ml (0.05 M) sodium acetate buffer (pH 5.2) and 2.5 mM EDTA. The homogenates were centrifuged at 8000 rpm 10 min, 4° C. Crude enzyme extract, 62.5 µl, was added to a 62.5 µl laminarin 4% (0.1 M sodium acetate buffer, pH 5.2) and incubated at 40°C for 10 min. The reaction was stopped by adding 375 µl of dinitrosalicylic reagent and boiled for 5 min in a water bath. The resulting coloured solution was diluted with 4.5 ml of water, vortexed and its absorbance at 500 nm was measured (Shanmugam *et al*., 2013). The glucanase activity was calculated using the standard curve of glucose. The unit activity was expressed as µmoles of glucose formed in mg protein^{-1} min^{-1}.

3.3.6.8 Determinationof H_2O_2 and superoxide by staining

H_2O_2 was determined by 3,3′-Diaminobenzidine (DAB) staining technique described by (Kumar *et al*., 2014). The diseased and control leaves after 2nd day of symptom development were dipped in DAB solution prepared by dissolving 50 mg DAB in 45 ml distilled water in an amber colored bottle. The pH was adjusted to 3.8 using 0.1

N HCl while mixing properly on a magnetic stirrer. The volume was made to 50 ml to get 1 mg ml^{-1} solution. DAB staining solution was prepared freshly before use.

Superoxide was by Nitro blue tetrazolium chloride (NBT) staining technique as described by (Kumar *et al*., 2014). In an amber coloured bottle, 0.1 g of NBT was dissolved in 50 mM sodium phosphate buffer (pH 7.5) and volume was made up to 50 ml to get a 0.2% solution. The solution was mixed thoroughly using a magnetic stirrer. The control and treated leaves were placed in the falcon tubes and the tubes were wrapped with aluminium foil and kept overnight at room temperature. The solution was drained off the staining solution from the test tubes.

Chlorophyll was removed for proper visualization of the stain. This was done by immersing the leaves in 3:1 (ethanol: glacial acetic acid (85 %)) and heating in a boiling water-bath at 50-55°C. The stained leaves were photographed against a contrast background for proper documentation.

3.3.6.8 Cell death

Cell death was detected using trypan blue technique in the leaves on the 2nd day of symptom development. A trypan blue stock solution was prepared by mixing 10 g of phenol, 10 ml of glycerol, 10 ml of lactic acid, 10 ml of distilled water, and 0.02 g of trypan blue. This stock solution was further diluted with ethanol (96%; 1:2, v/v) to obtain trypan blue working solution. The control and treated leaves were immersed by soaking in trypan blue working solution in a falcon tube and were boiled in a water bath for 1 min, incubated in the working solution for 1 day and cleared by immersing the leaves in 3:1 (ethanol : glacial acetic acid (85 %)) by heating in a boiling water bath at 50 -55°C (Pogany *et al*., 2009).

3.3.7 Chlorophyll fluorescence signals

Chlorophyll fluorescence kinetics was measured after one week of inoculation in the intact leaves. The measurements were made using portable photosynthetic system (CIRAS-3, PP systems U.S.A) fitted with CFM-3 chlorophyll fluorescence module at ambient CO_2 concentration as per the manufacturer's manual. The leaves were dark

adapted for 20 min. The minimal fluorescence (F_0) was measured with sufficiently low intensity modulated light and a maximal fluorescence (Fm) was determined by a short (0.8 s) saturating pulse. The difference between F_0 and Fm is the variable fluorescence (Fv). Then, the leaves were continuously illuminated with white actinic light and the steady-state value of fluorescence (Fs) was recorded. Thereafter, a second saturating pulse similar to measuring Fm was applied to record maximal fluorescence in the light adapted leaf (Fm^1).

The actual PSII quantum efficiency (ΦPSII) was determined in the light-adapted state using the formulae

$$\phi PSII = (Fm^1 – Fs)/Fm^1$$

The apparent electron transport rate (ETR), was determined using the formulae

ETR = PAR x 0.84 x 0. 5 x ΦPSII

PAR= Photosynthetically active radiation

3.4 EXPERIMENT III

Molecular studies on the impact of elevated CO_2 on the response of ginger to *P. aphanidermatum*

3.4.1 Protein profile

The leaves of the varieties Aswathy and Athira, both diseased and control raised in ambient and elevated CO_2 were used for the profiling of proteins on the 2^{nd} day of symptom development (fourth day after inoculation).

3.4.1.2 Gel preparation and casting of gel

The gel unit was cleaned thoroughly using distilled water and ethanol and the dried glass plates were assembled properly. Resolving gel (10%) was prepared using 30% acrylamide stock solution, 1.5 M tris HCl (pH 8.8), 10% SDS, distilled water, 10% ammonium per sulphate solution and TEMED (N,N,N',N'-tetramethylethylene-1-diamine). It was mixed gently and the gel solution was carefully poured between the

glass plates to the three-forth level. Overlaid with distilled water to prevent contact with air for proper polymerization and allowed it to polymerize. Stacking gel (5%) was prepared using 30% acrylamide stock solution, 0.5 M tris HCl (pH 6.8), 10% SDS, distilled water, 10% ammonium per sulphate (APS) solution and TEMED. Overlaid water was removed and stacking gel was poured between glass plates. Comb was placed appropriately and allowed to set. Placed the gel plate in the Biorad electrophoresis apparatus and poured the electrode buffer containing tris base, glycine and SDS. The comb was carefully removed from the gel assembly.

3.4.1.3 Sample preparation and gel loading

The wells were flushed with the buffer using a syringe to remove residual gel remains before loading samples. Protein concentration in each sample was adjusted to 40 µg and it was mixed well with the sample buffer containing 0.5 M tris HCl (pH 6.8), 0.5% bromophenol blue, 0.5% β-mercaptoethanol, 0.1 ml glycerol and 10% SDS. Samples were heated at 93°C for 3 min before loading to ensure complete interaction between proteins and SDS. The protein samples were allowed to cool and samples along with a pre-stained protein ladder (5 µl) were loaded in the wells.

3.4.1.4 Electrophoresis

Electrode buffer was poured into the buffer tank and electrophoresis was conducted at 60 V until dye front reached the stacking gel and 75 V for 1 to 1.5 h until dye front reached the bottom.

3.4.1.5 Gel staining and destaining

After the run was complete, gel was removed carefully from the glass plates and immersed in the staining solution (0.1%) for two to three h with occasional shaking. Staining solution was prepared by mixing Coomassie brilliant blue R 250 (0.1 g), glacial acetic acid (10 ml), methanol (40 ml) and distilled water (50 ml). After staining, gel was transferred to a destaining solution containing all components except dye and allowed to destain till the bands were visible. Dye that did not bind to the proteins was removed. The gel was photographed after proper destaining.

3.4.2 Expression pattern of *PAL, CAD, CHS*

3.4.2.1 Isolation of RNA

The total RNA was isolated from leaves of varieties, Aswathy and Athira (two biological replicates) on the 2nd day of symptom development utilizing TRI reagent (Merck). Mortar and pestle, microtips, microfuge tubes, forceps and reagents sterilised by double autoclaving. DEPC (Diethyl pyrocarbonate) treated water was used for reagent preparation.

Chilled mortar and pestle wiped with RNase away was used for grinding the leaf samples into a fine powder using liquid nitrogen. One ml of TRIzol reagent was added to the powdered tissue in mortar and gently mixed to homogenize the mixture and incubated at room temperature for 5 min. The homogenate was transferred to a 2 ml pre chilled microfuge tube. To remove protein contamination, 0.2 ml chloroform was added and shaken vigorously for 15 sec and incubated at room temperature for 5 min. The samples were kept on ice for 10 min and then centrifuged at 12,000 g for 15 min at 4°C. Aqueous phase of the sample was transferred to a fresh tube. Then 0.5 ml ice cold isopropanol (100 %) was added to each tube and incubated at room temperature for 10 min for precipitation of RNA. The tubes were mixed by inverting slowly and the samples were centrifuged at 12000 g for 10 min at 4°C. The supernatant was removed and the pellet was washed with 1 ml of 75 % alcohol (in DEPC treated water). The sample was vortexed briefly and centrifuged at 7500 g for 5 min at 4°C and dried for 30-40 min in RNase free laminar air flow chamber. The RNA pellet was re suspended in 30 µl RNase free water and incubated at 55-60°C for 10 min and stored at -20°C.

Quality of the total RNA was determined by agarose gel electrophoresis. RNA (5 µl) mixed with 6X gel loading dye (2 µl) and DEPC treated water (5 µl) was poured into wells using a micropipette on agarose gel (1.2 percent). An absorbance value of 1.0 at 260 nm indicates that 40 ng μl^{-1} of RNA is present in the solution. The concentration of RNA in the sample was determined by the formula:

$$\text{Concentration of RNA (ng } \mu l^{-1}) = A_{260} \times 40 \times \text{dilution factor}$$

RNA purity was estimated by taking the ratio of absorbance at 260 nm to 280 nm (A_{260}/A_{280}).

The relative value RNA ~2.0 is generally accepted as "pure" for RNA.

3.4.2.6 Preparation of cDNA

cDNA was synthesised from the isolated total RNA (Verso cDNA synthesis kit, Thermo Fisher Scientific, catalog number: AB1453A). It includes verso reverse transcriptase enzyme, RNase inhibitor, oligo dT primer, Random hexamer and RT enhancer for removing DNA contamination.

The composition of reaction mixture (20 µl) for cDNA synthesis is as follows:

SI. No	Components	Quantity
1	5X cDNA buffer	4 µl
2	dNTP mix	2 µl
3	Oligo dT	0.5 µl
4	Random Hexamer	0.5 µl
5	RT enhancer	1 µl
6	Verso enzyme mix	1 µl
7	RNA	4µl
8	Nuclease free water	7 µl
	Total volume	20 µl

The contents were mixed well and incubated at 42°C for 30 min followed by incubation at 92°C for 2 min in order to inactivate the RT enhancer and the cDNA samples were stored at -20°C.

3.4.2.7 Quality check of cDNA

cDNA synthesised was checked by PCR using gene specific primers for *PAL, CAD, CHS*. A standard PCR mix was prepared for 20 µl total volume containing 50 ng of

template DNA, 0.2 mM dNTPs, and 400 nM each of primers, 1 unit of Taq polymerase and 1X PCR buffer.

Thermal profile for amplification of genes (*PAL, CAD, CHS)*

Step	Stage	Temperature (°C)	Duration
1	Initial denaturation	95°C	2 min
2	Denaturation	92°C	15 sec
3	Annealing	60°C	15 sec
4	Extension	72°C	45 sec
5	Final extension	72°C	5 min

The steps 2-4 were allowed to repeat 40 times.

The amplified products were separated on agarose gel (1.2 percent) and were observed in a gel documentation system.

3.4.2.8 Differential expression analysis using real-time quantitative PCR

To study the differential expression of defense responsive genes *viz.*, *PAL*, *CAD* and *CHS* in the above mentioned varieties at different CO_2 concentration levels and pathogen inoculation, real-time quantitative PCR was done. Housekeeping gene *Actin* and non-template (without cDNA) were kept as control.

SYBR green, an intercalating dye was used for generating fluorescence. Reaction mixture (20 µl) was prepared as follows:

SI. No	Components	Quantity
1	2X Real time PCR Smart Mix	10 µl
2	Forward primer (10 pmol/µl)	1 µl
3	Reverse primer (10 pmol/µl)	1 µl
4	Template cDNA	1.5 µl
5	Nuclease free water	6.5 µl

RT-qPCR was performed on BIO-RAD CFX^{TM} real time system. The thermal profile of the real time PCR programme was set with initial denaturation temperature of 95°C for 2 min, followed by 40 cycles of denaturation at 92°C for 15 sec, annealing at 60°C for 15 sec, and extension at 72°C for 45 sec. The final extension was set at 72°C for 5 min.

Results were expressed as relative fold change in expression. A house keeping gene *i.e.,* β-Actin was used as reference gene for normalisation of RT-qPCR data. All the reactions were conducted in three replications along with No Template Control (NTC). Threshold cycle (Cq) values were determined for all the reactions from Bio-Rad CFX manager (Version 1.6,Bio-Rad) and fold changes were calculated by using ΔΔCq method (Rao *et al*., 2013). Threshold cycle (Cq) is the cycle at which the fluorescence value of a sample reaches an arbitrary threshold fluorescence. The difference between Cq values of control or infected sample and reference gene was considered as ΔCq value.

$$\Delta Cq = Cq\ (Control/Infected) - Cq\ (Reference\ gene)$$

The difference between ΔCq values of control and infected samples were considered as ΔΔCq value. The fold change in expression was calculated using 2^ (-ΔΔCq).

. $$\Delta\Delta Cq = \Delta Cq\ (Infected\ Sample) - \Delta Cq\ (Healthy\ Sample)$$

Relative CO_2 effect

The relative CO_2 effect was calculated using the formula:

$$\frac{\textbf{OTC-Control}}{\textbf{Control}} * 100$$

OTC- Open Top Chamber

Results

4. RESULTS

The experiment entitled "Evaluation of CO_2 enrichment on growth, development and soft rot tolerance in ginger (*Zingiber officinale* Rosc.)" was undertaken to elicit information on the impact of elevated CO_2 on rhizome development, yield, quality and tolerance to soft rot in ginger through morphological, physiological, biochemical and molecular analysis.

The results based on statistically analyzed data pertaining to the experiment conducted during the course of investigation are presented below.

Assessing the impact of elevated CO_2 on growth, development and quality of ginger

The three varieties of ginger namely Aswathy, Athira and Maran were grown under ambient and elevated CO_2 for 8 months. Observations on growth, physiological and biochemical parameters were recorded at 4^{th}, 5^{th}, 7^{th} and 8^{th} MAP. Observations on yield parameters, quality parameters and tissue nutrient status were assessed at the time of final harvest.

Impact of elevated CO_2 on growth parameters

Plant height

Effect of elevated CO_2 on plant height at all the selected stages of plant growth is presented in the Table 1. Elevated CO_2 significantly enhanced the plant height of all the three varieties at different growth stages. However, the increase in plant height was higher at 4 MAP and 5 MAP; whereas the increase in plant height has been slowly declined at 7 MAP and 8 MAP under elevated CO_2. Maximum plant height was recorded in Aswathy (38.60 cm) at 4 MAP. Under elevated CO_2, there was no significant difference in plant height among varieties at 5 MAP. At elevated CO_2, Aswathy recorded maximum plant height of 46.50 cm and 50.18 cm at 7 MAP and 8 MAP respectively (Plate 4).

Number of leaves

Elevated CO_2 had profound effect on number of leaves which significantly increased at all stages of plant growth (Table 2). Among the varieties, Aswathy exhibited

maximum number of leaves under elevated CO_2. The number of leaves recorded were 90.04, 119.66, 150.00, and 230.66 at 4 MAP, 5 MAP, 7 MAP, and 8 MAP respectively. Athira exhibited lower number of leaves at all the selected growth stages.

The maximum number of leaves was recorded at 8 MAP under elevated CO_2 with 230.66, 157.00 and 189.66 for Aswathy, Athira and Maran respectively.

Specific leaf area

Effect of elevated CO_2 on specific leaf area at all the selected stages of plant growth is presented in the Table 3. Significant differences were evident for all the three varieties, and exhibited a decrease in specific leaf area under CO_2 enrichment compared to ambient conditions at different stages of the crop growth. Under elevated CO_2, maximum specific leaf area was observed in variety Aswathy (250.66 cm^2 g^1), Maran (241.35 cm^2 g^{-1}), Aswathy (231.32 cm^2 g^{-1}) and Athira (262.43 cm^2 g^{-1}) at 4 MAP, 5 MAP, 7 MAP and 8 MAP respectively.

Root fresh weight

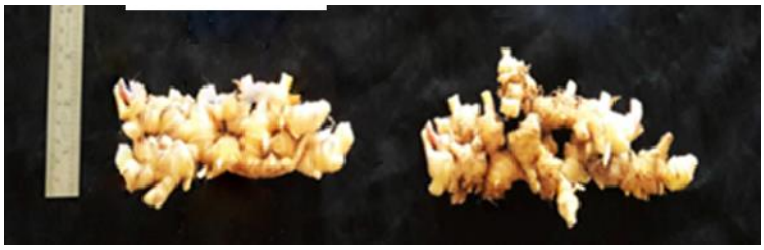
Effect of elevated CO_2 on root FW at all the selected stages of plant growth is presented in the Table 4. Significant differences were evident for all the three varieties. Upon CO_2 enrichment, maximum enhancement of the root growth was observed in the varieties Aswathy (16.07 g $plant^{-1}$) at 4 MAP and Maran (19.18 g $plant^{-1}$) at 5 MAP. At later stages of growth, the elevated CO_2 significantly enhanced the root growth for all the three varieties. The maximum root weight was evident for variety Aswathy (61.64 g $plant^{-1}$) at 7 MAP and also at 8 MAP (48.86 g $plant^{-1}$). Reduction in the root weight was evident at 8 MAP for all the three varieties compared to root weight at 7 MAP.

Shoot fresh weight

The effect of elevated CO_2 on shoot FW at 4 MAP, 5MAP, 7MAP, and 8 MAP is shown in Table 5. Upon CO_2 enrichment, the shoot weight significantly increased for all the three varieties selected for the study at different growth stages. Maximum shoot weight was observed under elevated CO_2 for Aswathy at all the time periods and was highest at 8 MAP (188.83 g $plant^{-1}$) followed by Maran (126.48 g $plant^{-1}$).

Plate 4. Effect of elevated CO_2 on growth and development of ginger varieties at different time intervals

Aswathy



Athira

Maran

Plate 5. Effect of elevated CO_2 on yield and yield parameters of ginger varieties

Stem diameter

Effect of elevated CO_2 on stem diameter at all the selected stages of plant growth is presented in the Table 6. Significant differences were evident in all the three varieties, which exhibited an increase in stem diameter under CO_2 enrichment compared to ambient conditions. Stem diameter was maximum under elevated CO_2 for the variety Aswathy with 1.00, 1.26, 1.31, 1.16 cm at 4 MAP, 5 MAP, 7 MAP and 8 MAP respectively. Stem diameter exhibited increase in trend, peaked at 7 MAP and thereafter declined at 8 MAP.

Tiller number

Effect of elevated CO_2 on number of tillers at all the selected stages of plant growth is presented in the Table 7. Elevated CO_2 stimulated more number of tillers, and significant variations among the varieties were also evident. The tiller number was highest for the variety Aswathy during all the selected growth stages with 10.00, 15.00, 22.66 and 23.00 at 4 MAP, 5MAP, 7MAP, and 8 MAP respectively under CO_2 enriched conditions.

Impact of elevated CO_2 on yield parameters

Total FW of rhizome

The influence of elevated CO_2 on FW of rhizome at 8 MAP is presented in the Table 8. Elevated CO_2 significantly enhanced the FW of rhizome in the varieties studied. Maximum FW of rhizome was recorded in Aswathy with 228.75 g plant^{-1} followed by Maran (192.25 g plant^{-1}) and Athira (169.19 g plant^{-1}) under elevated CO_2 (Plate 5).

Number of fingers per rhizome

Effect of elevated CO_2 on number of fingers per rhizome at 8 MAP is presented in the Table 8. Elevated CO_2 significantly stimulated the production of fingers in all the three varieties. The maximum number of fingers were produced in the variety Aswathy (7.33) followed by Maran (6.33) and Athira (4.66).

Table 1. Effect of elevated CO_2 on plant height of ginger varieties at different growth stages

	Plant height (cm)											
	4 MAP			5 MAP			7 MAP			8 MAP		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	34.33	38.60	36.46	37.16	42.40	39.78	41.23	46.50	43.86	49.26	50.18	49.72
Athira	28.16	32.06	30.11	37.33	42.50	39.91	41.13	43.41	42.27	45.13	47.13	46.13
Maran	27.00	35.46	31.23	39.16	42.00	40.58	41.16	43.20	42.18	49.30	49.36	49.33
Mean	29.83	35.37	-	37.88	42.30	-	41.17	44.37		47.90	48.89	
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.41	1.30		0.40	NS		0.42	1.31		0.36	1.14	
Treatment	0.34	1.06		0.32	1.02		0.34	1.07		0.30	0.93	
Var X Treat	0.59	1.84		0.56	NS		0.59	1.86		0.52	NS	

Table 2. Effect of elevated CO_2 on number of leaves of ginger varieties at different growth stages

	Number of leaves/ Plant											
	4 MAP			5 MAP			7 MAP			8 MAP		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	76.20	90.04	83.12	100.00	119.66	109.83	126.66	150.00	138.33	148.33	230.66	189.50
Athira	37.21	54.66	45.94	64.00	87.33	75.66	95.66	135.00	115.33	106.33	157.00	131.66
Maran	54.00	69.66	61.83	93.00	106.00	99.50	114.33	142.33	128.33	139.00	189.66	164.33
Mean	55.80	71.46		85.66	104.33		112.22	142.44		131.22	192.444	
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.55	1.72		0.49	1.52		0.77	2.39		0.73	2.28	
Treatment	0.45	1.40		0.40	1.24		0.62	1.95		0.59	1.86	
Var X Treat	0.78	NS		0.69	2.16		1.08	3.39		1.03	3.22	

Table 3. Effect of elevated CO_2 on specific leaf area of ginger varieties at different growth stages

	Specific leaf area ($cm^2 g^{-1}$)											
	4 MAP			5 MAP			7 MAP			8 MAP		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	273.00	250.66	261.83	266.07	235.52	250.79	255.89	231.32	243.60	266.82	253.48	260.15
Athira	268.39	249.11	258.75	258.17	240.09	249.13	250.93	230.09	240.51	271.31	262.43	266.87
Maran	260.94	243.55	252.24	254.84	241.35	248.09	244.84	230.88	237.86	243.81	235.00	239.41
Mean	267.44	247.77	-	259.69	238.98	-	250.55	230.76	-	260.65	250.30	
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.68	2.12		0.61	1.92		0.605	1.88		0.47	1.48	
Treatment	0.55	1.73		0.50	1.57		0.49	1.53		0.38	1.21	
Var X Treat	0.96	NS		0.87	2.72		0.85	2.66		0.67	2.10	

Table 4. Effect of elevated CO_2 on root weight of ginger varieties at different growth stages

	Root fresh weight (g $plant^{-1}$)											
	4 MAP			5 MAP			7 MAP			8 MAP		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	13.13	16.07	14.60	21.37	19.40	20.39	34.36	61.64	48.00	26.61	48.86	37.74
Athira	5.77	5.77	5.77	15.47	13.50	14.48	30.32	32.66	31.49	23.72	34.90	29.31
Maran	11.26	7.84	9.55	14.80	19.18	16.99	31.22	41.76	36.49	21.97	41.17	31.57
Mean	10.05	9.89		17.21	17.36		31.96	45.35		24.10	41.64	
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.37	1.16		0.54	1.687		0.45	1.41		0.63	1.96	
Treatment	0.30	NS		0.44	NS		0.37	1.15		0.51	1.60	
Var X Treat	0.52	1.64		0.76	2.386		0.64	1.99		0.89	2.78	

Table 5. Effect of elevated CO_2 on shoot weight of ginger varieties at different growth stages

	Shoot fresh weight (g plant^{-1})											
	4 MAP			5 MAP			7 MAP			8 MAP		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	59.34	65.08	62.21	77.00	125.54	101.27	103.67	167.88	135.78	99.15	188.83	143.99
Athira	24.75	33.41	29.08	57.64	63.66	60.65	66.72	89.03	77.88	74.74	95.64	85.19
Maran	57.54	60.94	59.24	76.82	109.75	93.28	109.96	155.84	132.90	87.31	126.48	106.90
Mean	47.214	53.14		70.48	85.87		93.45	137.58		87.07	136.98	
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.61	1.90		0.64	2.00		0.59	1.84		0.55	1.74	
Treatment	0.49	1.55		0.52	1.64		0.48	1.50		0.45	1.42	
Var X Treat	0.86	2.69		0.91	2.84		0.83	2.61		0.79	2.46	

Table 6. Effect of elevated CO_2 on stem diameter of ginger varieties at different growth stages

	Stem diameter (cm)											
	4 MAP			5 MAP			7 MAP			8 MAP		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	0.91	1.00	0.95	1.09	1.26	1.18	1.11	1.31	1.19	1.07	1.16	1.11
Athira	0.82	0.81	0.81	1.01	1.05	1.03	1.05	1.10	1.07	1.00	0.99	1.00
Maran	0.88	1.00	0.94	1.09	1.23	1.16	1.10	1.25	1.16	1.03	1.10	1.06
Mean	0.87	0.94		1.07	1.18		1.08	1.19		1.03	1.08	
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.005	0.016		0.011	0.035		0.013	0.040		0.011	0.034	
Treatment	0.004	0.013		0.009	0.029		0.010	0.032		0.009	0.028	
Var X Treat	0.007	0.022		0.016	0.049		0.018	0.056		0.016	0.048	

Table 7. Effect of elevated CO_2 on tiller number of ginger varieties at different growth stages

	Tiller Number/Plant											
	4 MAP			5 MAP			7 MAP			8 MAP		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	6.00	10.00	8.00	11.00	15.00	13.00	15.00	22.66	18.83	19.33	23.00	21.16
Athira	4.33	6.66	5.50	7.33	12.66	10.00	12.66	16.00	14.33	15.00	18.00	16.50
Maran	7.00	8.66	7.83	11.66	13.00	12.33	13.66	17.66	15.66	18.00	21.33	19.66
Mean	5.77	8.44		10.00	13.55		13.77	18.77		17.44	20.77	
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.33	1.03		0.40	1.27		0.56	1.74		0.43	1.34	
Treatment	0.27	0.84		0.33	1.03		0.45	1.42		0.35	1.09	
Var X Treat	0.47	NS		0.57	1.79		0.79	2.47		0.60	NS	

Table 8. Effect of elevated CO_2 on yield and yield parameters of ginger varieties at 8 MAP

	Fresh weight of rhizome (g plant^{-1})			Number of fingers per rhizome			Rhizome thickness (cm)			Rhizome spread (cm)		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	165.35	228.75	197.05	5.33	7.33	6.33	2.47	3.74	3.10	14.60	18.13	16.36
Athira	150.90	169.19	160.04	3.66	5.66	4.66	2.00	3.28	2.64	18.76	18.38	18.57
Maran	160.20	192.25	176.23	5.00	6.33	5.66	2.13	2.41	2.27	18.04	20.70	19.37
Mean	158.81	196.73		4.66	6.44		2.20	3.14		17.13	19.07	
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.65	2.02		0.198	0.61		0.10	0.319		0.26	0.83	
Treatment	0.53	1.65		0.16	0.50		0.08	0.26		0.21	0.68	
Var X Treat	0.92	2.87		0.28	NS		0.14	0.45		0.37	1.17	

Table 9. Effect of elevated CO_2 on dry weight of ginger varieties

	Dry weight of rhizome (g plant^{-1})		
	Ambient CO_2	**Elevated CO_2**	**Mean**
Aswathy	44.73	61.78	53.25
Athira	34.82	43.02	38.92
Maran	42.51	55.64	49.08
Mean	40.69	53.48	
	SEm±	**CD(0.05)**	
	0.47	1.48	
	0.39	1.21	
	0.67	2.10	

Rhizome thickness

Effect of elevated CO_2 on rhizome thickness at 8 MAP is presented in the Table 8. Elevated CO_2 significantly increased the rhizome thickness in all the three varieties; however variety Aswathy had the maximum thickness of 3.74 cm compared to Athira (3.28 cm) and Maran (2.41 cm).

Rhizome spread

Effect of elevated CO_2 on rhizome spread at 8 MAP is presented in the Table 8. Significant varietal variations were evident with maximum spread for Maran (20.70 cm) followed by Athira (18.38 cm) and Aswathy (18.13 cm) under elevated CO_2.

Total DW of rhizome

Effect of elevated CO_2 on DW of rhizome at 8 MAP is presented in the Table 9. Significant increase in the DW was evident under elevated CO_2 and was more pronounced in the variety Aswathy which had maximum DW of rhizome (61.78 g plant^{-1}) followed by Maran (55.64 g plant^{-1}) and Athira (43.02 g plant^{-1}) .

Impact of elevated CO_2 on physiological and biochemical parameters

Total chlorophyll content

The data on effect of elevated CO_2 on total chlorophyll content at all the selected stages of plant growth is presented in Table 10. Although there was significant increase in the total chlorophyll content under elevated CO_2 in all the three varieties, the increase was not significant enough between the varieties at 4 MAP and 7 MAP.

At 7 MAP and 8 MAP, it is noteworthy that there was significant reduction in chlorophyll content under ambient condition and elevated CO_2 condition; but the reduction was higher at elevated CO_2 condition. Among the three varieties, Aswathy recorded maximum chlorophyll content of 2.90 mg g^{-1} FW, 3.98 mg g^{-1} FW, 2.43 mg g^{-1} FW and 1.98 mg g^{-1} FW at 4 MAP, 5 MAP, 7 MAP, and 8 MAP respectively under CO_2 enriched conditions.

Stomatal conductance

Effect of elevated CO_2 on stomatal conductance at different stages of plant growth is presented in the Table 11. The results indicate that at ambient CO_2, the rate of stomatal conductance was significantly high at all stages as compare to high CO_2 levels.

Among the three varieties, highest rate of stomatal conductance was recorded for Aswathy at 5 MAP with 204.05 mmol m^{-2} s^{-1} and 186.36 mmol m^{-2} s^{-1} both at ambient and elevated CO_2 respectively. While, the lowest rate for Athira at 8 MAP with 138.48 and 129.74 mmol m^{-2} s^{-1} under ambient and elevated CO_2 respectively.

Photosynthetic rate

The data on effect of elevated CO_2 on photosynthetic rate of ginger at all the selected stages of plant growth is reported in Table 12. The mean photosynthetic rate exhibited increase in trend upto 5 MAP; thereafter it decreased. There was significant difference between the varieties at all the stages of study.

Among the three varieties, the maximum photosynthetic rate was evident for Aswathy which recorded 10.17 µmol CO_2 m^{-2} s^{-1}, 11.21 µmol CO_2 m^{-2} s^{-1}, 8.63 µmol CO_2 m^{-2} s^{-1} and 6.06 µmol CO_2 m^{-2} s^{-1} at 4 MAP, 5 MAP, 7 MAP and 8 MAP respectively under CO_2 enriched conditions.

Transpiration rate

The result of transpiration rate of ginger at different stages of plant growth with respect to influence of elevated CO_2 is presented in the Table 13. At ambient CO_2, the rate of transpiration increased significantly from 4 MAP to 5 MAP; peaked at 5 MAP and declined with the lowest rate evident at 8 MAP. However, at elevated CO_2 significant decrease in the transpiration rate was evident for all the three varieties at different stages of growth compared to ambient condition.

Among the three varieties highest transpiration rate was recorded in Aswathy at 5 MAP with 1.69 mmol H_2O m^{-2} s^{-1} and 1.41 mmol H_2O m^{-2} s^{-1} while the lowest rate in

Athira at 8 MAP with 0.66 mmol H_2O m^{-2} s^{-1} and 0.52 mmol H_2O m^{-2} s^{-1} under ambient and elevated CO_2 respectively.

Total protein

Effect of elevated CO_2 on total protein content at all the studied stages of plant growth is presented in the Table 14. Significant differences were evident among the varieties, which exhibited increase in total protein content at 4 MAP followed by decrease at 5 MAP, 7 MAP, and 8 MAP under CO_2 enrichment. Whereas, at atmospheric CO_2, protein content gradually increased as the plant growth progressed. Among the three varieties the maximum protein content was evident for Aswathy at 4 MAP (19.31 mg g^{-1} DW) and 5 MAP (18.12 mg g^{-1} DW) at elevated CO_2. At 7 MAP and 8 MAP, maximum protein content was evident for Maran with 16.93 mg g^{-1} DW and 17.07 mg g^{-1} DW respectively under elevated CO_2 condition.

Total sugars

Influence of elevated CO_2 on total sugar content at different growth stages of ginger is presented in the Table 15. Total sugar content exhibited a steady increase throughout the growth stages at ambient CO_2. However, at elevated CO_2, significant increase of total sugar content was evident upto 7 MAP after which it declined at 8 MAP.

The significantly higher sugar content was recorded at 8 MAP with 119.27, 83.54, and 99.31 mg g^{-1} DW for Aswathy, Athira and Maran respectively at ambient CO_2. Reduction in the total sugar was evident at 8 MAP which was 142.17, 86.51, 116.51 mg g^{-1} DW for Aswathy, Athira and Maran respectively compared to 7 MAP under CO_2 enrichment.

Tissue nutrient status

Influence of elevated CO_2 on N, P, and K status of rhizome at 8 MAP is presented in the Table 16. Significant decrease in N content in the rhizome was observed in the CO_2 enriched condition. However, there was no significant difference between the varieties. Among the varieties Maran (0.34 mg g^{-1} DW) had the highest amount of N followed by Aswathy (0.33 mg g^{-1} DW) and Athira (0.29 mg g^{-1} DW) under elevated

CO_2. There was no significant difference between the varieties and CO_2 levels in ginger rhizomes.

For K, the results were similar to that of N. Significant decrease in K content in the rhizome was observed in the CO_2 enriched conditions. However, there was no significant difference between the varieties. Among the varieties Maran had the highest amount of K at both ambient (3.33 mg g^{-1} DW) and elevated CO_2 (2.41 mg g^{-1} DW).

Effect of elevated CO_2 on Fe, Cu, and Zn status of rhizome of ginger at 8 MAP is presented in the Table 17. Significant decrease in the Fe content was evident under elevated CO_2 and was more pronounced in the variety Aswathy (66.34 ppm) followed by Maran (79.42 ppm) and Athira (77.02 ppm). Although significant varietal differences existed, influence of elevated CO_2 in Cu content was not evident in the rhizomes. From Table 17, it is clear that significant decrease in Zn content is evident under elevated CO_2 and was more pronounced in the variety Aswathy (19.09 ppm) followed by Maran (18.69 ppm) and Athira (18.38 ppm).

Impact of elevated CO_2 on quality parameters

Fiber content

Exposure of plants to elevated CO_2 resulted in increased fiber content in ginger rhizomes. From the Table 18, it is clear that significant difference exists for fiber content between varieties. Maximum fiber content was recorded for Maran (103.33 mg g^{-1} DW) followed by Aswathy (86.66 mg g^{-1} DW) and Athira (76.66 mg g^{-1} DW) under elevated CO_2.

Ascorbic acid

The effect of elevated CO_2 on ascorbic acid content at 8 MAP is as presented in Table 18. Elevated CO_2 did stimulate ascorbic acid production in the varieties studied. Slight increase in ascorbic acid under elevated CO_2 was not significant among the varieties.

Starch content

The effect of elevated CO_2 on starch content at 8 MAP is presented in Table 18. Significant increase in the starch content was evident under elevated CO_2 and was more pronounced in the variety Aswathy which had maximum starch content in the rhizome (56.85 % DW) followed by Maran (51.62% DW) and Athira (39.57% DW).

6-Gingerol

The results of effect of elevated CO_2 on gingerol content is as presented in the Table 19. Significant increase in the gingerol content was evident under elevated CO_2 for Aswathy (5.05 mg g^{-1} FW) and Maran (4.22 mg g^{-1} FW). In the variety Athira, gingerol content (1.93 mg g^{-1}FW) decreased significantly under elevated CO_2 compared to ambient (3.97 mg g^{-1} FW) which was the highest among all the three varieties.

Phenol content

The effect of elevated CO_2 on phenol content in rhizome at 8 MAP is presented in Table 19. Significant increase in the phenol content was evident under elevated CO_2 for Aswathy (13.03 mg g^{-1} DW) and Maran (9.11 mg g^{-1} DW). In the variety Athira, phenol content (5.56 mg g^{-1} DW) decreased significantly under elevated CO_2 compared to ambient.

Total oleoresin

The results of effect of elevated CO_2 on total oleoresin content at 8 MAP in ginger rhizome is presented in the Table 19. Elevated CO_2 stimulated oleoresin content with significant increase in the variety Aswathy (12.38 % DW) and Maran (10.24 % DW). In the variety Athira oleoresin content (6.42 % DW) decreased under elevated CO_2 compared to ambient condition.

Table 10. Effect of elevated CO_2 on total chlorophyll content in ginger varieties at different growth stages

	Total chlorophyll (mg g^{-1} FW)											
	4 MAP			5 MAP			7 MAP			8 MAP		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	1.97	2.90	2.44	2.75	3.98	3.36	2.99	2.43	2.71	2.77	1.98	2.37
Athira	1.92	2.48	2.20	2.24	2.90	2.57	2.56	2.08	2.32	2.32	1.95	2.13
Maran	2.11	2.55	2.33	2.32	2.70	2.51	2.37	1.64	2.01	1.89	1.84	1.86
Mean	2.00	2.64		2.44	2.77		2.64	2.05		2.32	1.92	
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.150	NS		0.18	0.15		0.18	NS		0.05	0.16	
Treatment	0.122	0.381		0.14	0.23		0.14	0.46		0.04	0.13	
Var X Treat	0.212	NS		0.25	0.22		0.25	NS		0.07	0.22	

Table 11. Effect of elevated CO_2 on stomatal conductance in ginger varieties at different growth stages

	Stomatal conductance (mmol m^{-2} s^{-1})											
	4 MAP			5 MAP			7 MAP			8 MAP		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	194.40	170.39	182.39	204.05	186.36	195.20	184.65	164.61	174.63	149.33	139.02	144.17
Athira	176.35	167.72	172.04	191.04	177.36	184.20	193.95	182.42	188.19	138.48	129.74	134.11
Maran	182.81	174.06	178.43	185.83	164.42	175.12	182.76	173.90	178.33	146.41	135.89	141.15
Mean	184.52	170.72	-	193.64	176.04	-	187.12	173.64	-	144.74	134.88	-
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.62	1.94		0.71	2.23		0.60	1.89		0.43	1.35	
Treatment	0.50	1.58		0.58	1.82		0.49	1.54		0.35	1.10	
Var X Treat	0.88	2.74		1.01	3.16		0.86	2.68		0.61	NS	

Table 12. Effect of elevated CO_2 on photosynthetic rate in ginger varieties at different growth stages

	Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})											
	4 MAP			5 MAP			7 MAP			8 MAP		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	7.98	10.17	9.08	9.14	11.21	10.17	8.05	8.63	8.34	7.09	6.06	6.58
Athira	6.91	7.86	7.38	7.07	7.88	7.48	6.90	7.32	7.11	5.95	5.50	5.73
Maran	7.29	9.00	8.14	8.13	9.93	9.03	7.17	7.27	7.22	5.06	4.10	4.58
Mean	7.39	9.01	-	8.11	9.67	-	7.37	7.74	-	6.03	5.22	-
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.11	0.37		0. 65	0.82		0.24	0.75		0.23	0.74	
Treatment	0.09	0.30		0.22	0.67		0.19	NS		0.19	0.60	
Var X Treat	0.16	0.52		0.37	NS		0.34	NS		0.33	NS	

Table 13. Effect of elevated CO_2 on transpiration rate in ginger varieties at different growth stages

	Transpiration rate (mmol H_2O m^{-2} s^{-1})											
	4 MAP			5 MAP			7 MAP			8 MAP		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	1.46	0.92	1.19	1.69	1.41	1.55	1.57	0.76	1.17	0.72	0.63	0.68
Athira	1.31	0.72	1.01	1.65	1.18	1.41	1.55	0.88	1.22	0.66	0.52	0.59
Maran	1.36	1.08	1.22	1.47	0.87	1.17	1.48	0.84	1.16	0.69	0.62	0.65
Mean	1.37	0.91	-	1.60	1.15	-	1.53	0.83	-	0.69	0.59	-
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.02	0.07		0.06	0.20		0.06	NS		0.01	0.03	
Treatment	0.02	0.06		0.05	0.17		0.04	0.15		0.00	0.02	
Var X Treat	0.03	0.11		0.09	NS		0.08	NS		0.01	NS	

Table 14. Effect of elevated CO_2 on total protein in ginger rhizomes at different growth stages

	Total protein (mg g^{-1} DW)											
	4 MAP			5 MAP			7 MAP			8 MAP		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	17.34	19.31	18.32	19.56	18.12	18.84	20.51	16.85	18.68	23.73	15.12	19.42
Athira	15.44	17.19	16.31	17.66	16.47	17.06	17.38	14.51	15.95	18.55	16.52	17.53
Maran	15.48	18.43	16.95	17.41	15.51	16.46	20.06	16.93	18.49	22.29	17.07	19.68
Mean	16.09	18.31	-	18.21	16.70	-	19.32	16.10	-	21.52	16.24	-
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.35	1.11		0.44	1.37		0.44	1.37		0.60	NS	
Treatment	0.29	0.90		0.36	1.12		0.36	1.12		0.49	1.55	
Var X Treat	0.50	NS		0.62	NS		0.62	NS		0.86	2.68	

Table 15. Effect of elevated CO_2 on total sugars in ginger rhizomes at different growth stages

	Total sugars (mg g^{-1} DW)											
	4 MAP			5 MAP			7 MAP			8 MAP		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	69.99	90.46	80.22	89.49	131.21	110.35	102.19	151.17	126.68	119.27	142.17	130.72
Athira	50.35	60.82	55.58	69.38	89.37	79.37	79.62	90.90	85.26	83.54	86.51	85.02
Maran	59.60	83.71	71.65	78.03	111.95	94.99	91.42	119.48	105.45	99.31	116.51	107.91
Mean	59.98	78.33	-	78.96	110.84	-	91.08	120.51	-	100.71	115.06	-
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.35	1.11		0.37	1.18		0.58	1.83		0.46	1.45	
Treatment	0.29	0.91		0.31	0.96		0.48	1.49		0.38	1.19	
Var X Treat	0.50	1.58		0.53	1.67		0.83	2.59		0.66	2.06	

Table 16. Effect of elevated CO_2 on tissue nutrient status (N, P, K) in ginger rhizomes

	Tissue nutrient status (mg g^{-1} DW)								
	Nitrogen			**Phosphorous**			**Potassium**		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	0.43	0.33	0.38	0.32	0.29	0.30	2.93	2.06	2.49
Athira	0.34	0.29	0.31	0.29	0.32	0.30	2.86	2.20	2.53
Maran	0.38	0.34	0.36	0.34	0.30	0.32	3.33	2.41	2.87
Mean	0.38	0.32		0.31	0.30	-	3.04	2.22	-
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.02	NS		0.01	NS		0.12	NS	
Treatment	0.01	0.05		0.01	NS		0.10	0.32	
Var X Treat	0.03	NS		0.02	NS		0.17	NS	

Table 17. Effect of elevated CO_2 on tissue nutrient status (Fe, Cu, Zn) in ginger rhizomes

	Tissue nutrient status (ppm)								
	Iron			**Copper**			**Zinc**		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	79.99	66.34	73.16	12.66	11.65	12.15	24.98	19.09	22.04
Athira	83.97	77.02	80.49	10.43	9.96	10.19	21.02	18.38	19.70
Maran	88.58	79.42	84.00	11.64	11.07	11.35	22.97	18.69	20.83
Mean	84.18	74.26		11.57	10.89		22.99	18.72	
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.45	1.42		0.39	1.21		0.45	1.41	
Treatment	0.37	1.16		0.31	NS		0.37	1.15	
Var X Treat	0.64	2.01		0.55	NS		0.64	NS	

Table 18. Effect of elevated CO_2 on fiber, ascorbic acid and starch content of ginger rhizomes

	Fiber content (mg g^{-1})			Ascorbic acid (mg $100g^{-1}$ DW)			Starch content (%)		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	46.66	86.66	66.66	1.91	2.06	1.98	31.70	56.85	44.28
Athira	36.66	76.66	56.66	1.91	1.99	1.95	30.93	39.57	35.25
Maran	80.00	103.33	91.66	1.63	1.81	1.72	39.58	51.62	45.60
Mean	54.44	88.88	-	1.82	1.95	-	34.07	49.35	
V	**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	4.30	13.40		0.12	NS		0.06	0.19	
Treatment	3.51	10.94		0.09	NS		0.05	0.15	
Var X Treat	6.08	NS		0.17	NS		0.08	0.27	

Table 19. Effect of elevated CO_2 on total oleoresin, essential oil, phenol content and gingerol in ginger rhizomes

	Total oleoresin (%)			Essential oils (%)			Phenol content (mg g^{-1} DW)			Gingerol (mg g^{-1} DW)		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	9.20	12.38	10.79	2.87	3.62	3.24	8.12	13.03	10.58	3.28	5.05	4.16
Athira	7.94	6.42	7.18	2.71	3.78	3.25	7.68	5.56	6.62	3.97	1.93	2.95
Maran	8.89	10.24	9.56	2.03	2.58	2.30	7.41	9.11	8.26	3.02	4.22	1.54
Mean	8.68	9.68	-	2.54	3.33	-	7.74	9.23	-	3.42	3.73	-
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.15	0.49		0.03	0.11		0.15	0.48		0.03	0.06	
Treatment	0.12	0.40		0.02	0.09		0.12	0.39		0.01	0.05	
Var X Treat	0.22	0.69		0.05	0.15		0.21	0.68		0.02	0.09	

Essential oils

The effect of elevated CO_2 on essential oils at 8 MAP is as presented in Table 19. Significant increase in the essential oils content was recorded under elevated CO_2 and was more pronounced in the variety Athira which had maximum essential oils in the rhizome (3.78% DW) followed by Aswathy (3.62% DW) and Maran (2.58% DW).

Assessing the impact of elevated CO_2 on the response of ginger to *P. aphanidermatum*

The effect of elevated CO_2 on three varieties of ginger Aswathy, Athira and Maran and its wild congener, *Z. zerumbet* were evaluated for tolerance to soft rot. The results obtained are presented below.

Disease incidence and severity

***Percentage Disease Incidence* (DI)**

The effect of elevated CO_2 on disease incidence after one week of pathogen inoculation in *Zingiber* species is presented in Table 20. The DI varied between 0 to 100 per cent. DI of 100 per cent was recorded for all the three varieties. The disease incidence in *Z. zerumbet* exhibited only 1 per cent at both ambient and CO_2 enriched conditions.

***Percentage Disease Index* (PDI)**

The effect of elevated CO_2 on PDI at 20 days after inoculation on *Zingiber* species is presented in Table 20. The PDI varied between 16.66 to 100 per cent. PDI significantly reduced under elevated CO_2 for all the *Zingiber* species except for the variety Aswathy. Maxiumum PDI was evident for Aswathy (100 per cent) under both ambient and elevated CO_2, whereas least for *Z. zerumbet* with 16.66 and 25.55 per cent under ambient and elevated CO_2 respectively (Plate 6).

Number of days taken for symptom development

The effect of elevated CO_2 on number of days taken for symptom development on *Zingiber* species is presented in Table 20. Significant differences were evident for number of days taken for symptom development among the *Zingiber* species. The number of days taken for the symptom development was less under elevated CO_2. The

symptom was first evident in the variety Aswathy after 2.58 and 3 days post inoculation under elevated and ambient CO_2 respectively. Maximum number of days was taken for *Z. zerumbet* (9.16) under elevated CO_2, where as it was 10.33 days under ambient CO_2.

Post harvest incidence / severity of the disease

Post harvest incidence of the disease was not evident during the period of storage.

Growth parameters

Number of leaves

Effect of elevated CO_2 on number of leaves in *Zingiber* species due to under *P. aphanidermatum* infection is represented in Table 21. Elevated CO_2 significantly increased the number of leaves. Among the *Zingiber* species, *Z. zerumbet* exhibited maximum number of leaves under both ambient and elevated CO_2. However, there was no significant difference between the control and inoculated plants.

Plant height

Effect of elevated CO_2 on plant height in *Zingiber* species under *P. aphanidermatum* infection is presented in Table 22. Elevated CO_2 significantly increased the plant height compared to ambient CO_2. The pathogen inoculation did not significantly vary the plant height. Significant varietal variations were evident with maximum plant height for *Z. zerumbet* with 52.52 cm and 52.79 cm for control and inoculated plants under CO_2 enrichment. Under ambient CO_2, maximum plant height was recorded in *Z. zerumbet* with 48.21 cm and 48.20 cm under control and inoculated conditions respectively.

Number of tillers

Effect of elevated CO_2 on number of tillers in *Zingiber* species under *P. aphanidermatum* infection is presented in Table 23. Significant difference was evident among varieties. The maximum number of tillers was evident for *Z. zerumbet* under both elevated CO_2 (8.33-inoculated) and ambient (7.33-inoculated) conditions. The pathogen inoculation did not significantly affect the number of tillers.

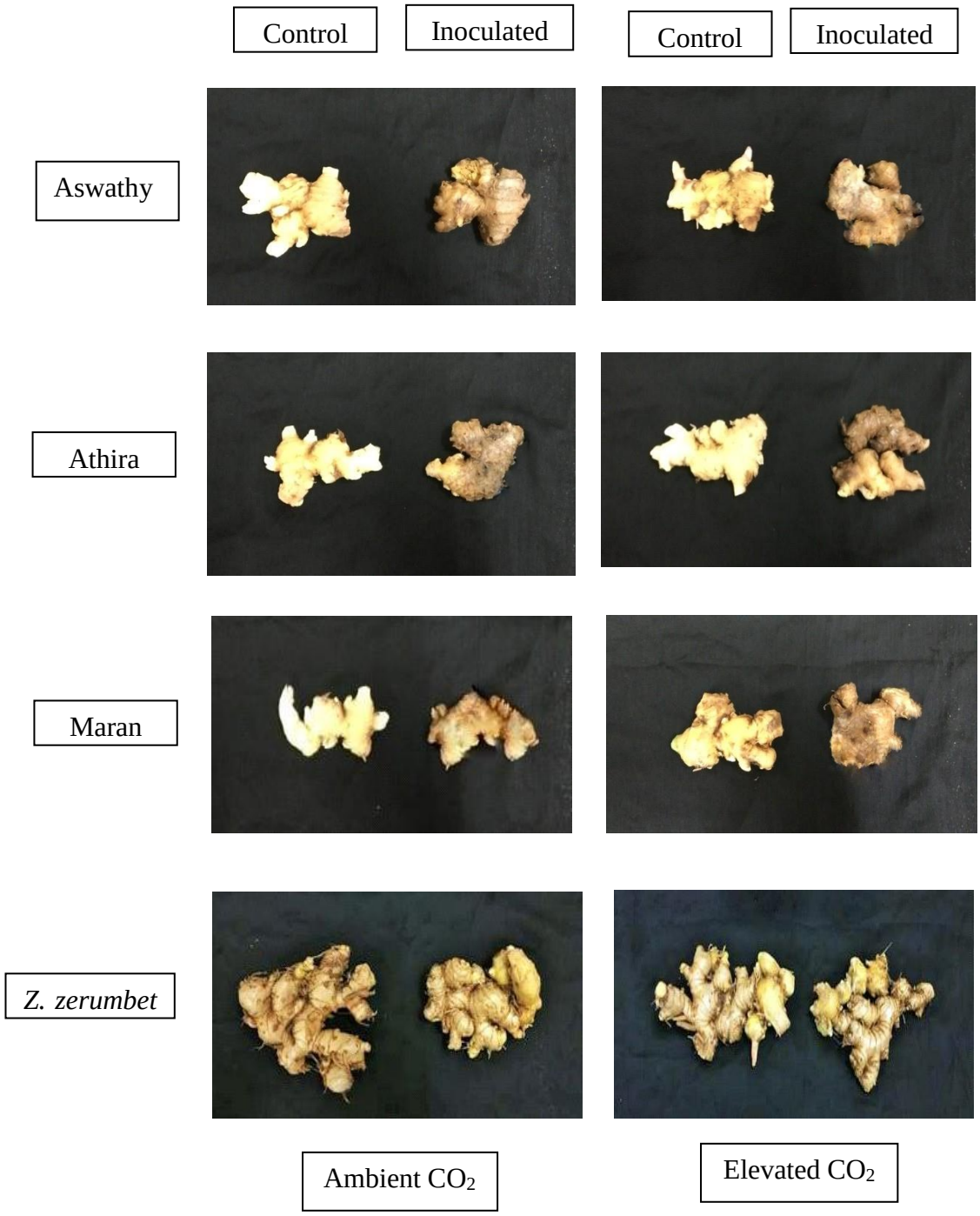


Plate 6. Soft rot of rhizomes under ambient and elevated CO_2

Table 20. Effect of elevated CO_2 on percentage disease incidence, percentage disease index and number of days taken for symptom development in *Zingiber* species upon *P. aphanidermatum* inoculation

	Percentage Disease Incidence (%)			Percentage Disease Index (%)			Number of days taken for symptom development		
	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean	Ambient CO_2	Elevated CO_2	Mean
Aswathy	100 (10.05)	100 (10.05)	10.05	100.00	100.00	100.00	3.00	2.58	2.79
Athira	100 (10.05)	100 (10.05)	10.05	83.33	66.66	75.00	3.83	3.50	3.66
Maran	100 (10.05)	100 (10.05)	10.05	91.66	83.33	87.50	3.33	2.83	3.08
Z. zerumbet	0 (1.00)	0 (1.00)	1.00	25.00	16.66	20.83	10.33	9.16	9.75
Mean	7.78	7.78	-	75.00	66.67		5.12	4.52	-
	SEm±	**CD(0.05)**		**SEm±**	**CD(0.05)**		**SEm±**	**CD(0.05)**	
Varieties	0.00	0.00		0.24	0.73		0.20	0.60	
Treatment	0.00	NS		0.17	0.51		0.14	0.43	
Var X Treat	0.00	0.00		0.34	1.03		0.28	NS	

Data in the parenthesis indicate transformed values

Table 21. Effect of elevated CO_2 on number of leaves in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Number of leaves/ plant				
	Ambient CO_2		Elevated CO_2		
	Control	Inoculated	Control	Inoculated	Mean
Aswathy	46.33	47.33	51.33	53.00	49.50
Athira	33.66	34.00	43.00	41.33	38.00
Maran	41.66	39.33	49.00	52.00	45.50
Z. zerumbet	57.00	55.33	64.33	66.33	60.75
Mean(T)	44.66	44.00	51.91	53.16	-
Mean (C)	44.33		52.54		48.43
CD (0.05)	T= NS	V=1.16	C*T=1.16	V*C=1.64	T*V= NS
SEm±	T=0.28	V=0.40	C*T=0.40	V*C= 0.57	T*V= 0.57

V= Varieties, C= CO_2 conditions, T= Pathological treatments

Table 22. Effect of elevated CO_2 on plant height in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Plant height (cm)				
	Ambient CO_2		Elevated CO_2		
	Control	Inoculated	Control	Inoculated	Mean
Aswathy	31.06	32.00	35.56	36.07	33.67
Athira	27.00	26.18	30.36	30.95	28.62
Maran	27.00	26.67	33.56	34.69	30.48
Z. zerumbet	48.21	48.20	52.52	52.79	50.43
Mean(T)	33.32	33.26	38.00	38.62	-
Mean (C)	33.29		38.31		35.80
CD(0.05)	T= NS	V=0.90	C*T= NS	V*C=1.27	T*V= NS
SEm±	T=0.22	V=0.31	C*T=0.31	V*C=0.44	T*V= 0.44

V= Varieties, C= CO_2 conditions, T= Pathological treatments

Table 23. Effect of elevated CO_2 on number of tillers in *Zingiber* species upon *P. aphanidermatum* inoculation

	Number of tillers				
	Ambient CO_2		Elevated CO_2		
Varieties	Control	Inoculated	Control	Inoculated	Mean
Aswathy	5.66	5.66	7.66	7.66	6.66
Athira	4.66	4.66	6.00	6.66	5.50
Maran	5.00	4.66	6.66	7.00	5.83
Z. zerumbet	6.66	7.33	8.00	8.33	7.58
Mean(T)	5.50	5.58	7.08	7.41	-
Mean (C)	5.54		7.25		6.39
CD(0.05)	T= NS	V=0.50	C*T= NS	V*C= NS	T*V= NS
SEm±	T=0.12	V=0.17	C*T=0.17	V*C=0.25	T*V=0.25

V= Varieties, C= CO_2 conditions, T= Pathological treatments

Table 24. Effect of elevated CO_2 on fresh weight of rhizome in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	**Fresh weight of rhizome (g plant^{-1})**					
	Ambient CO_2			Elevated CO_2		
	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	42.03	27.58	-34.38	50.69	35.24	-30.47
Athira	35.17	26.20	-25.50	41.91	33.46	-20.16
Maran	37.56	27.16	-27.68	46.37	33.89	-26.91
Z. zerumbet	151.72	138.54	-8.68	160.90	154.27	-4.12
Mean(T)	66.62	54.89		74.96	64.21	
Mean (C)	60.75			69.59		
CD(0.05)	T=0.71	V=1.01		C*T= NS	V*C=1.43	T*V= 1.43
SEm±	T=0.24	V=0.35		C*T=0.35	V*C=0.49	T*V= 0.49

V= Varieties, C= CO_2 conditions, T= Pathological treatments

Fresh weight of rhizome

Inoculation of *P.aphanidermatum* decreased the FW of rhizome both under ambient and elevated CO_2 condition (Table 24). However, *Z. zerumbet* showed least reduction in FW even after inoculation (Plate 6). Whereas the decline in the FW of rhizome was significantly higher under ambient compared to the elevated CO_2 up on the disease development.

Dry weight of rhizome

P. aphanidermatum inoculation decreased the DW of rhizome significantly both under ambient and elevated CO_2 conditions in Aswathy, Athira and Maran (Table 25). However, no significant effect of *P. aphanidermatum* was observed in *Z. zerumbet*. The effect of *P. aphanidermatum* was less under elevated CO_2 condition.

Physiological and biochemical observations

Chlorophyll a, Chlorophyll b and total Chlorophyll content

The chlorophyll content (a, b and total) were decreased due to inoculation of *P. aphanidermatum* in all the varieties and *Z. zerumbet* (Table 26, 27 and 28). The effect of *P. aphanidermatum* was decreased due to elevated CO_2 concentration. Among the species *Z. zerumbet* was least affected by of *P. aphanidermatum* inoculation.

Caroteniods

The carotenoids content was significantly higher under elevated CO_2 (0.65 mg g^{-1} FW) compared to the ambient CO_2 (0.52 mg g^{-1} FW). Significant difference was evident among the varieties (Table 29). Upon disease development, the decline in the carotenoids content was significantly higher under ambient CO_2 as compared to the elevated CO_2.

Among the *Zingiber* species under elevated CO_2, the maximum carotenoids content was evident in Aswathy recording 0.93 mg g^{-1} FW and 0.71 mg g^{-1} FW for control and inoculated plants respectively. Under ambient CO_2, the maximum carotenoids content was evident in *Z. zerumbet* recording 0.66 mg g^{-1} FW and 0.65 mg g^{-1} FW for control and inoculated plants respectively.

Xanthophyll

P. aphanidermatum inoculation decreased the xanthophyll content under both ambient and elevated CO_2 condition (Table 30). However, elevated CO_2 decreased the effect of *P. aphanidermatum* inoculation on xanthophyll content content.

Among the *Zingiber* species under elevated CO_2, the maximum xanthophyll content was evident in Aswathy recording 0.60 mg g^{-1} FW and *Z. zerumbet* recording 0.57 mg g^{-1} FW for control and inoculated plants respectively. Under ambient CO_2, the maximum xanthophyll content was recorded in *Z. zerumbet* with 0.47 mg g^{-1} FW and 0.45 mg g^{-1} FW for control and inoculated plants respectively.

Photosynthetic rate

Upon disease development, the rate of reduction in photosynthetic rate was significantly higher under the ambient CO_2 compared to the elevated CO_2, and is represented in Table 31. Significant difference was evident among the *Zingiber* species.

Among the *Zingiber* species under elevated CO_2, the maximum photosynthetic rate was evident in Aswathy recording 9.24 µmol CO_2 m^{-2} s^{-1} and *Z. zerumbet* recording 7.76 µmol CO_2 m^{-2} s^{-1} for control and inoculated plants respectively. Under ambient CO_2, the maximum photosynthetic rate was recorded in *Z. zerumbet* with 8.18 µmol CO_2 m^{-2} s^{-1} and 8.20 µmol CO_2 m^{-2} s^{-1} for control and inoculated plants respectively.

Membrane integrity

Membrane integrity measured in terms of per cent leakage of solutes was significantly higher after the pathogen inoculation. Significantly higher per cent leakage was registered for varieties under ambient CO_2 compared to the elevated CO_2 and is represented in Table 32. Among the *Zingiber* species under elevated CO_2, the maximum per cent leakage of solutes was observed in Aswathy recording 4.50% and 10.15% for control and inoculated plants respectively. Under ambient CO_2, the maximum per cent leakage of solutes was recorded in Aswathy with 4.45% and 11.85% for control and inoculated plants respectively.

Table 25. Effect of elevated CO_2 on dry weight of rhizome in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Dry weight of rhizome (g plant^{-1})					
	Ambient CO_2			Elevated CO_2		
	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	9.15	6.62	-27.65	10.27	-10.22	-11.39
Athira	7.91	6.31	-20.22	9.22	-5.74	-6.10
Maran	8.39	6.30	-24.91	9.77	-7.67	-8.31
Z. zerumbet	34.14	36.45	6.76	38.63	-1.68	-1.71
Mean(T)	14.90	13.92		16.97	16.23	
Mean (C)	14.41			16.60		
CD(0.05)	T=0.40	V=0.57		C*T= NS	V*C= NS	T*V= 0.80
SEm±	T=0.14	V=0.19		C*T=0.19	V*C=0.28	T*V= 0.28

V= Varieties, C= CO_2 conditions, T= Pathological treatments

Table 26. Effect of elevated CO_2 on chlorophyll a concentration in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Chlorophyll a (mg g^{-1} FW)					
	Ambient CO_2			Elevated CO_2		
	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	1.17	0.43	-63.25	1.39	0.90	-35.25
Athira	0.96	0.48	-50.00	1.16	0.81	-30.17
Maran	1.06	0.45	-57.55	1.21	0.68	-43.80
Z. zerumbet	1.10	1.08	-1.82	1.26	1.19	-5.56
Mean(T)	1.07	0.61		1.25	0.90	
Mean (C)	0.84			1.07		
CD(0.05)	T=0.04	V=0.06		C*T=0.06	V*C=0.09	T*V= 0.09
SEm±	T=0.01	V=0.02		C*T=0.02	V*C=0.03	T*V= 0.03

V= Varieties, C= CO_2 conditions, T= Pathological treatments

Table 27. Effect of elevated CO_2 on chlorophyll b concentration in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Chlorophyll b (mg g^{-1} FW)					
	Ambient CO_2			Elevated CO_2		
	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	1.04	0.34	-67.31	1.16	-46.55	-87.10
Athira	0.89	0.48	-46.07	0.81	-19.75	-24.62
Maran	0.93	0.47	-49.46	1.00	-27.00	-36.99
Z. zerumbet	1.00	1.00	0.00	0.94	2.13	2.08
Mean(T)	0.96	0.57		0.98	0.74	
Mean (C)	0.77			0.86		
CD(0.05)	T=0.04	V=0.05		C*T=0.05	V*C=0.08	T*V= 0.08
SEm±	T=0.01	V=0.02		C*T=0.02	V*C=0.02	T*V= 0.02

V= Varieties, C= CO_2 conditions, T= Pathological treatment

Table 28. Effect of elevated CO_2 on total chlorophyll concentration in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Total chlorophyll (mg g^{-1} FW)					
	Ambient CO_2			Elevated CO_2		
	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	2.22	0.77	-65.31	2.55	1.53	-40.00
Athira	1.85	0.97	-47.56	1.98	1.46	-26.26
Maran	2.00	0.92	-54.00	2.21	1.41	-56.74
Z. zerumbet	2.10	2.09	-0.47	2.20	2.1	-4.54
Mean(T)	2.04	1.19		2.24	1.64	
Mean (C)	1.57			1.94		
CD(0.05)	T=0.05	V=0.08		C*T=0.08	V*C=0.11	T*V= 0.11
SEm±	T=0.02	V=0.02		C*T=0.02	V*C=0.04	T*V= 0.04

Table 29. Effect of elevated CO_2 on carotenoids concentration in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Carotenoids (mg g^{-1} FW)					
	Ambient CO_2			Elevated CO_2		
	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	0.60	0.29	-51.66	0.93	0.71	-23.65
Athira	0.57	0.46	-19.29	0.62	0.51	-17.74
Maran	0.59	0.36	-38.98	0.58	0.41	-29.31
Z. zerumbet	0.66	0.65	-1.51	0.71	0.70	-1.40
Mean(T)	0.60	0.44		0.63	0.58	
Mean (C)	0.52			0.65		
CD(0.05)	T=0.03	V=0.04		C*T= NS	V*C=0.06	T*V= 0.06
SEm±	T=0.01	V=0.01		C*T=0.01	V*C=0.02	T*V= 0.02

V= Varieties, C= CO_2 conditions, T= Pathological treatment

Table 30. Effect of elevated CO_2 on xanthophyll concentration in *Zingiber* species upon *P. aphanidermatum* inoculation

	Xanthophyll (mg g^{-1} FW)					
	Ambient CO_2			Elevated CO_2		
Varieties	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	0.38	0.22	-42.10	0.60	0.40	-33.33
Athira	0.41	0.27	-34.14	0.49	0.36	-26.53
Maran	0.31	0.20	-35.48	0.37	0.26	-29.2
Z. zerumbet	0.47	0.45	-4.25	0.58	0.57	-1.72
Mean(T)	0.39	0.28		0.51	0.40	
Mean (C)	0.34			0.45		
CD(0.05)	T=0.02	V=0.03		C*T= NS	V*C=0.04	T*V= 0.04
SEm±	T=0.00	V=0.01		C*T=0.01	V*C=0.01	T*V= 0.01

V= Varieties, C= CO_2 conditions, T= Pathological treatment

Table 31. Effect of elevated CO_2 on photosynthetic rate in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})					
	Ambient CO_2			Elevated CO_2		
	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	6.71	2.78	-58.56	9.24	5.86	-36.58
Athira	5.72	3.62	-36.71	6.53	4.76	-27.10
Maran	6.62	3.77	-43.05	7.58	5.29	-30.21
Z. zerumbet	8.18	8.20	0.24	8.43	7.76	-7.94
Mean(T)	6.81	4.59		7.94	5.92	
Mean (C)	5.70			6.93		
CD(0.05)	T=0.33	V=0.47		C*T= NS	V*C=0.67	T*V= 0.67
SEm±	T=0.11	V=0.16		C*T=0.16	V*C=0.23	T*V=0.23

Table 32. Effect of elevated CO_2 on membrane integrity (% leakage) in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Membrane integrity (%)					
	Ambient CO_2			Elevated CO_2		
	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	4.45	11.85	166.29	4.50	10.15	125.55
Athira	2.54	5.81	128.74	2.81	5.71	103.20
Maran	3.53	8.22	132.86	2.86	5.86	104.89
Z. zerumbet	2.31	2.37	2.59	2.12	2.24	5.66
Mean(T)	3.21	7.06		3.07	5.99	
Mean (C)	5.13			4.53		
CD(0.05)	T=0.17	V=0.24		C*T=0.24	V*C=0.34	T*V= 0.34
SEm±	T=0.06	V=0.08		C*T=0.08	V*C=0.12	T*V= 0.12

V= Varieties, C= CO_2 conditions, T= Pathological treatments

Oxidative stress

Malondialdehyde content

P. aphanidermatum inoculation enhanced the MDA content both under ambient and elevated CO_2 conditions. The pathogen inoculation triggered the production of MDA which was significantly higher than the uninoculated plants under both ambient and elevated CO_2 conditions (Table 33). Among the *Zingiber* species *Z. zerumbet* accumulated lower MDA content as compared to the varieties Aswathy, Athira and Maran.

Catalase activity

Significantly higher CAT activity was evident under ambient CO_2 compared to the elevated CO_2 as shown in Table 34. Disease development significantly declined the CAT activity under both ambient and elevated CO_2 conditions. Among the *Zingiber* species under elevated CO_2, the highest CAT activity was observed in Aswathy recording 0.19 Units mg^{-1} total protein and 0.11 Units mg^{-1} total protein for control and inoculated plants respectively. Under ambient CO_2, the highest CAT activity was recorded in Aswathy with 0.30 Units mg^{-1} total protein and *Z. zerumbet* recording 0.13 Units mg^{-1} total protein for control and inoculated plants respectively.

Ascorbate peroxidase activity

As shown in Table 35, disease development significantly enhanced the APX activity under both ambient and elevated CO_2 conditions. Significant difference was evident among the *Zingiber* species with the highest APX activity in *Z. zerumbet* recording 6.40 Units mg^{-1} total protein and 6.40 Units mg^{-1} total protein for control and inoculated plants respectively. The highest APX activity was observed in *Z. zerumbet* both under ambient and elevated CO_2 conditions and in control and inoculated plants.

Phenylalanine ammonia lyase activity

PAL activity was significantly higher under elevated CO_2 as compared to the ambient CO_2 (Table 36). Disease development significantly enhanced the PAL activity. Significant differences among the varieties and *Z. zerumbet* were recorded and the highest PAL activity was recorded in Athira with 23.83 Units mg^{-1} total protein and 32.75

Units mg^{-1} total protein for control and inoculated plants respectively. Also, under ambient CO_2, the highest PAL activity was observed in Athira recording 16.47 Units mg^{-1} total protein and 20.03 Units mg^{-1} total protein for control and inoculated plants respectively.

Ascorbic acid content

The ascorbic acid content was higher under elevated CO_2 (6.71 mg 100 g^{-1} FW) compared to the ambient CO_2 (5.47 mg 100 g^{-1} FW). Significant difference in ascorbic acid content was noticed among the varieties and is represented in Table 37. Among the *Zingiber* species, the maximum ascorbic acid content was recorded in variety Athira with 7.33 mg 100 g^{-1} FW and 8.84 mg for control and inoculated plants respectively upon CO_2 enrichment. Similar results were found under ambient CO_2 with maximum ascorbic acid content in *Z. zerumbet* (6.61 mg 100 g^{-1} FW) and Athira (7.00 mg 100 g^{-1} FW) for control and inoculated plants respectively.

Phenols

As shown in Table 38, higher phenol content was recorded under elevated CO_2 compared to the ambient. Furthermore, disease development triggered the production of phenols. The maximum phenol content was recorded in the variety Athira with 27.99 mg g^{-1} DW and 40.89 mg g^{-1} DW for control and inoculated plants respectively upon CO_2 enrichment. Similar results were noticed under ambient CO_2 with maximum phenol content evident for *Z. zerumbet* (27.44 mg g^{-1} DW) and Athira (31.67 mg g^{-1} DW) for control and inoculated plants respectively.

Glucanase activity

Higher glucanase activity was evident under elevated CO_2 compared to the ambient CO_2 as shown in Table 39. The disease development significantly enhanced the glucanase activity. Highest glucanase activity was found in the variety Athira with 4.34 Units mg^{-1} total protein and 6.03 Units mg^{-1} total protein for control and inoculated plants respectively upon CO_2 enrichment. Under ambient CO_2, the highest glucanase activity was recorded in *Z. zerumbet* (3.03 Units mg^{-1} total protein) and Athira (5.39 Units mg^{-1} total protein) for control and inoculated plants respectively.

Table 33. Effect of elevated CO_2 on malondialdehyde in *Zingiber* species upon *P. aphanidermatum* inoculation

	Malondialdehyde content (µg g^{-1} FW)					
	Ambient CO_2			Elevated CO_2		
Varieties	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	0.76	1.46	92.10	0.66	1.23	86.36
Athira	0.50	0.85	70.00	0.64	0.74	16.66
Maran	0.45	0.77	71.11	0.55	0.63	14.41
Z. zerumbet	0.34	0.46	35.29	0.40	0.41	2.5
Mean(T)	0.51	0.88		0.56	0.75	
Mean (C)	0.70			0.65		
CD(0.05)	T=0.03	V=0.04		C*T= NS	V*C=0.06	T*V= 0.06
SEm±	T=0.01	V=0.01		C*T=0.01	V*C=0.02	T*V= 0.02

V= Varieties, C= CO_2 conditions, T= Pathological treatments

Table 34. Effect of elevated CO_2 on catalase activity in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Catalase activity (Units mg^{-1} total protein)					
	Ambient CO_2			Elevated CO_2		
	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	0.30	0.12	-60.00	0.19	0.11	-42.10
Athira	0.14	0.11	-21.42	0.10	0.08	-20.00
Maran	0.20	0.11	-0.45	0.12	0.10	-16.67
Z. zerumbet	0.14	0.13	-7.14	0.09	0.09	0.00
Mean(T)	0.16	0.11		0.12	0.09	
Mean (C)	0.13			0.10		
CD(0.05)	T=0.08	V=0.12		C*T=0.01	V*C=NS	T*V= 0.01
SE± (m)	T=0.04	V=0.03		C*T=0.04	V*C=0.06	T*V= 0.06

V= Varieties, C= CO_2 conditions, T= Pathological treatments; **One unit-** decompose 1µmol H_2O_2 min^{-1}

Table 35. Effect of elevated CO_2 on ascorbate peroxidise activity in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Ascorbate peroxidase activity (Units mg^{-1} total protein)					
	Ambient CO_2			Elevated CO_2		
	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	2.58	2.84	10.07	3.03	3.01	-0.66
Athira	3.61	5.06	40.16	5.19	6.08	17.14
Maran	2.22	3.07	38.28	3.74	3.98	6.41
Z. zerumbet	5.34	5.64	5.61	6.40	6.40	0.00
Mean(T)	3.43	4.15		4.59	4.86	
Mean (C)	3.79			4.72		
CD(0.05)	T=0.23	V=0.33		C*T= NS	V*C= NS	T*V= 0.47
SEm±	T=0.08	V=0.08		C*T=0.11	V*C=0.16	T*V= 0.16

V= Varieties, C= CO_2 conditions, T= Pathological treatments ; One unit- oxidise 1µmol of ascorbic acid min^{-1}

Table 36. Effect of elevated CO_2 on phenylalanine ammonia lyase activity in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Phenylalanine ammonia lyase activity (Units mg^{-1} total protein)					
	Ambient CO_2			Elevated CO_2		
	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	12.91	14.80	14.63	16.48	18.54	12.50
Athira	16.47	20.03	21.61	23.83	32.75	37.43
Maran	13.13	15.71	19.64	14.17	17.26	21.80
Z. zerumbet	11.94	13.60	13.90	14.93	15.10	1.13
Mean(T)	13.61	16.04		17.35	20.91	
Mean (C)	14.82			19.13		
CD(0.05)	T=0.34	V=0.48		C*T=0.48	V*C= 0.68	T*V= 0.68
SEm±	T=0.11	V=0.16		C*T=0.16	V*C=0.23	T*V= 0.23

V= Varieties, C= CO_2 conditions, T= Pathological treatments ;One unit - nmoles of trans-cinnamic acid formed min^{-1}

Table 37. Effect of elevated CO_2 on ascorbic acid content in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Ascorbic acid content (mg 100 g^{-1} FW)					
	Ambient CO_2			Elevated CO_2		
	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	5.17	5.01	-3.01	6.40	7.03	9.84
Athira	5.77	7.00	21.31	7.33	8.84	20.60
Maran	3.80	4.01	5.52	4.18	5.48	31.10
Z. zerumbet	6.61	6.45	-2.42	7.21	7.23	0.27
Mean(T)	5.33	5.61		6.28	7.14	
Mean (C)	5.47			6.71		
CD(0.05)	T=0.26	V=0.37		C*T= NS	V*C=0.52	T*V=0.52
SEm±	T=0.09	V=0.13		C*T=0.13	V*C=0.18	T*V=0.18

V= Varieties, C= CO_2 conditions, T= Pathological treatments

Table 38. Effect of elevated CO_2 on phenols in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Phenols (mg g^{-1}DW)					
	Ambient CO_2			Elevated CO_2		
	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	21.51	24.55	14.13	25.30	28.61	13.08
Athira	24.99	31.67	26.73	27.99	40.89	46.08
Maran	21.62	27.25	26.04	27.50	32.12	16.80
Z. zerumbet	27.44	28.73	4.70	26.59	28.00	5.30
Mean(T)	23.89	28.05		26.84	32.40	
Mean (C)	25.97			29.62		
CD(0.05)	T=0.32	V=0.45		C*T=0.45	V*C=0.64	T*V= 0.64
SEm±	T=0.11	V=0.15		C*T=0.15	V*C=0.22	T*V= 0.22

V= Varieties, C= CO_2 conditions, T= Pathological treatments

Table 39. Effect of elevated CO_2 on gluconase activity in *Zingiber* species upon *P. aphanidermatum* inoculation

	Glucanase activity (Units mg^{-1} total protein)					
	Ambient CO_2			Elevated CO_2		
Varieties	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	1.06	1.32	24.52	4.31	5.26	22.04
Athira	2.09	5.39	157.89	4.34	6.03	38.94
Maran	1.60	1.82	13.75	2.06	2.96	43.68
Z. zerumbet	3.03	3.46	14.19	3.82	4.04	5.75
Mean(T)	1.95	3.49		3.63	4.57	
Mean (C)	2.47			4.10		
CD(0.05)	T=0.17	V=0.24		C*T=0.24	V*C=0.35	T*V= 0.35
SEm±	T=0.06	V=0.08		C*T=0.08	V*C=0.12	T*V= 0.12

One unit- µmoles of glucose formed min^{-1}; V= Varieties, C= CO_2 conditions, T= Pathological treatments

Determination of superoxide and H_2O_2 by staining

The stain used for the detection of superoxide was NBT. It is based on the principle that NBT reacts with O_2^- to form a dark blue insoluble formazan compound nitroblue tetrazolium. From Plate 7 and 8, it is inferred that the staining was higher under ambient CO_2 compared to the elevated CO_2 upon the inoculation. Among the varieties, Aswathy had higher region and intensity of staining followed by Maran, Athira and *Z. zerumbet.* This indicates higher accumulation of O_2^- in the variety Aswathy and least for *Z. zerumbet.*

The staining technique utilised for H_2O_2 detection was DAB staining. It is based on the principle that DAB gets oxidised in the presence of H_2O_2 to a brown coloured compound. From Plate 9 and 10, it is evident that the staining was higher under ambient CO_2 compared to the elevated CO_2 upon the inoculation. Among the varieties, Aswathy had higher region and intensity of staining followed by Maran, Athira and *Z. zerumbet.* This indicates higher accumulation of H_2O_2 for variety the Aswathy and least for *Z. zerumbet.*

Determination of cell death by staining

The staining technique utilised for cell death was tryphan blue staining. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes. The dead cells get stained blue in colour. From Plate 11 it is inferred that among the varieties, Aswathy had higher region and intensity of staining followed by Maran, Athira and *Z. zerumbet.* This indicates higher cell death in the variety Aswathy and least for *Z. zerumbet.*

Chlorophyll fluorescence signals

Fv/Fm ratio

The Fv/Fm ratio significantly decreased in diseased plants compared to the healthy ones as shown in Table 40. Upon disease development the retention of Fv/Fm ratio was higher for the plants raised under CO_2 enrichment. Fv/Fm remained stable in wild genotype even with inoculation of *P. aphanidermatum* but it decreased significantly in cultivated varieties.

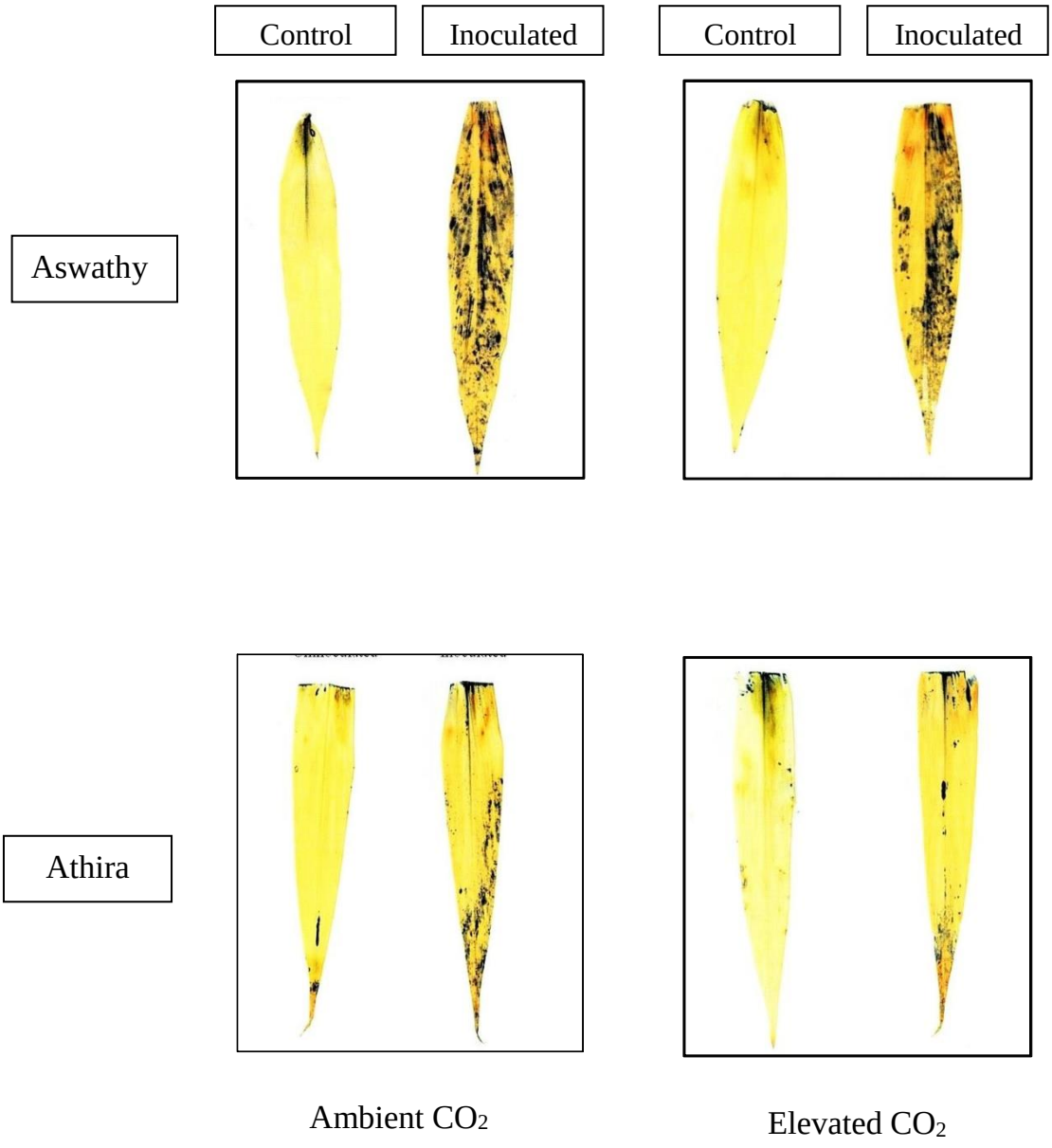


Plate 7. Determination of superoxide by Nitroblue tetrazolium chloride staining Technique (Variety Aswathy and Athira)

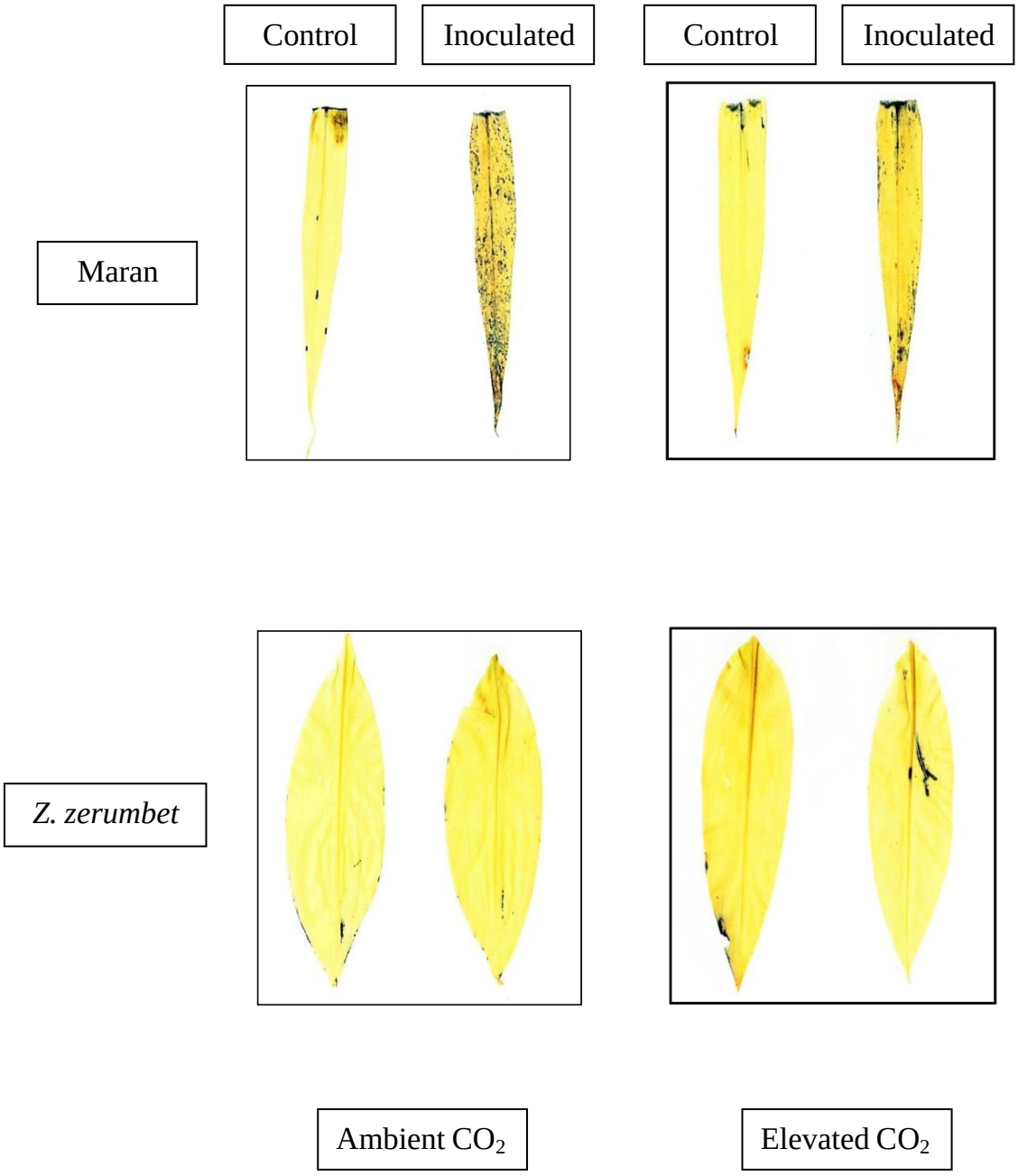


Plate 8. Determination of superoxide by Nitroblue tetrazolium chloride staining technique (Variety Maran and *Z. zerumbet*)

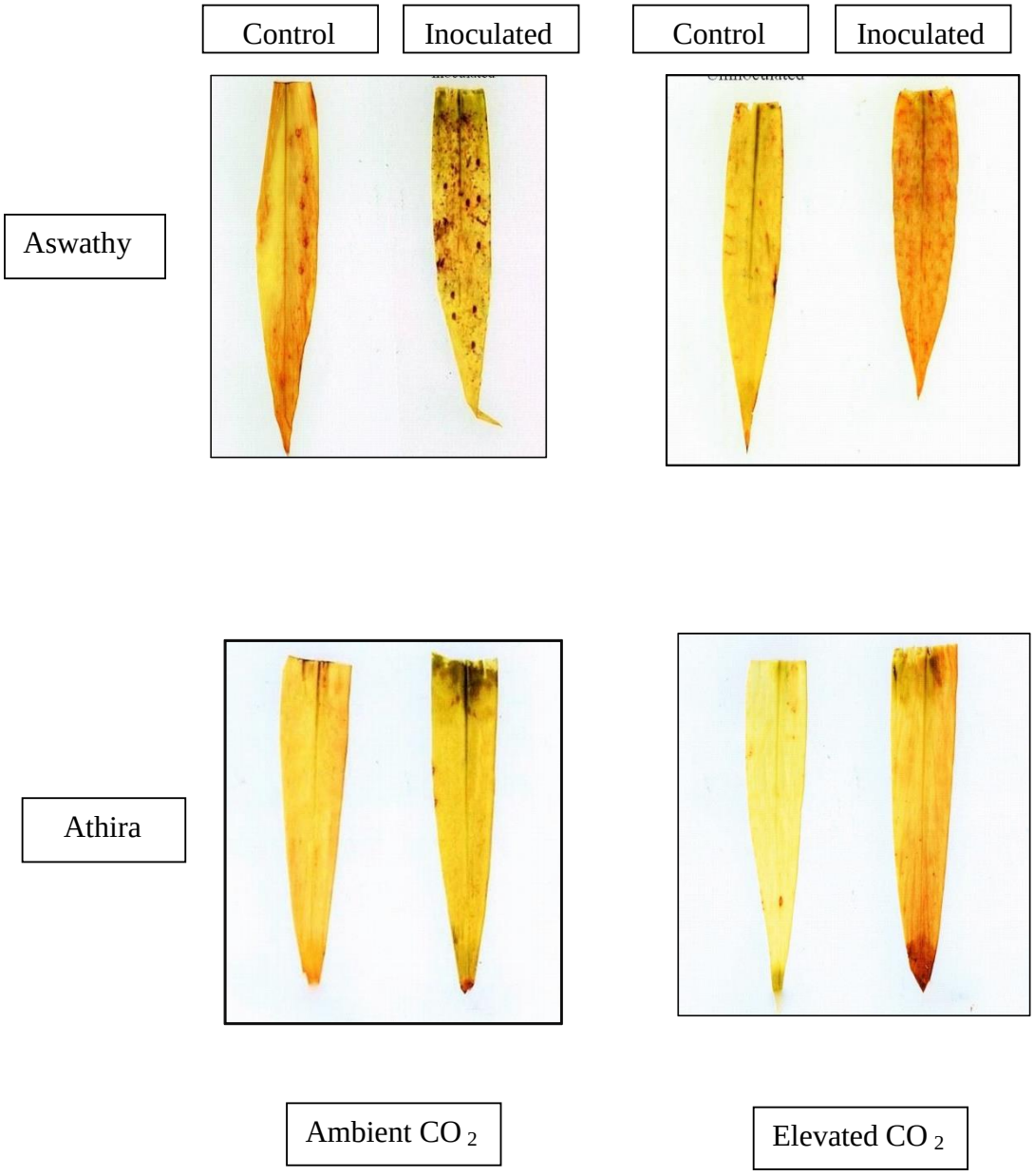


Plate 9. Determinationof H_2O_2 by 3,3- Diaminobenzidine staining technique (Variety Aswathy and Athira)

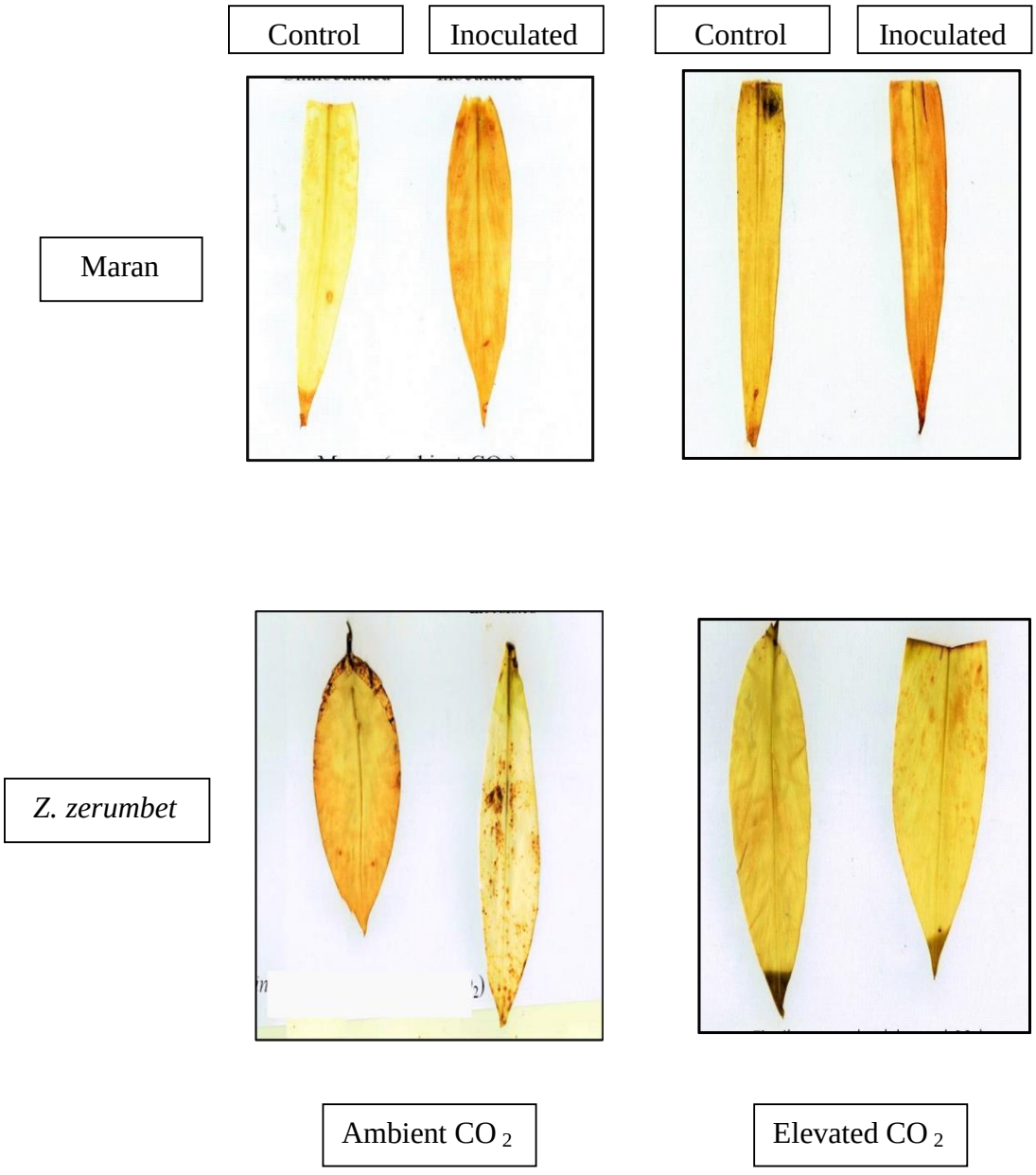


Plate 10. Determination of H_2O_2 by 3,3-Diaminobenzidine staining technique (Variety Maran and *Z. zerumbet*)

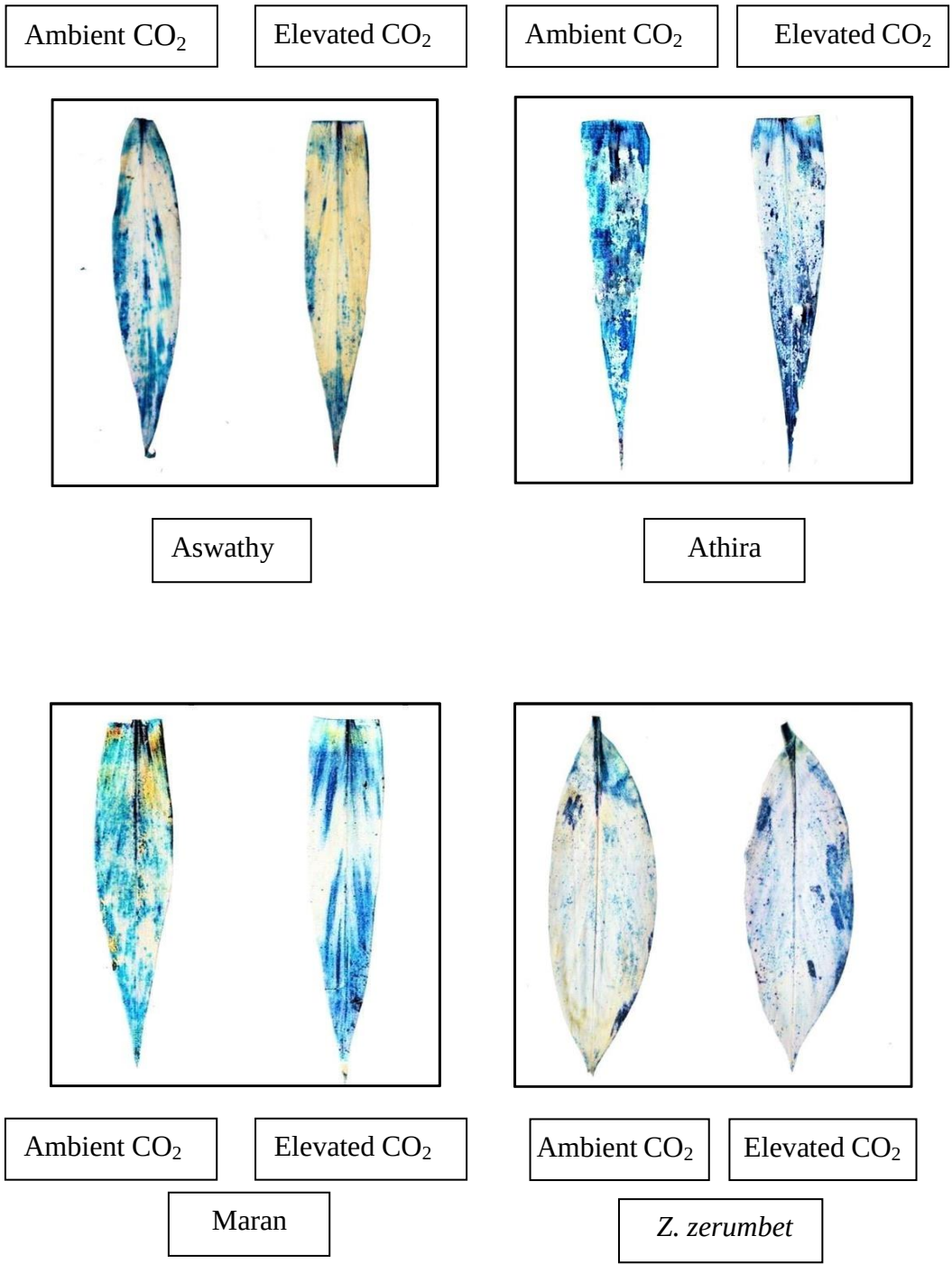


Plate 11. Determination of cell death by trypan blue technique

Photosynthetic electron transport rate

The photosynthetic electron transport rate decreased significantly upon *P. aphanidermatum* inoculation (Table 41). The plants under elevated CO_2 retained the photosynthetic electron transport rate relatively as compared to ambient. Significant difference was observed among the *Zingiber* species. The wild genotype was not affected much by *P. aphanidermatum* inoculation in terms of photosynthetic electron transport rate.

Quantum yield of PS II photochemistry

The quantum yield of PS II significantly decreased in the diseased plants compared to the healthy ones as shown in Table 42. The mean quantum yield did not differ markedly between ambient and elevated CO_2 conditions. However, inoculation with *P. aphanidermatum* decreased the quantum yield significantly compared to control both under ambient and elevated CO_2 conditions. Among the *Zingiber* species, *Z. zerumbet* was least affected and higher extent in Aswathy.

Molecular studies on the effect of elevated CO_2 on the response of ginger to soft rot tolerance

Expression pattern in *PAL, CAD, CHS*

Isolation of RNA

Since, among the selected varieties, Athira was affected to a lower extent and Aswathy exhibited greater symptom development. The expression analysis was performed for Aswathy and Athira to decipher the genes conferring tolerance. The leaf samples were collected on the 2^{nd} day of symptom development at four different conditions *viz*., with and without inoculation of *P. aphanidermatum* under both ambient and elevated CO_2. Three distinct intact bands with no apparent degradation of RNA were observed on agarose gel (2 %) (Plate 12).

Quantity of isolated RNA

Quantity of isolated RNA was recorded using absorbance value obtained by using spectropotometric method as presented in Table 43.

Table 40. Effect of elevated CO_2 on Fv/Fm ratio (variable to maximum fluorescence) in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Variable to maximum fluorescence					
	Ambient CO_2			Elevated CO_2		
	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	0.79	0.43	-45.56	0.81	0.48	-40.74
Athira	0.74	0.62	-16.21	0.75	0.65	-13.33
Maran	0.76	0.54	-28.94	0.78	0.61	-21.79
Z. zerumbet	0.82	0.81	-0.12	0.81	0.80	-1.23
Mean(T)	0.78	0.60		0.79	0.63	
Mean (C)	0.69			0.71		
CD(0.05)	T=0.02	V=0.03		C*T= NS	V*C= NS	T*V= 0.05
SEm±	T=0.01	V=0.01		C*T=0.01	V*C=0.01	T*V= 0.01

V= Varieties, C= CO_2 conditions, T= Pathological treatments

Table 41. Effect of elevated CO_2 on photosynthetic electron transport rate (micro equiv m^{-2} s^{-1}) in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Photosynthetic electron transport rate (micro equiv m^{-2} s^{-1})					
	Ambient CO_2			Elevated CO_2		
	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	112.88	52.16	-53.79	114.09	56.57	-50.41
Athira	94.57	76.64	-18.9	97.18	83.93	-13.63
Maran	97.56	64.22	-34.17	106.36	80.39	-24.41
Z. zerumbet	115.45	113.05	-2.08	112.18	109.52	-2.37
Mean(T)	105.11	76.52		107.45	82.60	
Mean (C)	90.81			95.03		
CD(0.05)	T=0.82	V=1.16		C*T=1.16	V*C=1.64	T*V= 1.64
SEm±	T=0.28	V=0.40		C*T=0.40	V*C=0.57	T*V= 0.57

V= Varieties, C= CO_2 conditions, T= Pathological treatments

Table 42. Effect of elevated CO_2 on quantum yield of PS II photochemistry in *Zingiber* species upon *P. aphanidermatum* inoculation

Varieties	Quantum yield of PS II photochemistry					
	Ambient CO_2			Elevated CO_2		
	Control	Inoculated	% change	Control	Inoculated	% change
Aswathy	0.39	0.12	-69.23	0.41	0.19	-53.65
Athira	0.32	0.28	-12.50	0.34	0.31	-8.82
Maran	0.37	0.25	-32.43	0.39	0.27	-30.76
Z. zerumbet	0.44	0.41	-6.81	0.40	0.40	0.00
Mean(T)	0.38	0.26		0.38	0.29	
Mean (C)	0.32			0.34		
CD(0.05)	T=0.02	V=0.03		C*T= NS	V*C= NS	T*V= 0.04
SEm±	T=0.00	V=0.01		C*T=0.01	V*C=0.01	T*V= 0.01

Table 43. Quantity and quality of isolated RNA

SI.No	Variety	CO_2 concentration	Treatment	RNA concentration (ng/µl)	A_{260}/A_{280}
1	Aswathy	Ambient CO_2	Unioculated	1239.5	1.85
2			Inoculated	1804.8	1.83
3		Elevated CO_2	Unioculated	1195.3	1.85
4			Inoculated	2248.1	1.90
5	Athira	Ambient CO_2	Unioculated	1784.2	1.80
6			Inoculated	1689.2	1.90
7		Elevated CO_2	Unioculated	2158.4	1.88
8			Inoculated	1984.2	1.95

Quality check of cDNA

The cDNA synthesised utilising PCR was checked for quality using *Actin* primer (Plate 13) and primers for defense responsive genes utilised in the study *viz.*, *CAD*, *PAL* and *CSH* (Plate 14, 15 and 16). Amplification of cDNA with all the gene primers produced amplicons of expected size indicating specificity of the primers.

q-RT PCR/ REAL TIME PCR

The cDNA obtained from both the varieties and different conditions were subjected to Real-time PCR (RT-qPCR) using primers for defence response genes with *Actin* as reference gene. Amplification plot was generated utilising 'CFX Maestro Software'. The raw expression data generated from the amplification plot of *PAL*, *CAD* and *CSH* are depicted in the Fig 1 and 2 respectively.

Analysis of RT-qPCR data

Differential expression of the three genes was evident in all the conditions and both the varieties. The relative fold change of *CAD* transcript level was significantly higher under elevated CO_2 compared to ambient CO_2 (Fig 3). However, there was no significant difference between the varieties Aswathy and Athira.

The relative fold change of *CSH* transcript level was significantly higher under elevated CO_2 compared to ambient CO_2 (Fig 4). The *CSH* transcript level of Athira had 1.22 fold changes which was significantly higher than that of Aswathy 0.38 under elevated CO_2. However, there was no significant difference evident between the varieties Aswathy and Athira at ambient CO_2.

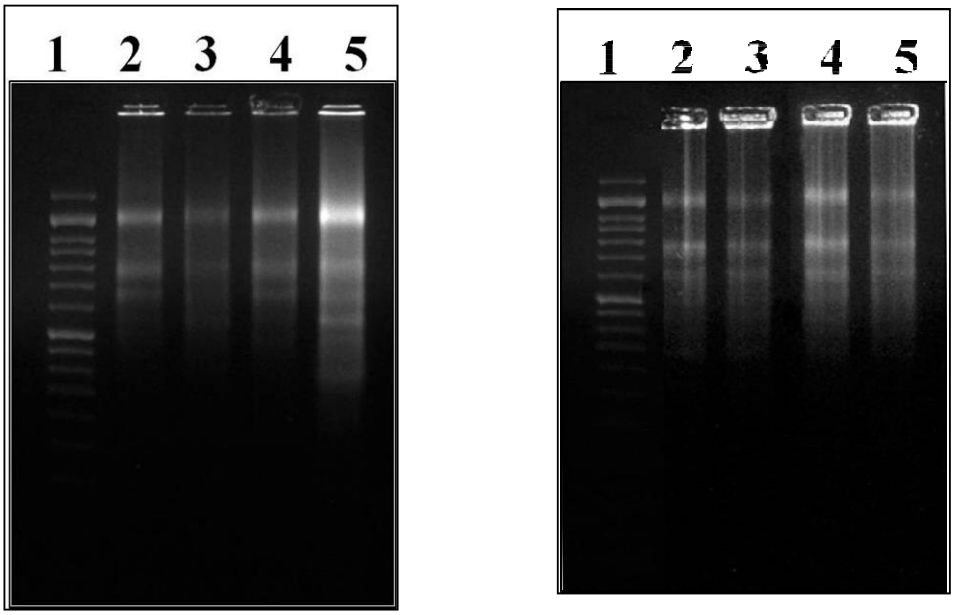
PAL gene, involved in the phenylpropanoid biosynthetic pathway varied markedly and exhibited higher levels of gene expression compared to *CAD* and *CSH* gene. Significant difference was evident between the varieties (Fig 5).

Protein profile

SDS mediated protein profiling was done in the leaves of variety Aswathy and Athira. The sample was collected on the 2^{nd} day of symptom development under both ambient and elevated CO_2 conditions. Differential expressions of proteins were evident.

The protein profile showed a large number of polypeptide bands. The major band resolved was of a 55 kDa polypeptide. This protein corresponds to the large subunit of Rubisco. This band was present in all the varieties; however the intensity of the band was found to vary with respect to the varieties and treatments. It is of interest to note that the intensity of the band corresponding to the large subunit reduced to a greater extent under pathogen inoculation (Plate 17).

The results showed that 25-35 kDa protein decreased in the variety Aswathy under ambient condition and *P. aphanidermatum* inoculation. Protein of size 20-25 kDa decreased in the leaves of both inoculated and uninoculated leaves of variety Aswathy under ambient CO_2. In the case of variety Athira, it is interesting to note that the protein of 63-75 kDa was present under ambient and uninoculated conditions.

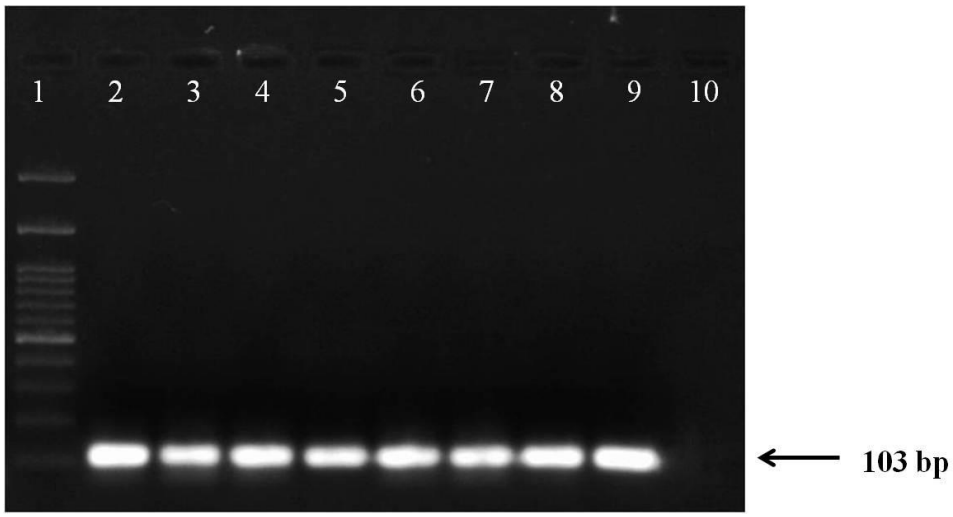


Aswathy

Athira

Lane 1: 50 bp Ladder
Lane 2: Control, ambient CO_2
Lane 3: Inoculated, ambient CO_2
Lane 4: Control, elevated CO_2
Lane 5: Inoculated, elevated CO_2

Plate 12. Gel profile showing total RNA isolated from the varieties Aswathy and Athira at different treatmental conditions

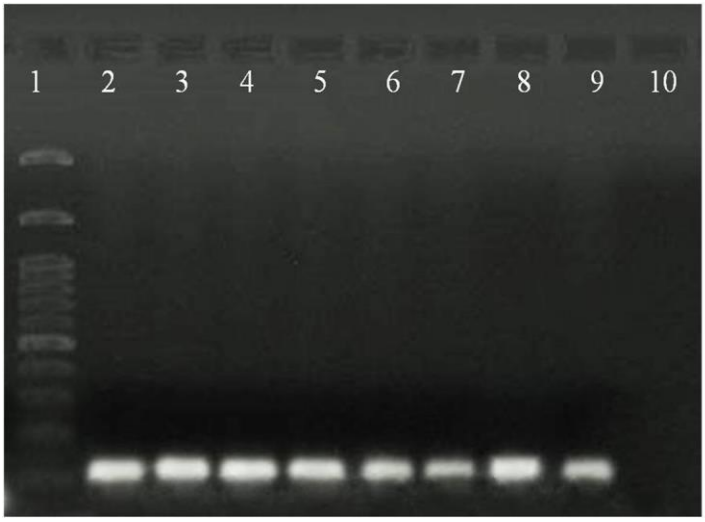


Lane 1: 100 bp Ladder
Lane 2: Aswathy uninoculated, ambient CO_2
Lane 3: Aswathy inoculated, ambient CO_2
Lane 4: Aswathy uninoculated, elevated CO_2
Lane 5: Aswathy inoculated, elevated CO_2
Lane 6: Athira uninoculated, ambient CO_2
Lane 7: Athira inoculated, ambient CO_2
Lane 8: Athira uninoculated, elevated CO_2
Lane 9: Athira inoculated, elevated CO_2
Lane10: Blank

Plate 13. Gel profile showing PCR amplicon obtained from cDNA from the varieties Aswathy and Athira at different treatmental conditions using the primer *Actin*

Lane 1: 100 bp Ladder
Lane 2: Aswathy uninoculated, ambient CO_2
Lane 3: Aswathy inoculated, ambient CO_2
Lane 4: Aswathy uninoculated, elevated CO_2
Lane 5: Aswathy inoculated, elevated CO_2
Lane 6: Athira uninoculated, ambient CO_2
Lane 7: Athira inoculated, ambient CO_2
Lane 8: Athira uninoculated, elevated CO_2
Lane 9: Athira inoculated, elevated CO_2
Lane 10: Blank

Plate 14. Gel profile showing PCR amplicon obtained from cDNA from the varieties Aswathy and Athira at different treatmental conditions using the primer *CAD*



Lane 1: 100 bp Ladder
Lane 2: Aswathy uninoculated, ambient CO_2
Lane 3: Aswathy inoculated, ambient CO_2
Lane 4: Aswathy uninoculated, elevated CO_2
Lane 5: Aswathy inoculated, elevated CO_2
Lane 6: Athira uninoculated, ambient CO_2
Lane 7: Athira inoculated, ambient CO_2
Lane 8: Athira uninoculated, elevated CO_2
Lane 9: Athira inoculated, elevated CO_2
Lane 10: Blank

Plate 15. Gel profile showing PCR amplicon obtained from cDNA from the varieties Aswathy and Athira at different treatmental conditions using the primer *PAL*

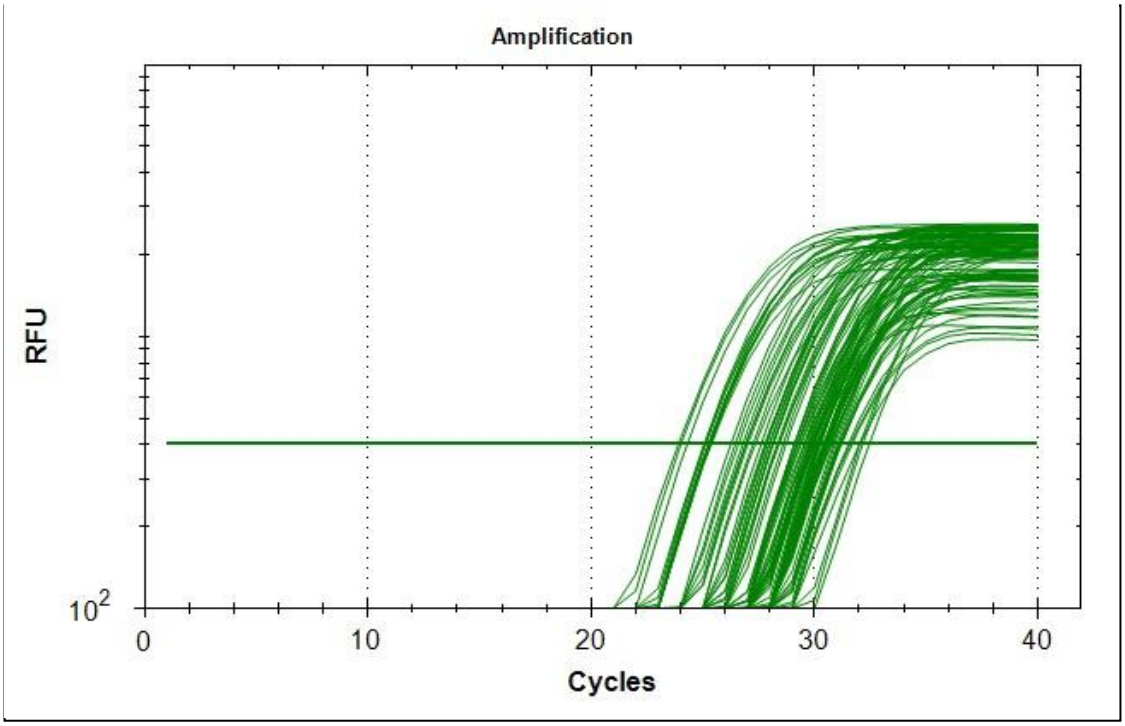


Fig. 1. Amplification plot of *CAD* by RT-qPCR

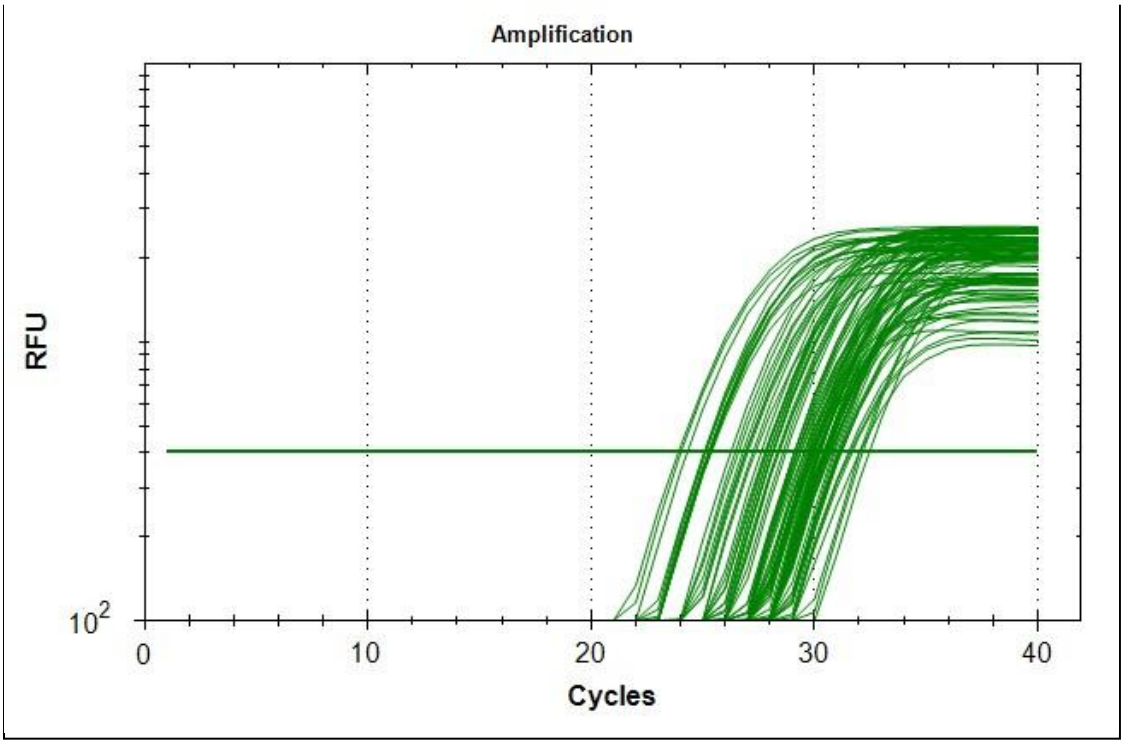


Fig. 2. Amplification plot of *PAL* and *CHS* by RT-qPCR

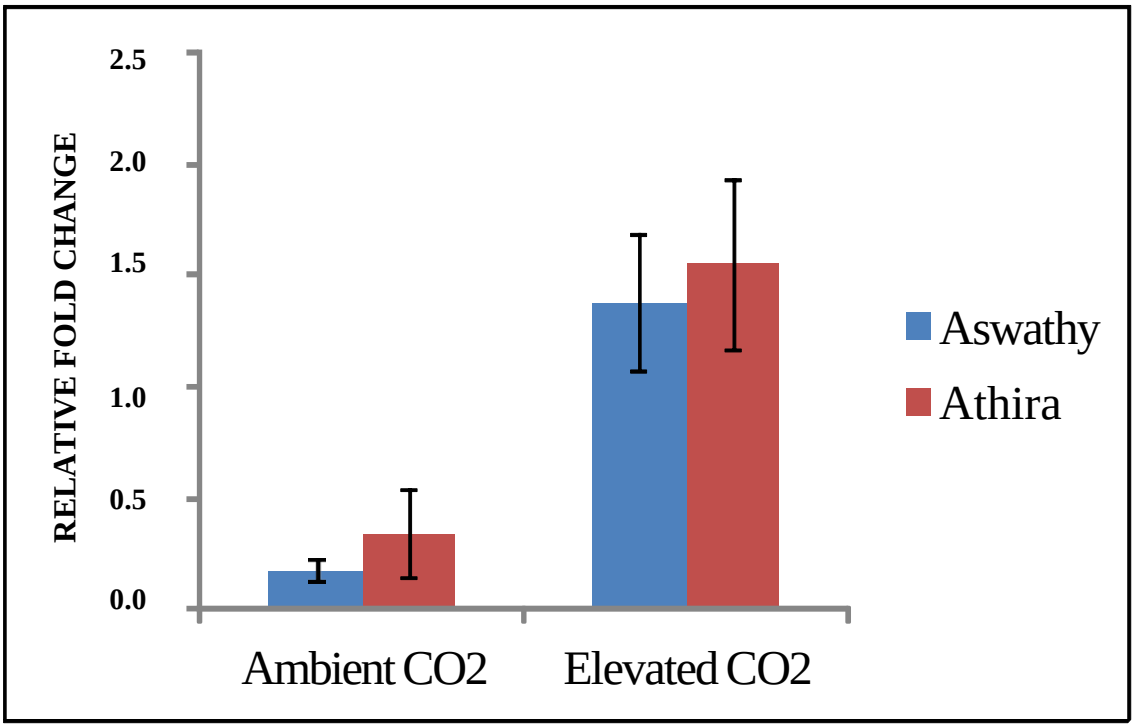


Fig. 3. Relative fold changes of *CAD* in Aswathy and Athira

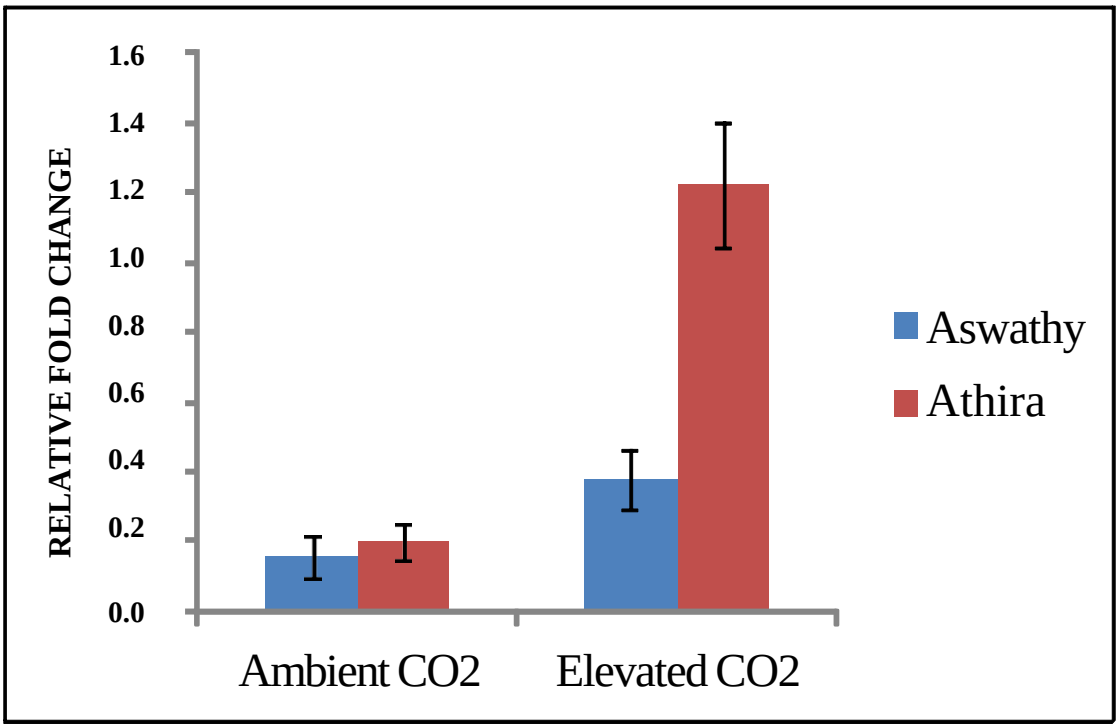


Fig. 4. Relative fold changes of *CSH* in Aswathy and Athira

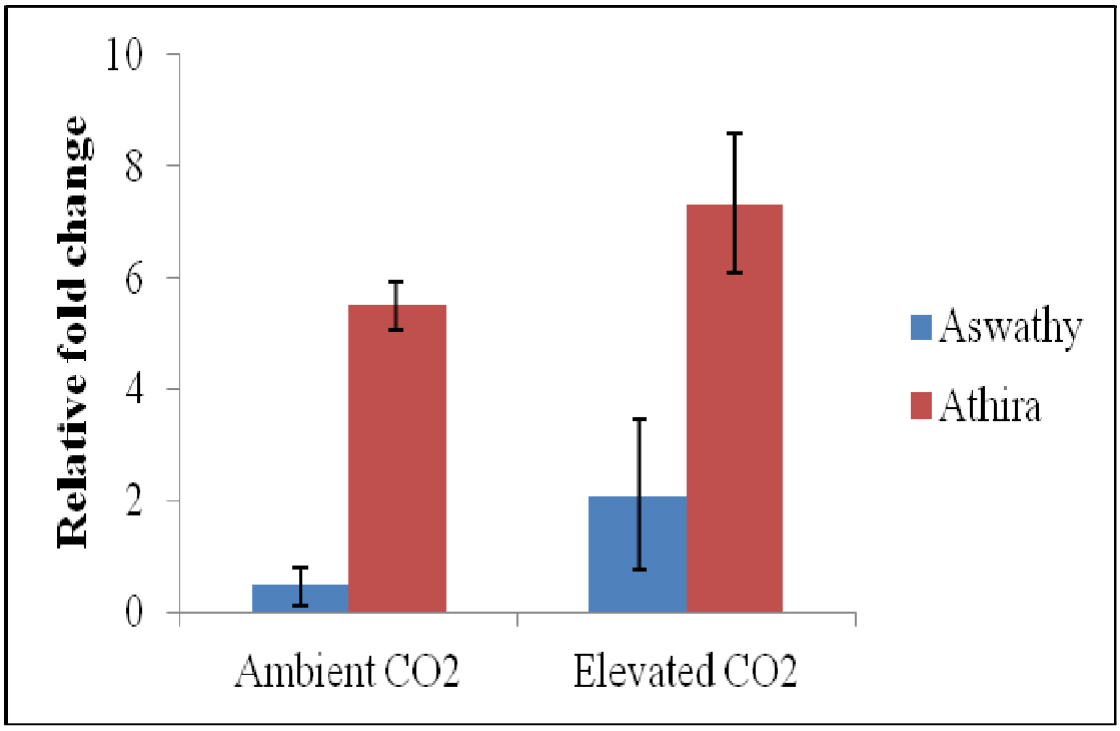
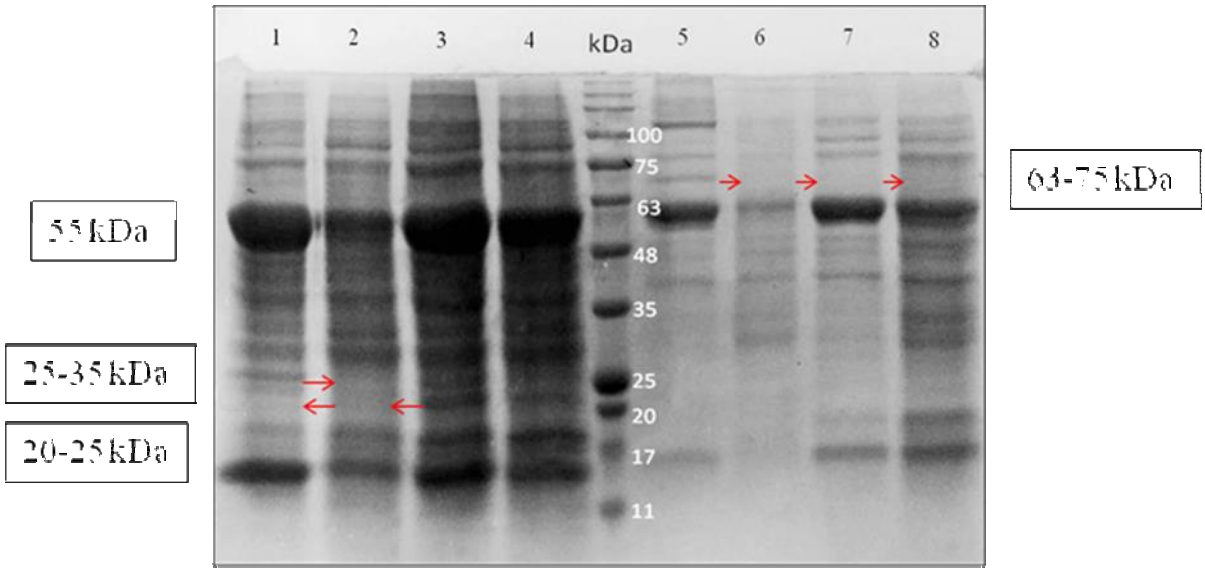


Fig. 5. Relative fold changes of *PAL* in Aswathy and Athira



Lane 1: Aswathy uninoculated, ambient CO_2
Lane 2: Aswathy inoculated, ambient CO_2
Lane 3: Aswathy uninoculated, elevated CO_2
Lane 4: Aswathy inoculated, elevated CO_2
Lane 5: Athira uninoculated, ambient CO_2
Lane 6: Athira inoculated, ambient CO_2
Lane 7: Athira uninoculated, elevated CO_2
Lane 8: Athira inoculated, elevated CO_2

Plate 17. SDS gel profile protein bands of Aswathy and Athira at different treatment conditions

Discussion

5. DISCUSSION

One of the most widely expected changes in climate change scenatio is the rise in atmospheric CO_2 concentration. Rising atmospheric CO_2 significantly influences plant growth, development and biomass. In this chapter focus is laid on the effect of enhanced carbon supply that influences a range of cellular functions and growth processes in ginger; and changes pertaining to their growth, development, biochemical and physiological parameters, yield parameters and its quality (Fig. 6).

5.1. Assessing the impact of elevated CO_2 on growth, development and quality of ginger

5.1.1 Impact of elevated CO_2 on growth parameters

In the present study, the positive response of plant height was higher at initial stages 4 MAP and 5 MAP ranging between 12.43 to 31.36% and 7.23 to 14.08% respectively. The gain in plant height reported here is in agreement with previous studies conducted by Mamatha *et al.* (2014) who reported that tomato plants grown at elevated CO_2 of 550 ppm recorded significantly higher plant height (115.0 cm), which was 40.7% more than those grown at control (92.3 cm). At 70 days after transplanting, UKMR-2 variety of *Hibiscus sabdariffa* with elevated CO_2 showed an increase in plant height by 15.4% compared to ambient CO_2 (Ali *et al.*, 2019).

Plant height had a positive and significant correlation with rhizome weight, rhizome thickness and leaves tiller^{-1}. Also it has been considered to be of prime importance in the selection program on the basis of its positive and significant correlation with rhizome yield (Sasikumar *et al.*, 1992). So increase in plant height will be useful. One of the approaches to improve plant height and associated yield could be through enrichment in CO_2 or increasing CO_2 in the changing climate scenario would have the positive impact on ginger yield.

Elevated CO_2 results in the altered plant structure which is due to change in pattern of primordium initiation, higher rates of cell division and increased cell expansion (Pritchard *et al.*, 1999). The growth stimulation of plants that are grown under elevated

CO_2 may be direct (based on substrate supply) or indirect (based on chemical signals) or both. For instance, increased photosynthate availability in meristems might have increased the proportion of rapidly dividing cells by stimulating cyclin activity, thus stimulating cell division (Jacobs, 1997).

In the present study, sharp decline in relative CO_2 effect of plant height was observed at 7 MAP (4.94% Maran) and 8 MAP (0.14 % Maran) (Fig. 7). This indicates that the period of vegetative phase could have been prolonged under elevated CO_2 in the present study.

The relative CO_2 effect was positive for number of leaves at different growth stages of the crop. It was highest at 8 MAP; 55.51% more in Aswathy, 47.65% more in Athira and 36.45% in Maran (Fig. 8). Similar results have been reported by Srikanth *et al*. (2019) in cowpea where number of leaves recorded was higher (78) under elevated CO_2 of 500 ppm as compared to open control (70.75). Under elevated CO_2, plants tend to show an increased rate of leaf initiation and accelerated elongation coupled with enhanced leaf area (Taylor *et al*., 2001). Changes in leaf anatomy and morphology, an increase in photo assimilate supply to the apical meristem increases and promotion in leaf primordial development was observed upon CO_2 enrichment (Pritchard *et al.,* 1999). Leaf number per plant and per tiller is another developmental index influenced by the CO_2 treatments. Similar results were evident in the present study, wherein positive correlation was observed between number of leaves per plant with tiller number under elevated CO_2 (Fig. 9). The result is in agreement with Jitla *et al*. (1997) who reported that in rice during the vegetative stage, a greater supply of carbohydrates accelerates the rate of development of the leaves on the main shoot and on each tiller, in addition to stimulate the number of new tillers.

Decrease in the SLA (leaf surface area per unit dry mass) was evident in the plants grown under CO_2 enriched conditions (Fig. 10). The decrease was higher at 5 MAP and 7 MAP; and was more pronounced in variety Aswathy (8.18, 11.48, 9.60 and 5.00 % at 4, 5, 7 and 8 MAP). The results are similar to the report of Li *et al.* (2019) wherein, the response of SLA to elevated CO_2 (550 ppm) decreased by an average of 6.9% in nine cultivars of soya bean. Radish plants grown at 740 ± 19 μmol mol^{-1} CO_2

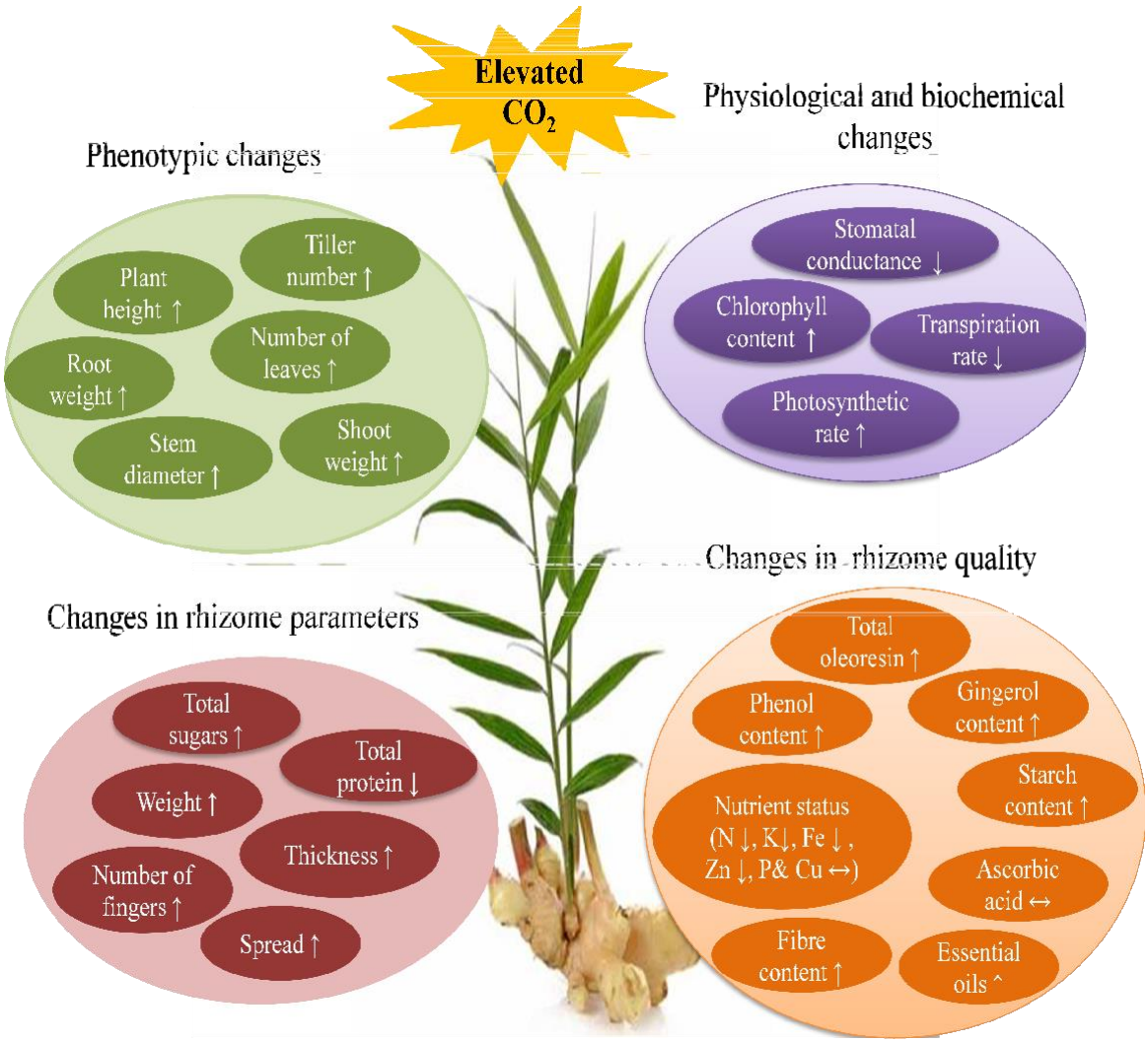
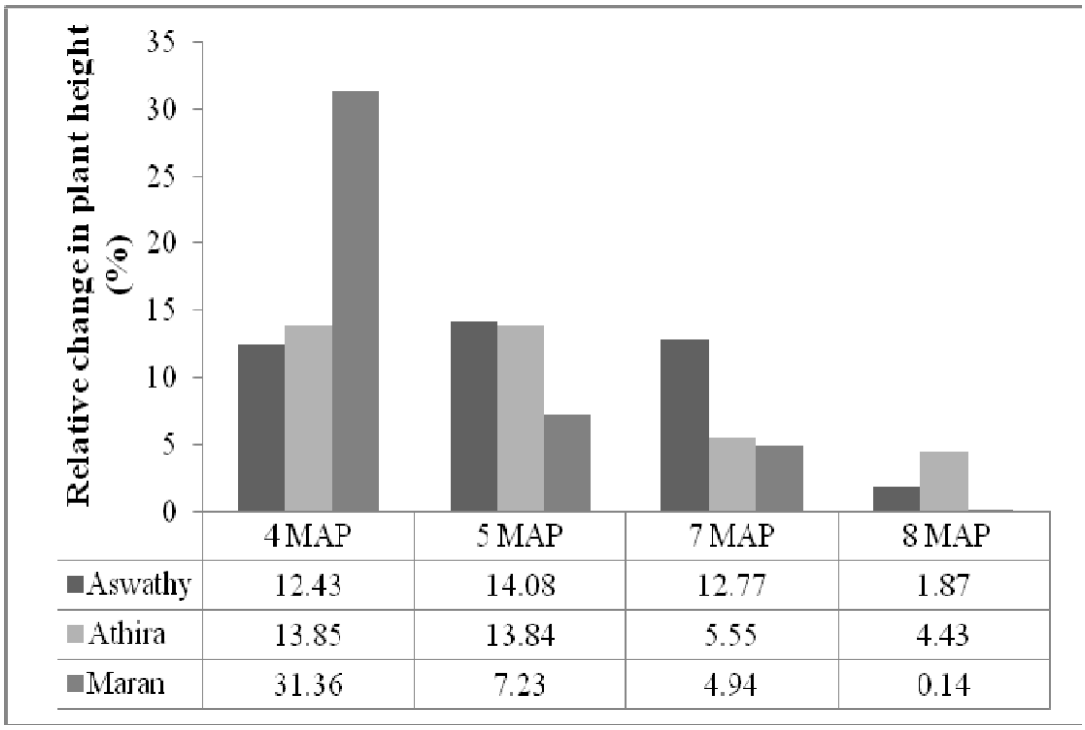
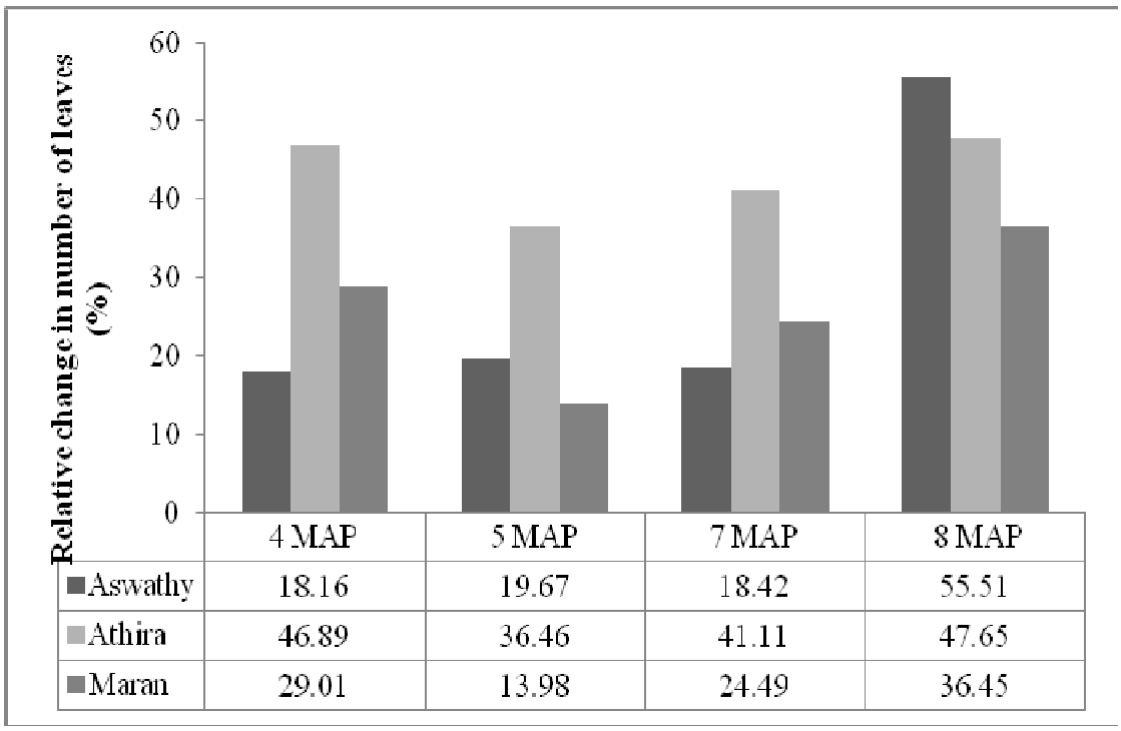


Fig. 6. Schematic representation on effect of elevated CO_2 on phenotypic parameters, physiological and biochemical parameters, rhizome parameters and its quality. ↑indicates increase, ↓indicates decrease, ↔indicates no change



	4 MAP	5 MAP	7 MAP	8 MAP
Aswathy	12.43	14.08	12.77	1.87
Athira	13.85	13.84	5.55	4.43
Maran	31.36	7.23	4.94	0.14

Fig. 7. Relative changes in plant height due to elevated CO_2 against ambient CO_2 at different time intervals



	4 MAP	5 MAP	7 MAP	8 MAP
Aswathy	18.16	19.67	18.42	55.51
Athira	46.89	36.46	41.11	47.65
Maran	29.01	13.98	24.49	36.45

Fig. 8. Relative changes in number of leaves due to elevated CO_2 against ambient CO_2 at different time intervals

and under high light conditions exhibited decrease in SLA by 15.35% at 15-17 days after planting, 19.86% at 17 -19 days after planting and 22.18% decrease at 19-21 days after planting (Usuda, 2006). Reported increase in leaf thickness and decrease in SLA are often due to the altered anatomy or enhanced starch accumulation when plants are exposed to elevated CO_2. In soybean leaves, elevated CO_2 induced an extra layer of palisade cells (Smith *et al.,* 2012), and in the leaves of *Pinus radiata*, elevated CO_2 increased both the mesophyll and the area of vascular tissue. Increase in mesophyll and vascular tissue area is considered as the important parameters that contribute to photosynthetic capacity and assimilate transport efficiency of the plant (Pritchard *et al.*, 1999).

Elevated CO_2 had profound effect on root weight from 8 MAP (Fig. 11) and highest effect was apparent in Aswathy with 83.59% increase over control followed by Maran (87.35%) and Athira (47.11 %). Results are in accordance with studies conducted by Raj *et al*.(2019) who reported that root weight in rice significantly increased under elevated CO_2 (550 ppm) (38.2 g pot $^{-1}$) as against ambient CO_2 (20.7 g pot $^{-1}$) with an increase of 84.3%. At the anatomical level, an increase in stele and cortex diameter, increased root diameter in the root maturation zone and increased total root volume had been observed in cotton exposed to elevated CO_2 (Rogers *et al.,* 1992). Increased CO_2 can increase the root growth to balance nutrient uptake with the rate of sugar production due to increased photosynthesis, or a larger root system can act as a sink to store excess sugar. Salsman *et al.* (1999) claim that the increase in root biomass at elevated CO_2 is associated with increased starch levels in roots and an increase in the levels of ABA that might have caused more carbon to be allocated for the root growth.

Relative changes in shoot weight under elevated CO_2 were found to be positive. A sharp increase was evident as the plant growth progressed and peaked at 8 MAP for variety Aswathy. However, this response was not observed in Maran and Athira indicating the differential varietal responses to the elevated CO_2. Results of the current study are similar to the findings of Ghasemzadeh and Jaafar (2011) when two ginger varieties Halia Bentong and Halia Bara were raised under elevated CO_2 (800 ppm) resulted in the enhancement of dry weight of leaves (54.62%) and stem (24.60%) in

variety Halia Bentong; where as in Halia Bara, the enhancement of dry weight of leaves was 85.18% and stems to 22%. Tomato plants grown at elevated CO_2 (600 ppm) upon recovery from drought stress exhibited 50.12% increase over control for shoot weight (Chatti and Manju, 2018). The meta-analysis performed by Ainsworth and Long (2005) in C_3 species indicated that above ground biomass increased by an average of 20% in response to elevated CO_2.

Elevated CO_2 increases photosynthetic rates and results in higher sugar production (Watanabe *et al.,* 2014; Aranjuelo *et al.,* 2011). These additional sugars are available for development of new sink organs such as leaves, stems, tillers, and seeds. Stimulation of early leaf growth has been identified as one of the key physiological traits associated with final biomass and grain yield (Thilakarathne *et al.,* 2015). Similar results were also evident in the present study, wherein the increase in shoot weight resulted from increased number of leaves, plant height and number of tillers under elevated CO_2 (Fig. 12).

Relative changes in stem diameter under elevated CO_2 were found positive except for variety Athira at 4 MAP and 8 MAP. A sharp increase in stem diameter was evident as the plant growth progressed; peaked at 7 MAP and a decrease was found at 8 MAP (Fig. 13). The results are in accordance to the output of many parallel studies for stem diameter. Conroy *et al.* (1990) reported that CO_2 enrichment increased tracheid wall thickness by 44% in *Pinus radiata*. The ability of stems to transport water to maintain favourable water relations in whole plant is governed not only by quantity, but also by the size of xylem conduits. Stem cross-sectional area is thought to be related to whole plant reproductive capacity and is directly proportional to the amount of leaf area which must be supplied with water and solutes (Atkinson and Taylor, 1996). Tyree and Alexander (1993) reported that improved photosynthesis and carbohydrate availability resulted in increase in the size and number of xylem elements (vessels and tracheids) in plants grown under elevated CO_2.

The relative CO_2 effect was positive for number of tillers at all stages of the growth of ginger plants. Decrease in trend of relative CO_2 effect on tiller number is evident as the plant growth progressed (Fig. 14). The results are in agreement with the

study of Raj *et al*. (2019) and they have reported 7.7% increase in tiller production upon CO_2 enrichment (550 ppm) in rice. The enhanced levels of carbohydrates generated by photosynthesis upon CO_2 enrichment are effectively used to develop additional sinks such as secondary shoots and new tillers (Makino and Mae, 1999). Under elevated CO_2 there is higher accumulation of ACC synthase for higher levels of ethylene biosynthesis (Finlayson and Reid, 1996). It has been reported that enhanced production of ethylene leads to accelerated axillary bud development and tiller production under elevated CO_2 that potentially leads to higher grain yield (Seneweera *et al.,* 2003).

5.1.2 Impact of elevated CO_2 on yield and yield parameters

One of the effects of increasing atmospheric CO_2 is the increase in growth and yield (Jaggard *et al*., 2010). For instance, in rice variety YD6 yield increased upto 29.5% under CO_2 enrichment of 590 μmol mol^{-1}. When the CO_2 concentration increased from 400 to 800 μmol mol^{-1}, rhizome yield enhanced by 66.20% in Halia Bentong and by 103.3% in Halia Bara ginger varieties (Ghasemzadeh and Jaafar, 2011). In the present study, the fresh weight of rhizome increased in Aswathy by 38.35%, Athira by 12.12% and Maran by 20.01%. Also the dry weight enhanced by 38.95% in Aswathy, 20.87% in Athira and 35.14% in Maran. The results showed the stimulation of elevated CO_2 on ginger yield varied among varieties.

Since yield is a complex character and associated with several yield contributing characters, an attempt was made to elucidate the effect of elevated CO_2 on the enhancement of rhizome yield and other yield components in ginger plants. Yield contributing characters in ginger are number of fingers, rhizome thickness and rhizome length. The number of fingers increased by an average of 39.57% under elevated CO_2 compared to ambient CO_2. Varieties had different responses to elevated CO_2 with 37.50% increase in Aswathy as compared to 54.5 % in Athira and 26.67% in Maran. The rhizome thickness increased under elevated CO_2 compared to ambient CO_2. Varieties had different responses to elevated CO_2 with a 51.36% increase in Aswathy in comparison to 64.23% in Athira and 13.14% in Maran.

The rhizome length increased under elevated CO_2 compared to ambient CO_2. Varieties had different responses to elevated CO_2 with 20.09% increase in Aswathy and 16.61% in Maran, excluding Athira which had 2.06% decrease. One of the probable reasons for yield increase in Maran and Athira is due to increase in rhizome length. However, the contribution of rhizome length to increase in yield of Aswathy was less compared to the other two varieties.

It is evident that a strong positive correlation exists between rhizome yield and photosynthetic rate (Fig. 15 b) with significant ($p \leq 0.05$) respective value of $R^2 = 0.95$ at elevated CO_2, where as $R^2 = 0.58$ under ambient CO_2 (Fig. 15 a) this demonstrates that elevated CO_2 stimulated rhizome yield, and the yield gain which greatly attributed to the higher photosynthetic capability. Therefore, we can look for photosynthetic rate as an important trait in determining the yield. Also, the photosynthetic traits such as number of leaves, tiller number, chlorophyll content and yield contributing characters significantly increased under elevated CO_2 thus increasing the yield substantially.

5.1.3 Impact of elevated CO_2 on physiological and biochemical parameters

In the present study, chlorophyll content increased upto 5 MAP, relative CO_2 effect was positive; and then a decline in trend was evident under CO_2 enrichment from 7 MAP conferring to negative relative CO_2 effect. The maximum relative CO_2 effect was found at 4 MAP accounting to 46.98% increase in chlorophyll content in Aswathy, 28.95% in Athira and 20.81% in Maran (Fig. 16). Apparently, the current work is in accordance to the results obtained by Li *et al*. (2019), the leaf chlorophyll concentration at initial pod filling stage significantly increased by 28.3% on an average in soyabean under elevated CO_2. Elevated CO_2 treatment of 550 ppm recorded significant increase in chlorophyll content (3.1 mg g^{-1}), compared to ambient CO_2 (2.6 mg g^{-1}) in cowpea (Dey *et al.,* 2017).

Also, CO_2 induced limitation of transpiration rate that might have negatively impacted the N uptake by the plant and potentially contributed to chlorophyll loss (Conroy and Hocking, 1993). Senescence is associated with a slower rate of photosynthesis with a decrease in photosynthetic pigments and protein contents (Ougham

et al. 2008). This might also be the reason for reduction of chlorophyll at later stages of growth in the present study.

The relative CO_2 effect on photosynthesis gradually increased with plant growth and decreased rapidly at later stages conferring to negative relative effect at 8 MAP. Maximum per cent increase was evident in variety Aswathy by 27.42%, 22.55%, 7.25% at 4, 5, 7 respectively and decreased by 14.51% at 8 MAP (Fig. 17). The results of the present study are consistent with the previous findings, which have proven that CO_2 enrichment enhances the photosynthetic capacity of ginger. Photosynthetic rate increased at elevated CO_2 concentration of 800 μmol mol^{-1} in ginger varieties, Halia Bentong (65%) and Halia Bara (46%) (Ghasemzadeh and Jaafar, 2011). Elevated CO_2 stimulated the photosynthetic carbon assimilation rates by an average of 31% across 40 species that have been investigated at 12 FACE experiments (Ainsworth and Long, 2005).

Elevated CO_2 increases photosynthetic rate, due to increased carboxylation efficiency of Rubisco, which was comparatively low at present atmospheric CO_2 (Gamage *et al.,* 2018). The efficiency of photosystems I and II (PSI and PSII) is enhandced at elevated CO_2 and correlates well with the rate of photosynthesis, producing more ATP and $NADPH_2$ to activate photosynthetic enzymes at enhanced CO_2 levels (Zhang *et al.*,2008).

However, the per cent increase in photosynthetic rate reduced from 7 MAP accounting for 7.25 % in Aswathy, 6.05% in Athira and 1.30% in Maran. The elevated CO_2 effect does not maintain for longer periods due to "photosynthetic acclimation" (Seneweera *et al.,* 2002) and ; hence, in the present experiment, the per cent increase in photosynthetic rate due to the increased CO_2 effect was low at later stages of crop growth. Furthermore, after acclimation, elevated CO_2 known to inhibit Rubisco activase and thus reduces photosynthetic rate (Bokhari *et al.,* 2007). This effect may be from higher starch levels, stomatal and internal resistances and diluted chlorophyll concentrations (Teng *et al.*, 2009). Also, the reduction in Rubisco content leading to corresponding increase in the accumulation of non-structural carbohydrates (Seneweera *et al.,* 2011) and repression of the *rbcS* and *rbcL* gene expression (Rolland *et al*., 2002).

Elevated CO_2 decreased both the stomatal conductance and transpiration at all the stages (Fig. 18 and Fig. 19) at the leaf and canopy scales (Leakey *et al*., 2009). The elevated CO_2, increases the malate concentrations which induce the closure of stomata by enhancing the activation potential of anion channels. *SLAC1* (Slow Anion Channel Associated 1) that encodes a protein mediating CO_2 induced stomatal closure through regulating S-type anion channels is activated by the gene *OST1* (open stomata 1), which is a SNF-1 related protein kinase 2, a positive regulator of CO_2 induced stomatal closure (Lind *et al.,* 2015). Activation of *OST1* is triggered by ABA signalling (Chater *et al.,* 2015). In addition, the expression of genes encoding aquaporins is suppressed in the stomatal guard cells of few plants upon CO_2 enrichment (Wei *et al.*, 2013).

Under elevated CO_2 concentration of 800 μmol mol^{-1} the stomatal conductance was decreased in ginger varieties, Halia Bara by 24.52% and Halia Bentong by 30.76% (Ghasemzadeh and Jaafar, 2011) and by 30–40% (Liu *et al*., 2008). The average reduction in stomatal conductance was 9.69% for Aswathy, 6.07% for Athira and 7.07% for Maran and; hence lower ratesof transpiration at elevated CO_2 levels.

At elevated CO_2 the protein content in the rhizomes increased at 4 MAP, followed by a decreaseing trend was evident from 5 MAP and the maximum reduction was evident at 8 MAP. The reduction was more pronounced in variety Aswathy by 36.26%, followed by Maran (23.39%) and Athira (10.96%) at 8 MAP (Fig. 20). The reduction in protein concentration could be due to enhanced carbon accumulation at later stages and constrained N uptake under elevated CO_2. Similar findings have been reported by Ghasemzadeh *et al*. (2014) when CO_2 concentration increased from 400 to 800 μmol mol^{-1}, the total protein content in ginger varieties decreased by 35.4% and 38.0% in rhizomes of Halia Bara and Halia Bentong respectively. The high CO_2 concentration enhanced the reduction in tuber protein concentration in potato by about 14% (Hogy and Fangmeier 2009).

The relative CO_2 effect on sugar content showed a steady increase and peaked at 7 MAP and; then decreased at 8 MAP. The increase was more pronounced in the variety Aswathy which on an average had 35.74 %, than Athira (16.82 %) and Maran (32.98 %) (Fig. 21). The results are similar to the study of Ghasemzadeh *et al*. (2014). Whereas,

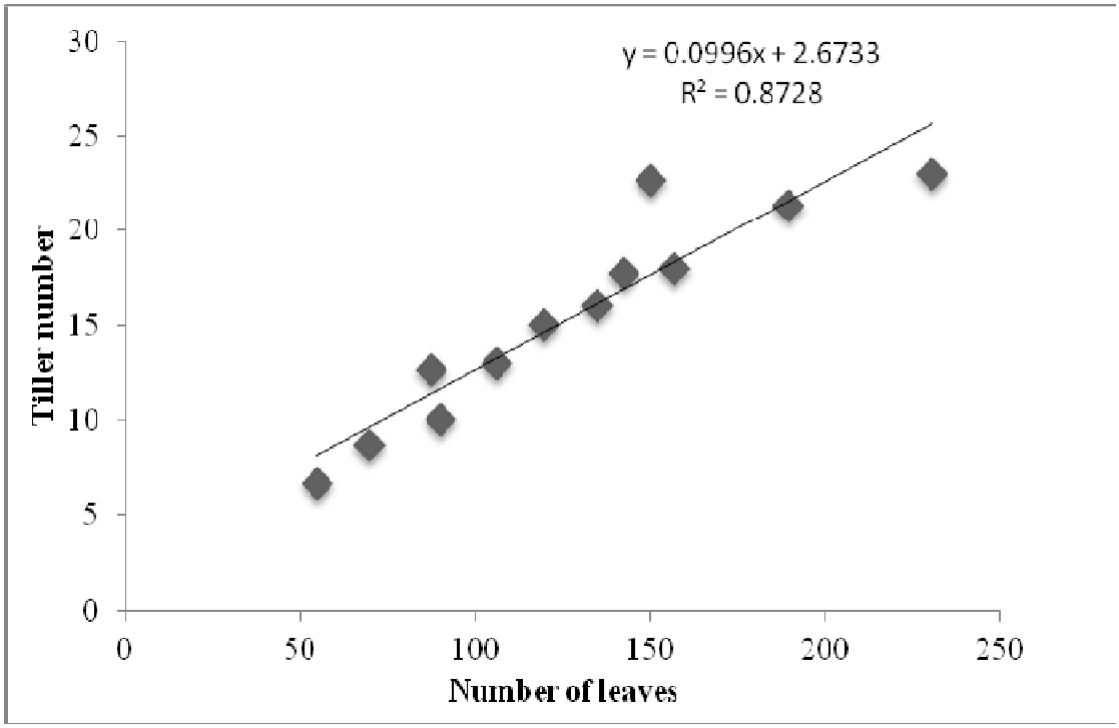
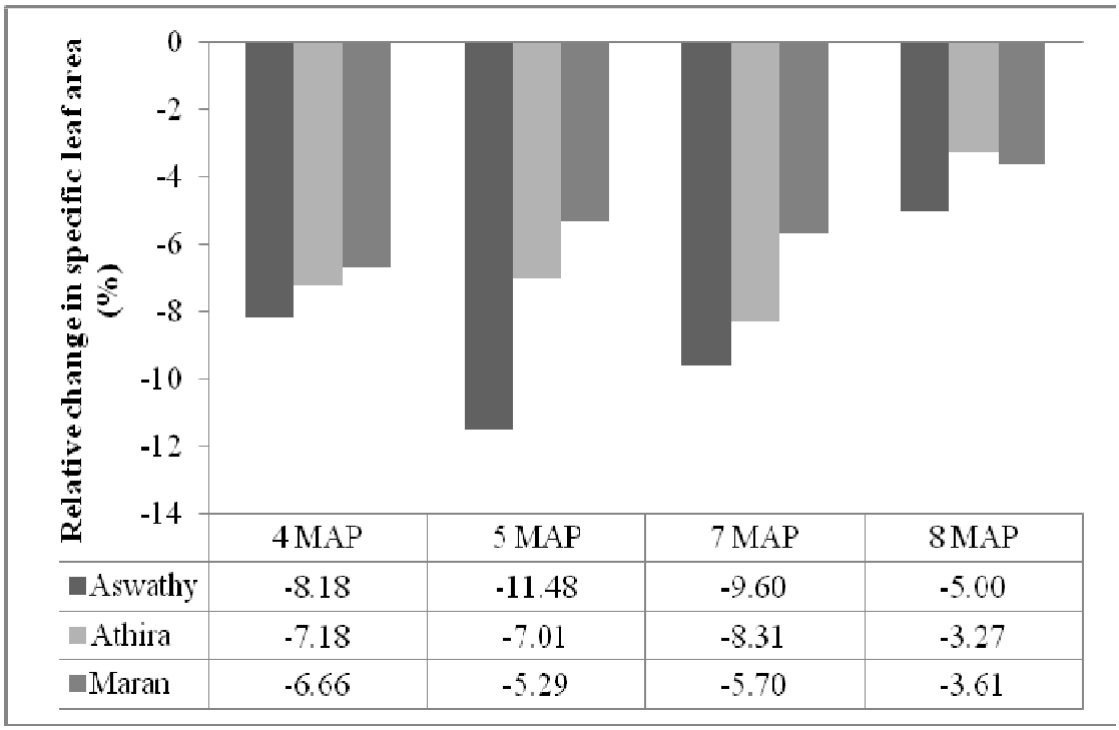
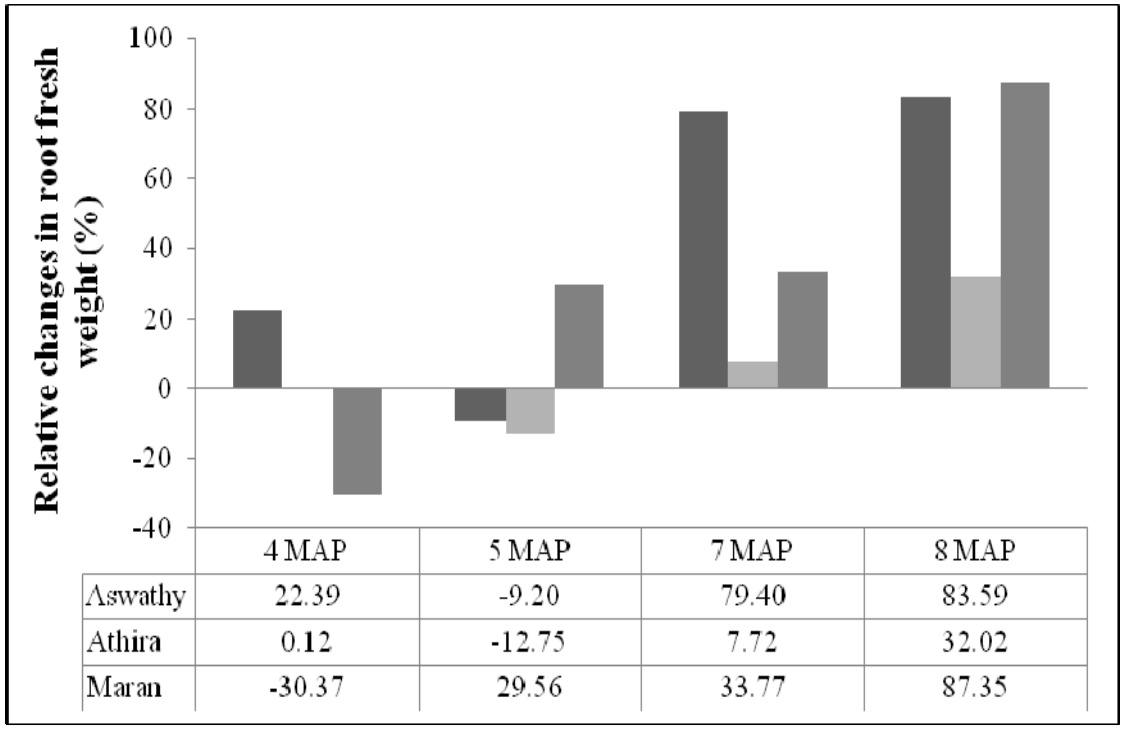


Fig. 9. Correlation of number of leaves with tiller number under elevated CO_2 (Mean values of all the varieties at 4 MAP, 5 MAP, 7 MAP and 8 MAP)



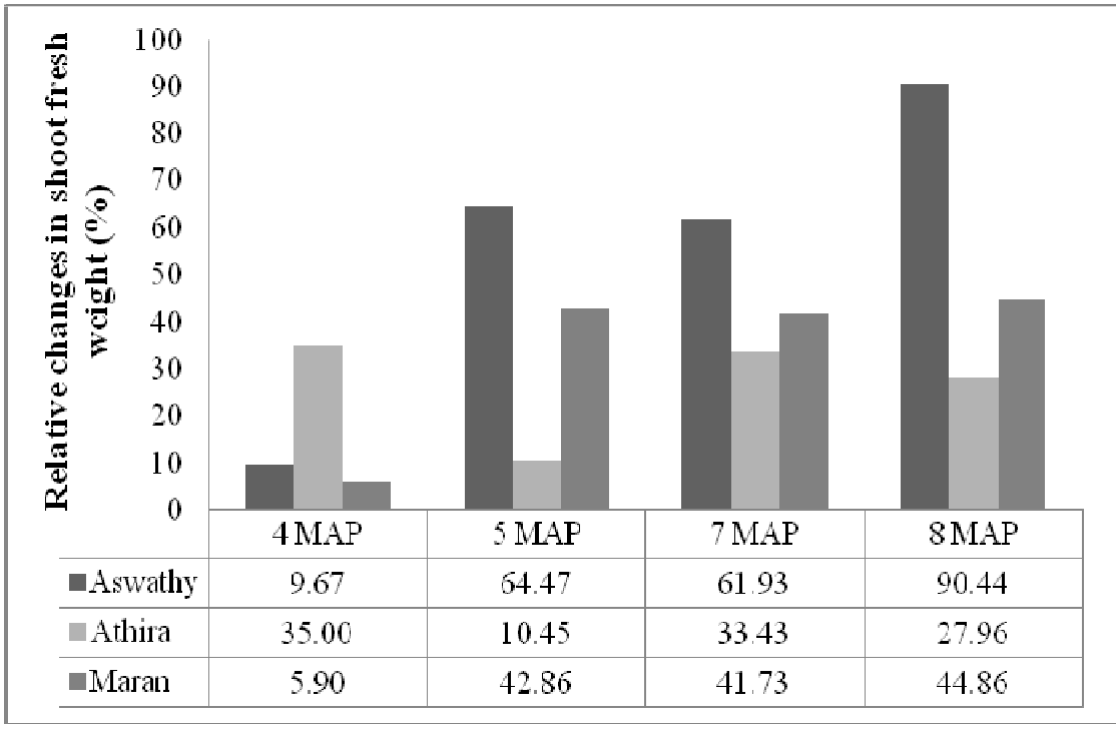
	4 MAP	5 MAP	7 MAP	8 MAP
Aswathy	-8.18	-11.48	-9.60	-5.00
Athira	-7.18	-7.01	-8.31	-3.27
Maran	-6.66	-5.29	-5.70	-3.61

Fig. 10. Relative changes in specific leaf area to elevated CO_2 against ambient CO_2 at different time intervals



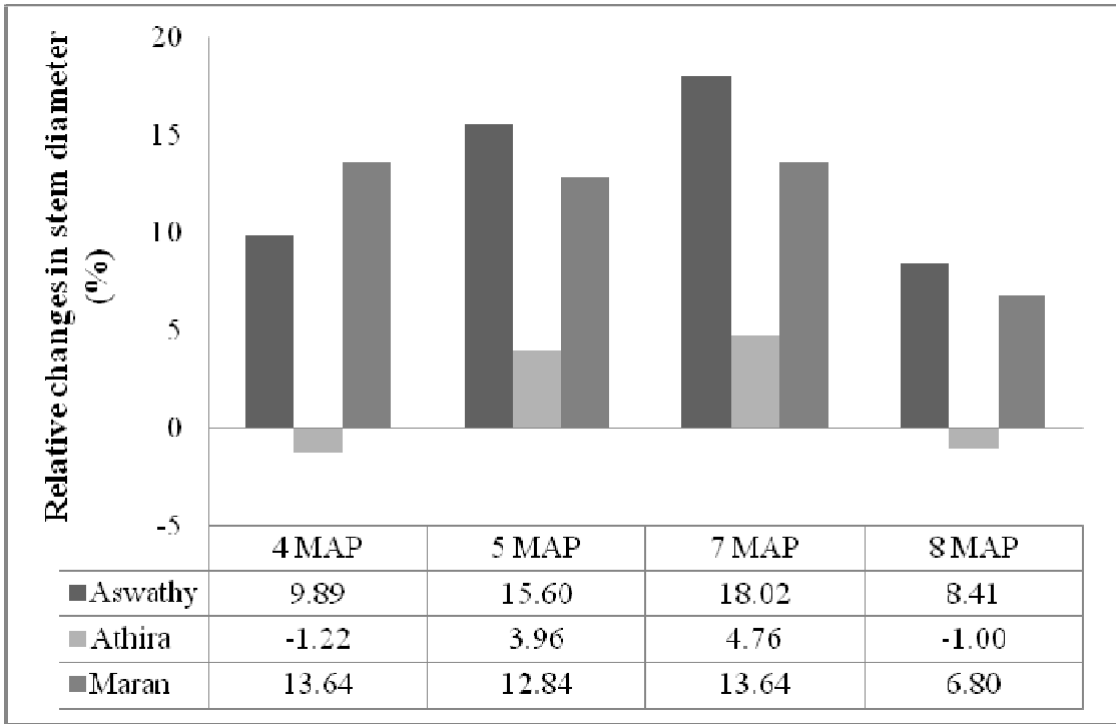
	4 MAP	5 MAP	7 MAP	8 MAP
Aswathy	22.39	-9.20	79.40	83.59
Athira	0.12	-12.75	7.72	32.02
Maran	-30.37	29.56	33.77	87.35

Fig. 11. Relative changes in root fresh weight to elevated CO_2 against ambient CO_2 at different time intervals



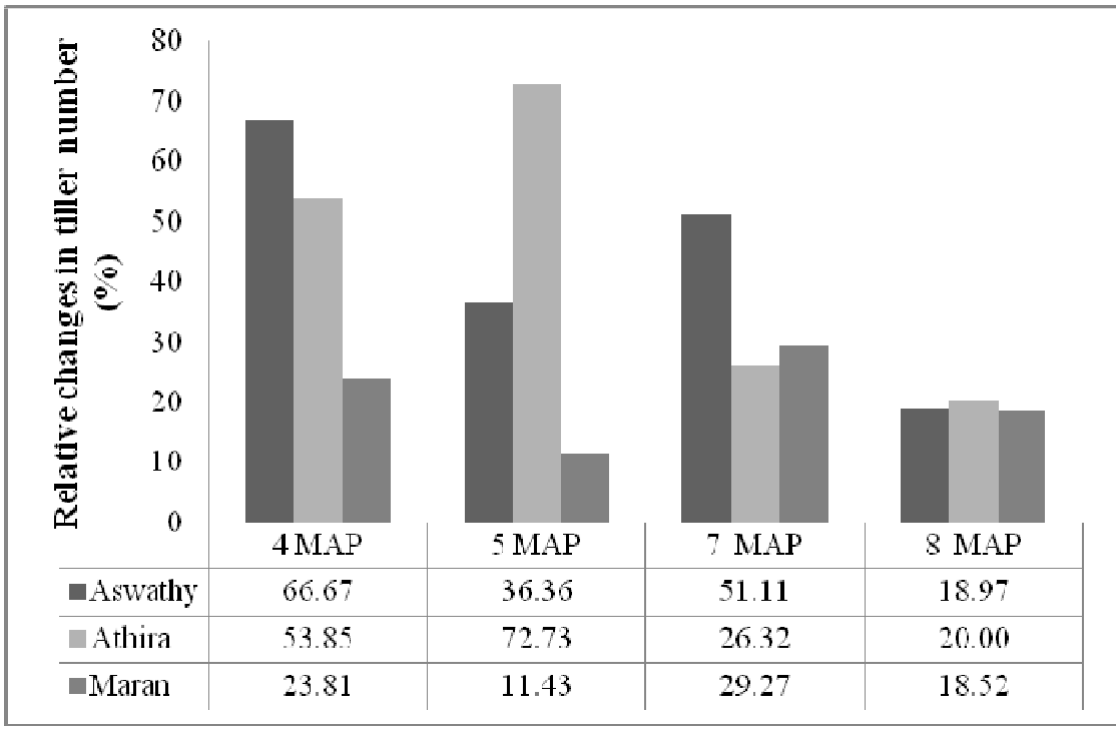
	4 MAP	5 MAP	7 MAP	8 MAP
Aswathy	9.67	64.47	61.93	90.44
Athira	35.00	10.45	33.43	27.96
Maran	5.90	42.86	41.73	44.86

Fig. 12. Relative changes in shoot fresh weight to elevated CO_2 against ambient CO_2 at different time intervals



	4 MAP	5 MAP	7 MAP	8 MAP
Aswathy	9.89	15.60	18.02	8.41
Athira	-1.22	3.96	4.76	-1.00
Maran	13.64	12.84	13.64	6.80

Fig. 13. Relative changes in stem diameter to elevated CO_2 against ambient CO_2 at different time intervals



	4 MAP	5 MAP	7 MAP	8 MAP
Aswathy	66.67	36.36	51.11	18.97
Athira	53.85	72.73	26.32	20.00
Maran	23.81	11.43	29.27	18.52

Fig. 14. Relative changes in number of tillers to elevated CO_2 against ambient CO_2 at different time intervals

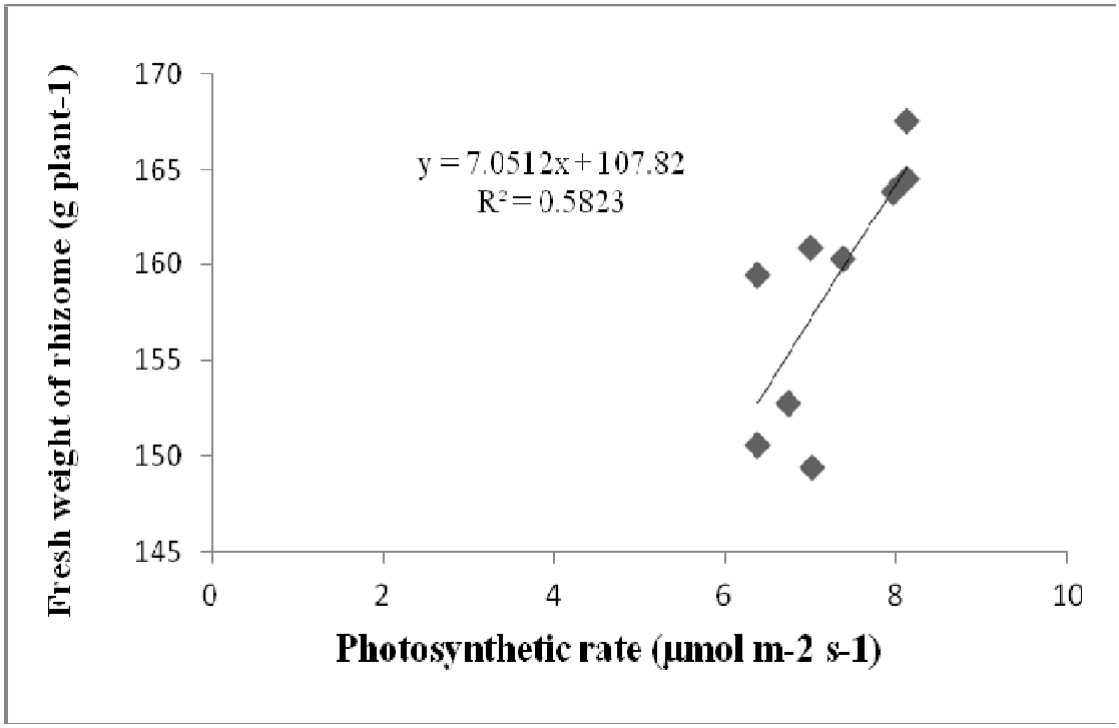


Fig. 15 a. Correlation between photosynthetic rate and fresh weight of rhizome under ambient CO_2 at 8 MAP

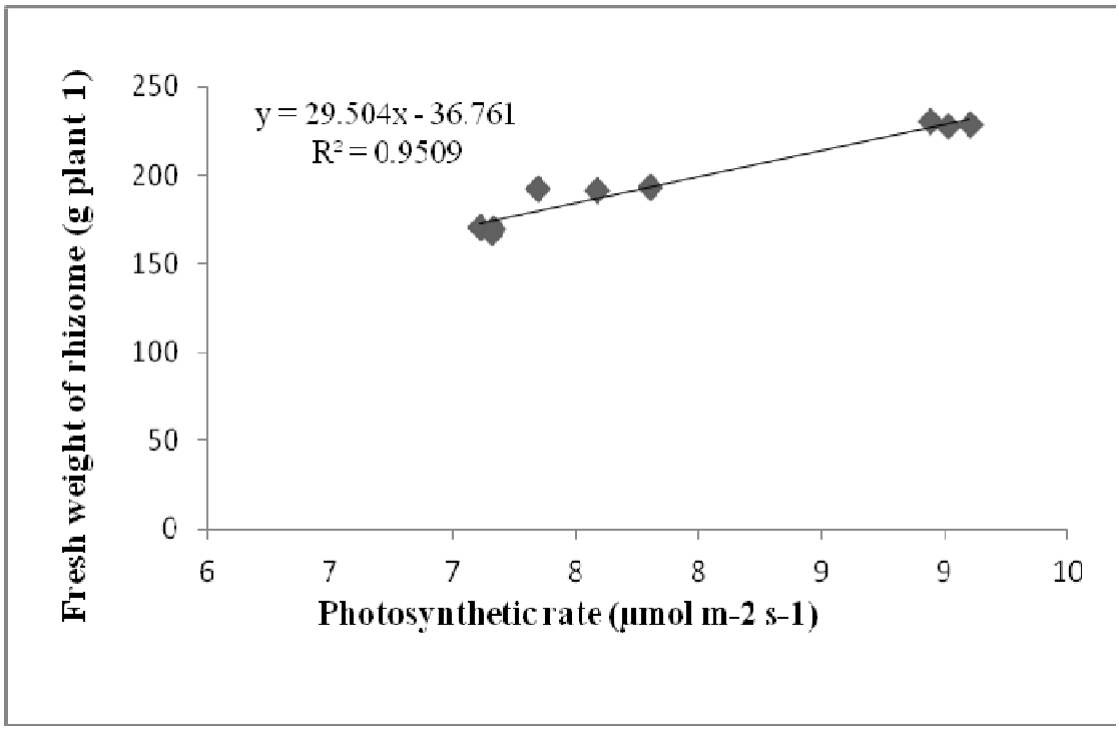
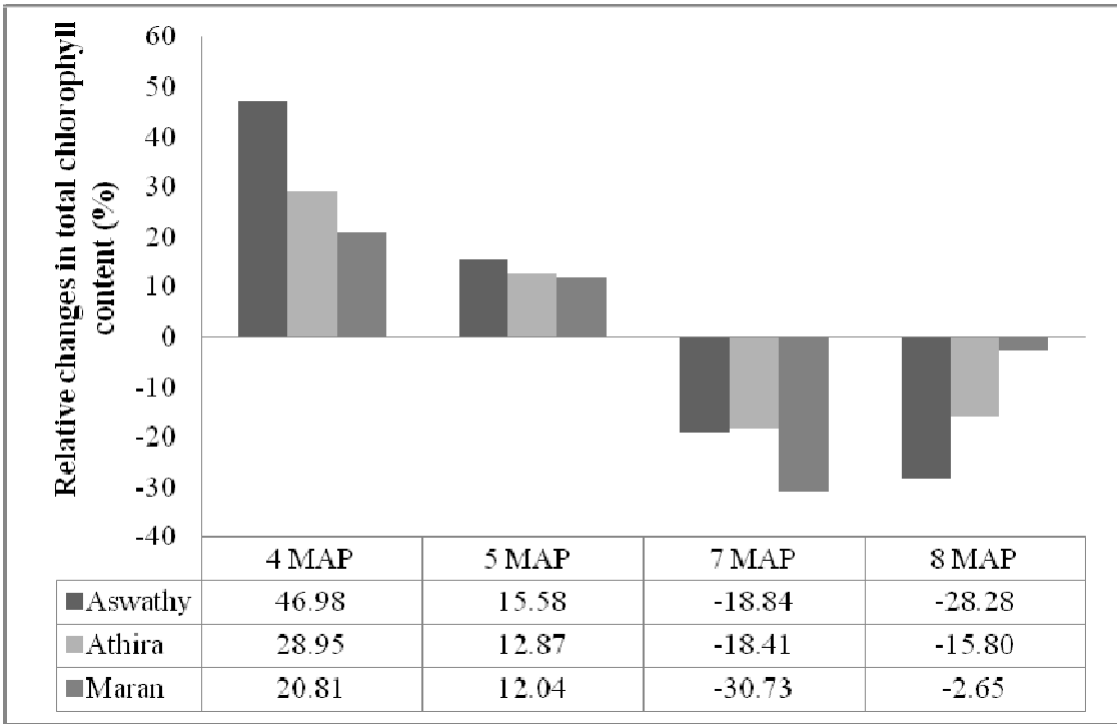
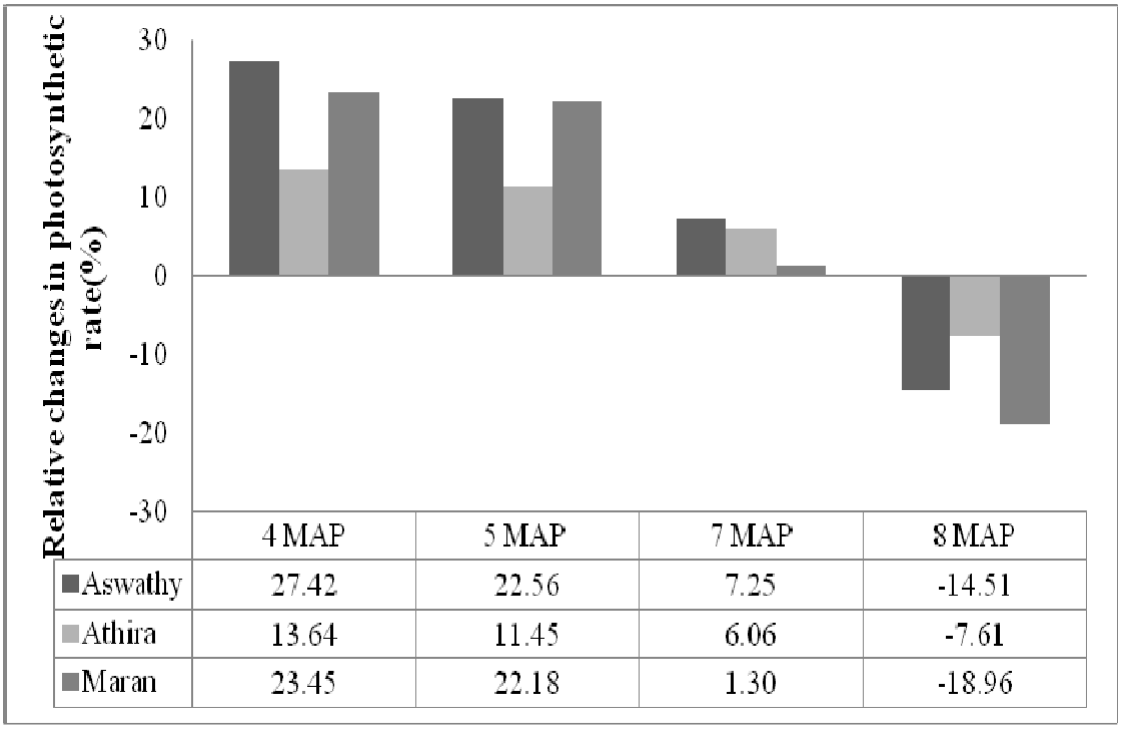


Fig. 15 b. Correlation between photosynthetic rate and fresh weight of rhizome under elevated CO_2 at 8 MAP



	4 MAP	5 MAP	7 MAP	8 MAP
Aswathy	46.98	15.58	-18.84	-28.28
Athira	28.95	12.87	-18.41	-15.80
Maran	20.81	12.04	-30.73	-2.65

Fig. 16. Relative changes in total chlorophyll content to elevated CO_2 against ambient CO_2 at different time intervals



	4 MAP	5 MAP	7 MAP	8 MAP
Aswathy	27.42	22.56	7.25	-14.51
Athira	13.64	11.45	6.06	-7.61
Maran	23.45	22.18	1.30	-18.96

Fig. 17. Relative changes in photosynthetic rate content to elevated CO_2 against ambient CO_2 at different time intervals

increase in CO_2 level from ambient (400 μmol mol^{-1}) to elevated (800 μmol mol^{-1}) resulted in increased content of sucrose, glucose and fructose in the rhizome of young ginger varieties Halia bentong 33.13%, 81.03% and 147.67% and in Halia bara 51.44%, 94.62% and 132.63% respectively. Increase in sucrose and glucose under CO_2 enriched conditions have also been reported in other plants such as orchids, sugarcane, tomatoes and potatoes (Hogy and Fangmeier, 2009; Chaum *et al*., 2011). Increase in sucrose and glucose could be due to an increase in hexose phosphate synthesis under high CO_2 concentrations (Paul and Foyer, 2001).

The decrease in the total sugar content with time due to elevated CO_2 with time might be due to acclimation to elevated CO_2 and; therefore minimal accumulation of sugar at later growth stage and limited translocation of photosynthates may be due to the long-term CO_2 exposure when less carbon can be fixed and translocated to sink (Islam *et al.,* 1996; Bindi *et al.,* 2001).It is likely that insufficient transport of sucrose under higher CO_2 may be associated with the N shortage (Zhang *et al.,* 2020). For example, N deficiency might alter the allocation of sucrose across plant organs (Lemoine *et al.,* 2013). Also, sugar accumulation in leaves can inhibit *SUT* activity and expression (Cordoba *et al.,* 2015).

The role of elevated CO_2 in altering nutrient composition is highly important in the present day context. Our results clearly show a decrease in rhizome nutrient concentration with elevated CO_2. N reduced by 22.36%, 15.37%, 10.23% and K by 29.77%, 23.26 % and 27.60% for Aswathy, Athira and Maran respectively. However, P reduction was evident in Aswathy (14.23%) and Maran (11.52%), not in Athira for which P increased by 9.23 %. Also the assessed micronutrients in the study, Fe reduced by 15.50%, 8.53%, 10.35%, Cu by 7.95%, 4.47%, 4.48% and Zn by 23.59%, 14.38%, 18.66% for Aswathy, Athira and Maran respectively.

Among varieties, high yielding variety Aswathy showed a higher reduction in all the elements and; to a lesser extent of reduction in low yielding variety Athira. These results indicate the compensation between rhizome yield and nutrient concentrations. More research in this regard are required.

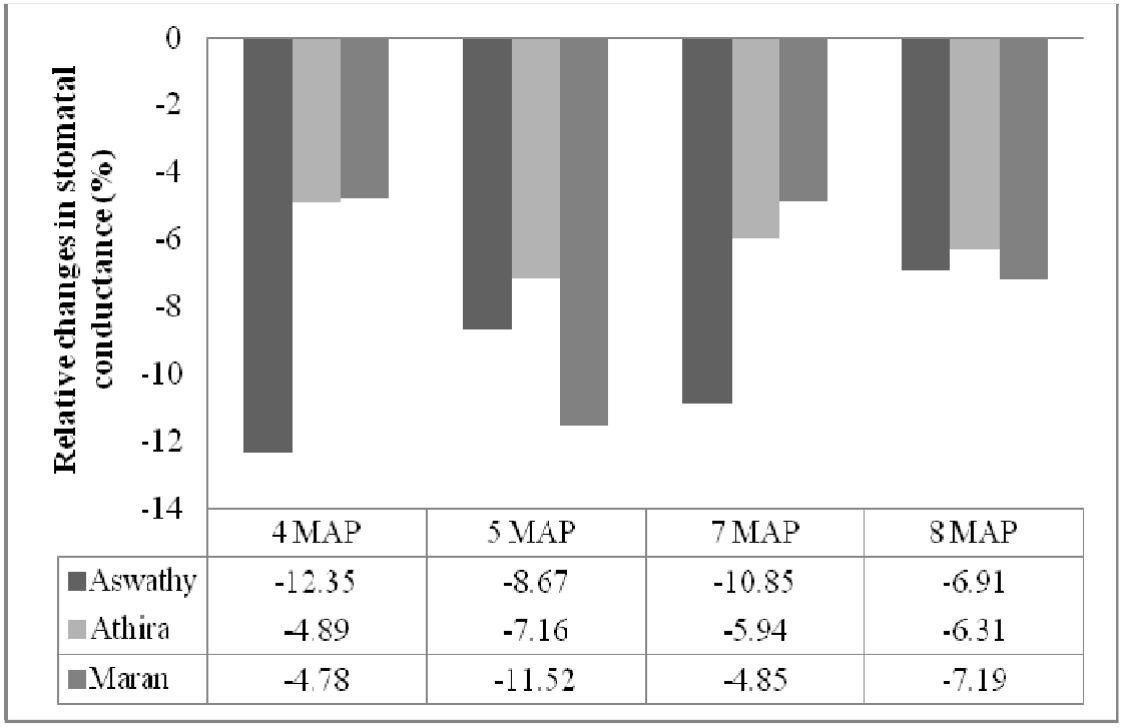
Elevated CO_2 concentration decreased the nitrogen concentrations in the grain of wheat, barley and rice (Kobayashi *et al*. 2006), Fe content by 5.2% (Myers *et al.*, 2014) and; protein and zinc in lettuce and spinach (Giri *et al*., 2016).

Reduced nutrient concentrations primarily attributed to dilution effect because of the high starch accumulation in elevated CO_2 (Riikonen *et al*., 2005). Because soluble nutrients are translocated with the flow of water, it is therefore possible that decreased nutrient concentrations in elevated CO_2 may be linked to reductions in stomatal conductance and transpiration flow (McGrath and Lobell, 2013). In the current study as well, leaf stomatal conductance and transpiration rates were reduced under elevated CO_2 (Fig. 18 and Fig. 19).

Taub and Wang (2008) had outlined other possible factors for low nitrogen concentration in plants at high CO_2. It is attributed to the decreased demand for nitrogen hence forth a decrease in uptake. Indeed, studies have shown that nitrate uptake by plants and its assimilation into organic compounds are known to be affected at elevated CO_2 in a wide variety of C_3 plants (Bloom *et al.*, 2012).

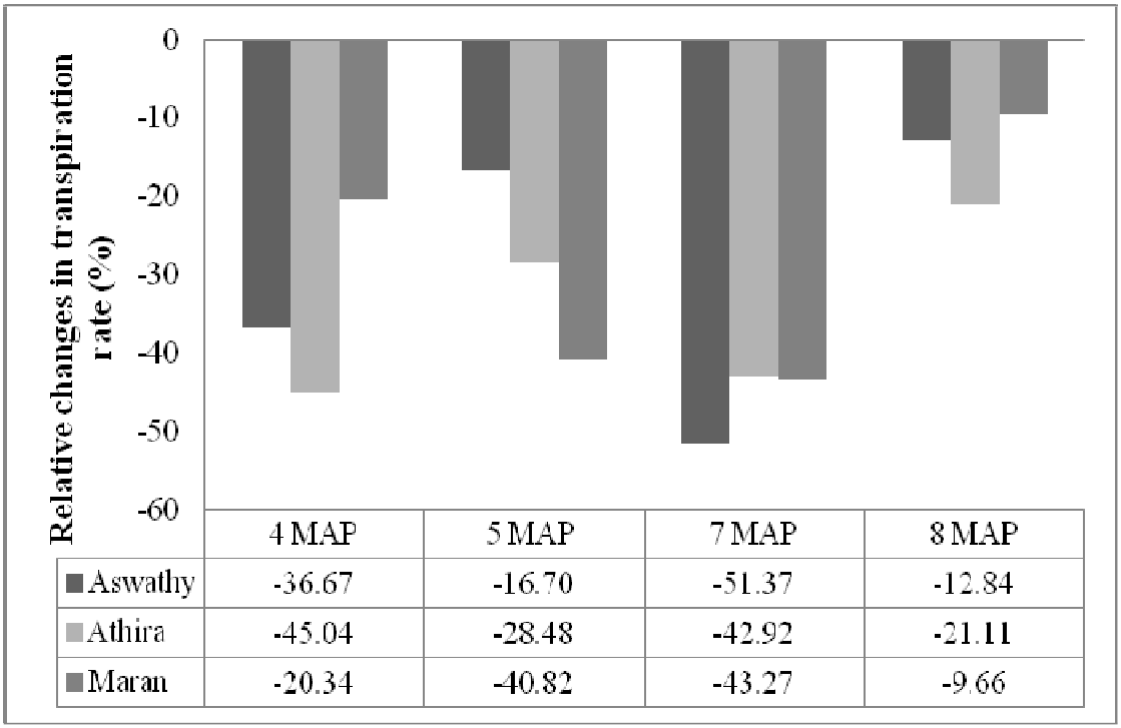
Ujiie *et al.* (2019) explained that in rice under CO_2 enrichment (700 ppm) the lower absorption and/or translocation of S and N is one of the reasons for lower elemental content in rice grains. During retranslocation, accumulated elements are transported along with carbohydrates in phloem sap which might affect elemental retranslocation via carbohydrate translocation.

Under elevated CO_2, gene expression profiles exhibited that genes encoding for Fe (*OsNAS3*) decreased by 2.66 folds, potassium channel SKOR (Stelar K(+) outward rectifying channel) decreased by 1.64 folds, Zinc transporter 11 precursor (ZRT/IRT-like protein 11) decreased by 0.57 fold, Zinc transporter protein *OsZIP5* which is a Fe and Zn regulated transporter like protein 5 decreased by 0.46 folds (Ujiie *et al.*, 2019). *OsZIP5* plays a major role under Zn deficiency and in the import of mineral ions such as Fe^{2+}, Cu^{2+}, and Mn^{2+} and is controlled at the transcriptional level in rice (Lee *et al* ., 2010). Therefore, elevated CO_2 might affect the expression of related membrane transporters which interferes with absorption and/or translocation of minerals.



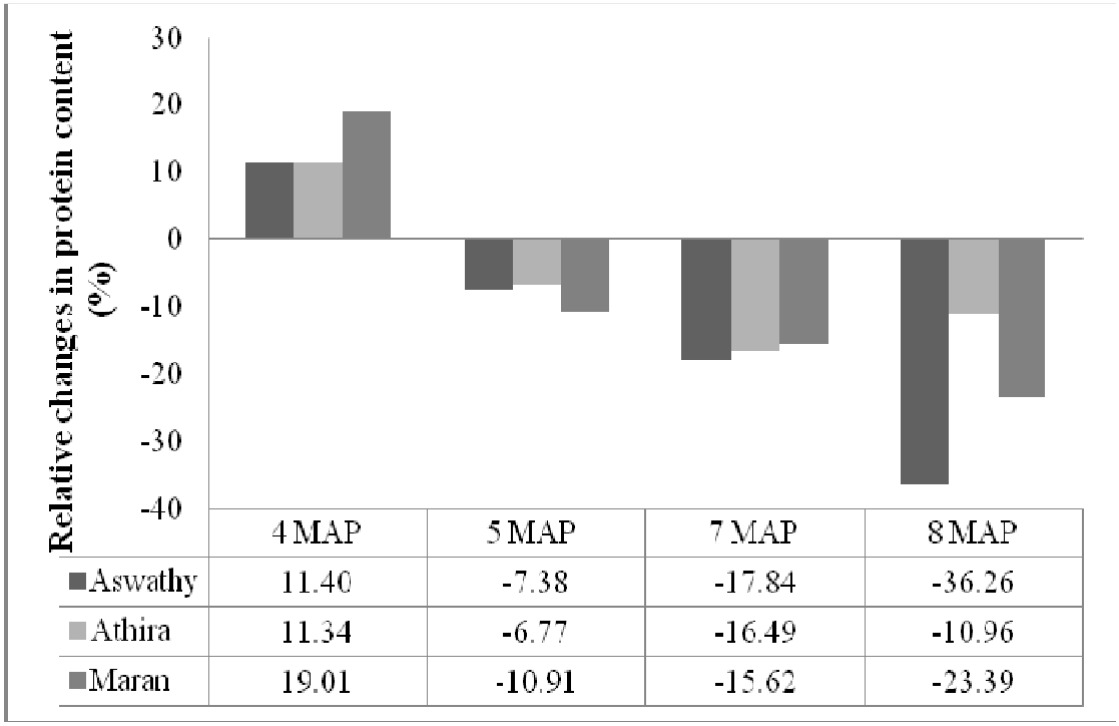
	4 MAP	5 MAP	7 MAP	8 MAP
Aswathy	-12.35	-8.67	-10.85	-6.91
Athira	-4.89	-7.16	-5.94	-6.31
Maran	-4.78	-11.52	-4.85	-7.19

Fig. 18. Relative changes in stomatal conductance to elevated CO_2 against ambient CO_2 at different time intervals



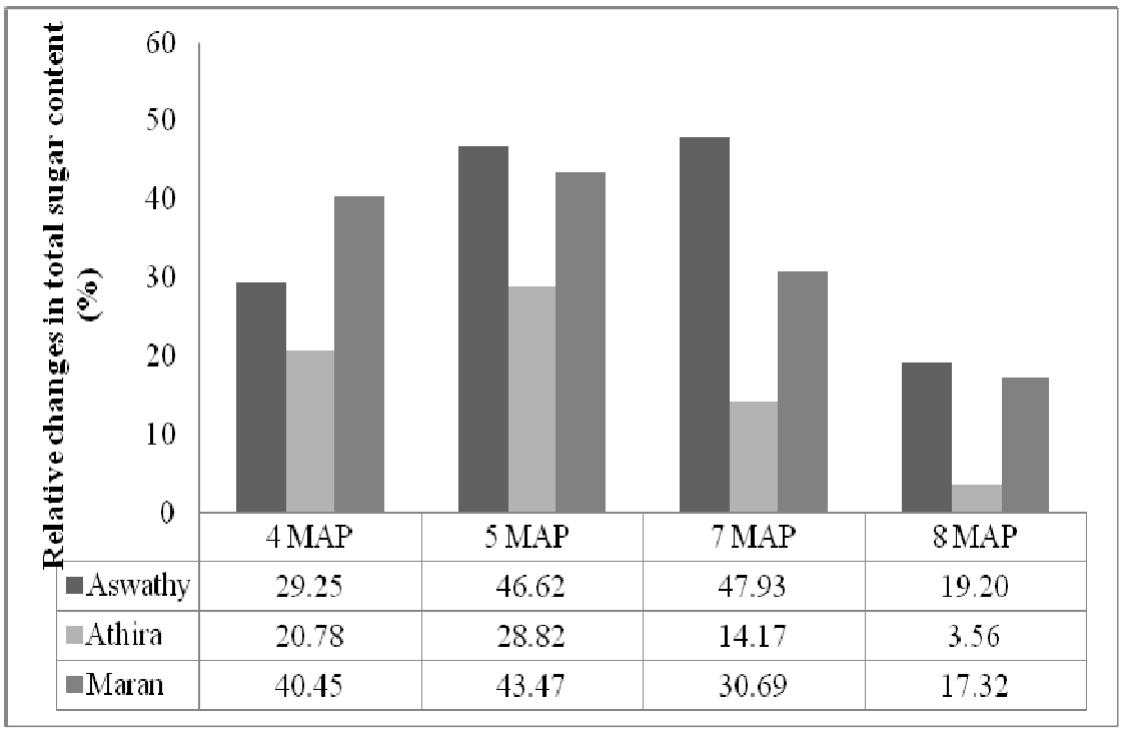
	4 MAP	5 MAP	7 MAP	8 MAP
Aswathy	-36.67	-16.70	-51.37	-12.84
Athira	-45.04	-28.48	-42.92	-21.11
Maran	-20.34	-40.82	-43.27	-9.66

Fig. 19. Relative changes in transpiration rate to elevated CO_2 against ambient CO_2 at different time intervals



	4 MAP	5 MAP	7 MAP	8 MAP
Aswathy	11.40	-7.38	-17.84	-36.26
Athira	11.34	-6.77	-16.49	-10.96
Maran	19.01	-10.91	-15.62	-23.39

Fig. 20. Relative changes in total protein content in rhizomes to elevated CO_2 against ambient CO_2 at different time intervals



	4 MAP	5 MAP	7 MAP	8 MAP
Aswathy	29.25	46.62	47.93	19.20
Athira	20.78	28.82	14.17	3.56
Maran	40.45	43.47	30.69	17.32

Fig. 21. Relative changes in total sugar content to elevated CO_2 against ambient CO_2 at different time intervals

1.1.4 Impact of elevated CO_2 on quality parameters

The major constituents in ginger rhizomes are carbohydrates (50–70%), lipids (3–8%), terpenes, flavanoids and phenolic compounds (Prasad and Tyagi 2015). Sreekala (2004) has reported that increase in volatile oil; oleoresin and starch with a decrease in crude fiber content improves the quality of ginger.

Dietary fibers of food play a crucial role in nutritional and health benefits. They prevent loss of nutrients in the digestive tract and reduce constipation (Trinidad *et al*., 2013). However, high quality rhizome (grade I) should possess less than 8% less crude fiber (Anon., 2004). For ginger improvement, the breeding objectives focus on superior vegetative growth with low fiber content (Ganapathy *et al.,* 2016)

The fiber content increased under the CO_2 enrichment in all the varieties, more prominantly in variety Athira (109.09%) then followed by Aswathy (85.71%) and Maran (29.17%). Similar results of increased fibre content with elevated CO_2 have been observed by Khan *et al.* (2013), fiber content in tomato varieties increased at 1,000 μmol mol^{-1}. In variety Astra the increase was 5.25% and for Eureka it was 4.40%. In rice the increase in soluble dietary fiber (82.10%) and dietary fiber (2.64%) were evident when grown under CO_2 enrichment (550 mol mol^{-1}) (Goufo *et al.,* 2014).

In the present study, starch constituted from 39-79% under elevated CO_2. Carbon dioxide increased the production and accumulation of starch in the rhizomes of Aswathy by 79.31%, followed by Maran 30.40% and Athira 37.94%. Starch content in the ginger varieties at elevated CO_2 concentration of 800 μmol mol^{-1} was 583.5 mg/g dry weight and 553.3 mg/g dry weight in rhizomes of Halia Bentong and Halia Bara rhizomes respectively (Ghasemzadeh and Jaafar 2011). Under elevated CO_2, there is an increase in the total non-structural carbohydrates, like starch and sugar (Markelz *et al.,* 2014) as their usage intensity is lower than their production (Moore *et al*., 1998).

In the current study ascorbic acid increased by 7.39% for Aswathy, 4.17 % for Athira and highest increase was evident for Maran 10.95%. However, there was no significant difference between treatment and varieties. Varied results were found for ascorbic acid content under elevated CO_2. Ascorbic acid content of tomato decreased

significantly with increase in atmospheric CO_2 concentration in tomato varieties Astra (24.72%) and Eureka (20.02%) (Khan *et al*., 2013). Enhanced atmospheric CO_2 decreased ascorbic acid content significantly in capsicum varieties. The decrease ranged between 11.84 and 15.84% for the matured stages and 8.98 to 12.12% for green stages (Azam *et al.,* 2017). Ascorbic acid was reported to accumulate in *Labisia pumila* in response to elevated CO_2 (Jaafar *et al.,* 2012).

Non-structural carbohydrates (starch and soluble sugars) increases under CO_2 enriched conditions due to enhanced photosynthesis and subsequent stimulation of secondary metabolism like glycolysis (Dizengremel *et al.,* 2012). The glycolysis pyruvate and inturn synthesize is requisite for phosphoenol pyruvic acid production and metabolism, which is required as a precursor for shikimic acid pathway (Herrmann, 1995). Elevated CO_2 enhances the partitioning of carbohydrates to secondary metabolites such as phenolics (Austen *et al.,* 2019).

Hence in the present study, elevated CO_2 increased the photosynthates and sugars and hence more partitioning of carbohydrates to phenolics, especially in variety Aswathy (60.42% as compared to ambient CO_2).

Enhanced CO_2 levels influenced PAL activity in oil palms. The highest PAL activity was at 1,200 μmol mol^{-1} (30.93 nM *trans*-cinnamic mg^{-1} protein h^{-1}), followed by 800 μmol· mol^{-1} (22.89 nM *trans*-cinnamic mg^{-1} protein h^{-1}). PAL had a positive relationship with total phenolics (R^2 = 0.744) which indicates an up-regulation of plant secondary metabolite production with increased PAL activity (Ibrahim and Jaafar, 2012). Probably the enhanced activity of PAL is one of the reasons for increase in phenol content in Aswathy and Maran.

The phenolic compounds are mainly gingerols, shogaols, and paradols, which account for the various bioactivities of ginger (Stoner, 2013). It was found that elevated CO_2 of 800 μmol mol^{-1} had significant increase in phenol content in ginger varieties with increase up to 182.66% in Halia Bara and 153.83% in Halia Bentong (Ghasemzadeh and Jaafar, 2011). In the present study the elevated CO_2 increased the gingerols and oleoresins. In fresh ginger, gingerols are the major polyphenols (Mao *et al.,* 2019) and

one of the major components of the oleoresin which accounts to $12.8 \pm 0.5\%$ (Murthy and Gautam 2015). Relative effect of CO_2 on gingerols and oleoresin production was high in Aswathy by 54.01%, and 34.01% respectively. Ghasemzadeh and Jaafar (2011) reported that CO_2 enrichment had improved the anticancer activity of two ginger varieties against human cancer cell lines (MDA–MB–231and MCF–7). Such elevated CO_2 induced improvement in biological activities was attributed to the paralleled increase in phenolics and flavonoids content.

Essential oils significantly increased up on CO_2 enrichment by 26.10% for Aswathy, 25.05% for Maran and was highest for Athira (39.44%). Upon CO_2 enrichment, one of the reasons for elevated seed oil percentage is the higher availability of substrate (acetyl CoA) through breakdown of sucrose via oxidative pentose phosphate pathway or glycolysis (Pal *et al*., 2014).

Ginger has been used as a spice for over 2000 years (Bartley and Jacobs, 2000). The medicinal properties of ginger are attributed to its spicy, pungent constituents, mainly gingerols. The essential oils, gingerols and shoagols possess a wide range of pharmacological and physiological effects, which include cardiovascular, gastro-intestinal (antiemetic, antinausea, antiulcer), antioxidant, anti-inflammatory, antimicrobial (analgesic, sedative, antipyretic, antibacterial) *etc*. In the present study elevated CO_2 enhanced phenol, oleoresin, gingerol and essential oils particularly in variety Aswathy and Maran. Therefore, CO_2 enrichment can be used as a strategy to improve the nutritional and health-promoting values of ginger.

5.2. Impact of elevated CO_2 on the response of ginger to *P. aphanidermatum*

5.2.1 Disease incidence and severity

All around the world, the major setback in ginger cultivation is the soft rot disease, caused by several species of the oomycete pathogen *Pythium*. In the present programme, the impact of increasing CO_2 concentration on the response of ginger to *P. aphanidermatum* was studied by exposing the plants to CO_2 concentration of 500 ppm, after inoculation in the OTC system and by recording the extent and severity of disease and biochemical and physiological plant responses.

In the present experiment the percent disease incidence was 100 per cent for all the three varieties at both ambient and elevated CO_2 indicating that all the selected varieties. However, the wild cultivar (*Z. zerumbet*) exhibited zero per cent disease incidence especially at the early stages although prevalence was recorded in the later periods. A severe host-response to *Pythium* occurs when the pathogen moves deeper into plant tissue and reaches the vascular system, resulting in severe wilting (Kamoun *et al.*, 1999).

The percent disease index was higher under ambient CO_2 and variety Aswathy recorded highest (100%) and least for *Z. zerumbet* (25%). Likewise under elevated CO_2 variety Aswathy exhibited 100% and *Z. zerumbet* (16.66%). Also the number of days taken for symptom development was lowest in the case of variety Aswathy. The last experimental system to develop disease symptom was *Z. zerumbet* (Table 20).

Amongst the varieties, Athira was found to be moderately tolerant while Aswathy was susceptible. Similar results were obtained by (Kavitha and Thomas 2007). When screening for tolerance for soft rot among the existing varieties and a wild relative of cultivated ginger. The pseudostems of improved ginger varieties 'Maran' and 'Varada' were totally wilted and their rhizomes were completely decayed within three weeks of inoculation with *P. aphanidermatum*. However, four of the five accessions of *Z. zerumbet*, showed no symptom of infection.

It is evident from the present study that elevated CO_2 brings down the disease index irrespective of varieties. The results are consistent with the study documenting reduced disease index under elevated CO_2 in the case of potato plants (*Solanum tuberosum* L. cv. Indira) which exhibited high susceptibility to the late blight pathogen *Phytophthora infestans.* Infected plants were exposed for 4 weeks to two different CO_2 concentrations (400/700 ppm). Increase in CO_2 from 400 to 700 ppm dramatically reduced symptom development, the area of sporulation and sporulation intensity (Plessl *et al,* 2007). When placed in erlenmeyer flasks containing water suspensions of oospores, the conversion of thick-walled oospores of *P. ultimum* to germinable thin-walled oospores decreased from 48 to 2% as the concentration of CO_2 increased from 2.9% to 100% (Johnson 1988). This reduced conversion of thick walled oospores might have also

reduced the symptom development under elevated CO_2. These results and literature suggests that the % disease index can be reduced by elevated CO_2.

5.2.2 Growth parameters

Growth responses to elevated CO_2 as affected by pathogen inoculation could not be illustrated appropriately in this experiment because of succumbence of experimental plants to the pathogen before realising significant growth. However, there was increase in plant height, tiller number and number of leaves when the plants were allowed to acclimatise for 15 days under elevated CO_2 before inoculation. CO_2 enriched environments typically enhances photosynthetic efficiency and growth by directly increasing the amount of carbon available for fixation, decreasing CO_2 lost to photorespiration, and reducing Oxygenase activity of Rubisco (Lawlor and Mitchell, 1991).

There was reduction in fresh weight of the rhizome upon disease development. Gradually rhizomes became soft and the internal tissues were completely rotten when examined on the 20^{th} day after inoculation. The decline in the weight was less under elevated CO_2 compared to the ambient condition. Maximum reduction was found for variety Aswathy (34.38%) and least for *Z. zerumbet* (8.68%) under ambient CO_2. Under elevated CO_2, highest reduction in weight of rhizome was recorded for Aswathy (30.47%) and least for *Z.* zerumbet (4.12%). Similar results were obtained in the case of dry weight also (Table 25).

Comparable results of varietal variation in growth responses under pathogen inoculation was evident when susceptibility of cultivars of ginger were tested against *P. aphanidermatum* under ambient CO_2 (Karmakar *et al*., 2003). Where in cultivar Kundali appeared to be least susceptible. Highest degree of susceptibility was shown by Varada, followed by Suprabha and Maran which exhibited reduction in fresh weight by 18.56% and 32% respectively and were found to be moderately susceptible. Ghosh (2015) reported *about* 25% reductions in fresh weight of rhizome in cultivar Suprabha (28 day old plants) after 21 days of inoculation with *P. aphanidermatum.*

5.2.3 Physiological and biochemical observations

Photosynthetic pigments chlorophylls a and b play an important role in light absorption during photosynthesis (Taiz and Zeiger, 2009). Other pigments such as carotenoids and xanthophylls are considered as accessory components in the photosynthetic complex which provide photo protection and stability of proteins present in the photo system under environmental stresses (Simkin *et al.,* 2008).

The chlorophyll and caroteniod contents decreased in inoculated plants to a greater extent under ambient CO_2 and more prominently in the variety Aswathy (Table 26, 27, 28, 29 and 30). The reduction in pigments due to infection could be due to chloroplast disorders during pathogen infection and loss in leaf photosynthetic area. In addition, other secondary factors of the infection, along with pathogen cause the accumulation ROS leading to inactivation/oxidation of pre-existing pigments in chloroplast (Radwan *et al.,* 2008). Similar findings of decreased chlorophyll content due to anthracnose infection in beans was obtained were obtained by (Lobato *et al.,* 2010) in beans plant infected by 6.4%, 20.6% and 21.3% on the 4th, 8th and 12th days respectively.

As components of antioxidants, carotenoids are capable of quenching the free radicals produced during a pathogen attack and decreases the effect of mycotoxins (Atroshi *et al.,* 2002). Also as a component of biomembranes they protect phospholipids from peroxidation caused by free radicals that are produced under abiotic and biotic stresses (Strzalka *et al.,* 2003). This maintenance of higher levels of carotenoids might be one among the reasons for the innate pathogen tolerance exhibited by *Z. zerumbet*. Such reduction in pigment systems would affect the photosynthetic rates.

The results are in agreement with the previous findings of Mali *et al.* (2000) ,who reported significantly higher contents of total phenols, increase in free proline accumulation and less reduction in total chlorophyll a, b, carotenoids, soluble carbohydrate and starch in yellow mosaic virus resistant moth bean genotype as compared to susceptible genotype.

The results show that reduction in the photosynthetic rate upon *P. aphanidermatum* inoculation is proportional to the leaf chlorophyll content. The reduction in the photosynthetic rate upon *P. aphanidermatum* inoculation was higher under ambient CO_2 which can be explained by the decrease in chlorophyll content and probably the damage to the photosynthetic apparatus. Among the varieties the highest reduction was found for Aswathy (58.56%) followed by Maran (43.05%) and least for Athira (36.71%). The reduction in chlorophyll was less under elevated CO_2 and the maximum reduction was found for Aswathy (57.68%) followed by Maran (43.29%) and least for Athira (37.18%).

It is suggested that plants switch off photosynthesis and other assimilatory metabolism to initiate respiration and other processes required for defence (Berger *et al.*, 2007). A decrease in photosynthetic capacity, CO_2 assimilation, and transpiration, a strong drop in the maximum fluorescence yield, and a reduction of total chlorophyll and a stable carotenoid content were reported when grape leaves were infected with esca disease (Petit *et al.*, 2006).

The membrane integrity which was measured in terms of membrane leakage (%) was higher under ambient CO_2 condition. The per cent leakage was least for *Z. zerumbet* (5.66%) followed by Athira (103.20%) and *Z. zerumbet* (2.59%) followed by Athira (128.74%) under elevated and ambient CO_2 increased the membrane leakage. However, elevated CO_2 decreased the production of ROS (H_2O_2, O_2^-, and cell death measured by DAB, NBT and tryphan blue staining respectively) (Plate 7 to 11). The elevated CO_2 might have increased the antioxidant enzymes to decrease the accumulation of ROS and protected the membrane integrity at higher CO_2 levels (Unyayar *et al.*, 2010). In the present study increased carotenoids under elevated CO_2 served to reduce the peroxidation of phospholipids from ROS. Also increase in the accumulation of phenols which reinforce the cell wall lignifications, suberization *etc.* at the penetration sites would have played a very important role to decrease membrane damage at elevated CO_2. Increase in ascorbate peroxidise activity and ascorbic acid content would have rendered to the maintenance of membrane integrity under elevated CO_2. Oliver *et al.* (2009) who

observed the deposition of phenolic compounds in cell walls of *Physcomitrella patens* infected with *P. debaryanum* and *Pythium irregular*

Fungal pathogens attack the host cell by penetrating the cell wall and hence, if this could be inhibited at this entry stage itself, cellular integrity can be maintained and damage to plant tissues could be reduced significantly (Belanger and Bushnell, 2002).

5.2.4 Oxidative stress

Generation of reactive oxygen molecules leading to oxidative stress has been identified as the main cause of the development and progression of several diseases. These highly active molecules cause rapid cell damage especially on the cell membranes. Plants have evolved an elaborate system of enzymatic and non enzymatic antioxidants which help to scavenge these indigenously generated oxygen molecules. Plants respond differently to elevated CO_2 with regard to the gene expression and activity of the enzymatic antioxidants. In the current programme an extensive study on the intensity of oxidative stress, enzymatic and non enzymatic antioxidants were made to study their modulations upon elevated CO_2 exposure.

Malondialdehyde is one of the final products of lipid peroxidation in the cells and it is an indicator of antioxidant status in living cells. In the present study, pathogen inoculation induced higher accumulation of MDA under ambient CO_2 as compared to elevated CO_2 (Table 33). Similarly, Koc *et al* (2011) reported that production of ROS, H_2O_2, and lipid peroxidation increased in pepper leaves when inoculated by *Phytophthora capsici* Leon. The lowest MDA was recorded in non-infected (control). Infected leaves in PM-702 which was a resistant cultivar recorded significantly lower values, compared to KM-hot and Demre-8 the susceptible cultivars This indicates higher accumulation of free radicals under ambient CO_2 leading to over production of MDA. This increase in ROS was evident from the staining techniques. Among the *Zingiber* species the per cent increase of MDA content over control was least for *Z. zerumbet* (2.5%) and highest for Aswathy (86.36%) under elevated CO_2 (Table 31). This indicates that *Z. zerumbet* could resist the pathogen aggression.

The most important antioxidant enzymes are CAT and APX which transforms H_2O_2 into H_2O for combating the stress. In the present study, when plants were inoculated with pathogen, the CAT activity decreased; the reduction was high under ambient CO_2 than the elevated CO_2. Among varieties, Aswathy had more reduction in CAT with inoculation of pathogen (Table 34). Further, CAT activity performed through for generation of H_2O_2 also proved higher staining under ambient CO_2. The reduced CAT activity could be due to enhanced proteolysis caused by peroxisomal peptidases under pathogen inoculation (Palma *et al.,* 2002). Similar results of reduced CAT activity under various stress have been reported (Koç *et al.*,2011; Nasir *et al.*,2017).

APX activity is important in detoxifying the H_2O_2 which is known to be produced under a given stress. Therefore, identifying a genotype with higher APX activity would be advantageous to over come the stress effect. In the present study APX activity was enhanced upon pathogen stress to combat ROS. Furthermore, under elevated CO_2 much more increased APX activity was observed (Table 35). Such increase could be due to enhanced expression of APX gene coding for APX at 700 ppm CO_2 to reduce the H_2O_2 in cabbage (Muthusamy *et al.,* 2019). Similarly, over expression of APX gene from capsicum in tobacco plants have shown two fold increase peroxidase transcript level upon *Phytophthora nicotianae* inoculation (Sarowar *et al.,* 2005). Also, large genotypic variability in APX activity was observed in rice genotypes and activity increased with *Magnaporthe oryzae* inoculation (Anushree *et al.,* 2016).

APX is very sensitive at high levels of peroxide and requires ascorbic acid in the medium for its acivity (Asada, 1999). This might be the reason for lesser ascorbate peroxidise activity for variety Aswathy which had lower ascorbic acid content accumulation upon pathogen inoculation and more susceptible for soft rot under ambient CO_2. These results suggest that, preliminary screening for rhizome rot tolerance could be possible through screening for APX activity.

Phenolic compounds assist in strengthening plant cell wall against pathogens and inhibit the mycelial growth of pathogenic fungi. These compounds also serve as important markers for resistance to pathogens (Witzell and Martin, 2008). For the synthesis of phenolic compounds, PAL has key regulatory role in conversion of L-

phenylalanine to trans-cinnamic acid, and the subsequent production of phenolic metabolites such as simple phenolics or coumarins, tannins salicylates, anthocyanins, lignins and flavonoids (Dixon *et al.,* 2002). In the present study, pathogen inoculation triggered the PAL activity for defense against pathogenesis. The PAL activity was high under elevated CO_2 compared to atmospheric CO_2, indicating more resistance can be achieved under elevated CO_2 concentrations and therefore, higher phenol contents were observed. The phenol content and PAL activity were higher in tolerant variety like Athira (Table 36 and 37). Similar genotypic variation with higher phenols was reported by Anushree *et al.* (2016). Higher total phenol content was recorded in the leaf samples of rice varieties infected by *Magnaporthe oryzae*. Highest in variety NLR-20104 (21.51 mg g^{-1}DW) followed by KJT-2 (18.62 mg g^{-1}DW) which was found to be resistant andlowest in both susceptible genotype EK-70 and Chimansal *i.e.* 12.92 and 13.70 mg g^{-1}DW respectively.

Ascorbic acid which is one of the major antioxidant molecules in plants provides the first line of defense against destructive ROS by playing an important role in detoxification of MDA and H_2O_2 (Boubakri, 2017). The ascorbic acid content increased under elevated CO_2 for both control and infected which was to a greater extent in the pathogen inoculated plants. Similar results were reported by Dong *et al.* (2018) in vegetables with 9.5% increase in ascorbic acid when plants were exposed to elevated CO_2. This indicated that ascorbic acid is a significant component of defence mechanism in ginger and its enhancement provides one of the reasons for reduced PDI % under CO_2 enrichment in the present study.

Ascorbic acid also plays a major role in the biosynthesis of certain plant hormones, such as gibberellic acid and ethylene, secondary metabolites with antimicrobial properties and cell wall glycoproteins (Boubakri, 2017). The per cent increase in ascorbic acid was higher for variety Athira 21.31% and Maran 31.10% under ambient and elevated CO_2 respectively which exhibited relatively higher tolerance to the disease (Table 37). Similar results were obtained by Fujiwara *et al.* (2013). When *Brassica rapa* resistant cultivars were infected with *Turnip mosaic virus,* an increase in ascorbic acid levels was evident. Transgenic potato plants overproduced 1.6–2-fold

ascorbic acid and exhibited tolerance to various abiotic stresses due to the enhanced ROS scavenging activity (Gururani *et al.,* 2012).

In addition to PAL, CAT and APX the pathogenesis-related proteins such as chitinase (PR-3 family) and ß-1, 3-glucanase (PR-2 family) the other defence enzymes which accumulate following pathogen attack and degrade the fungal cell wall. In the present study, *P. apanidermatum* inoculation was found to increase glucanase activity compared to the control plants and; more prominent in resistant variety Athira. Similar results were reported by Mohammadi *et al.* (2012) who reported that ß-1,3-glucanase have a key role to defense responses to *S. tritici* in wheat because it rapidly accumulated in the resistant genotype and the fungal cell walls were degraded and pathogen growth was inhibited.

Also the CO_2 enrichment enhanced the activity of ß-1, 3-glucanase, which was higher for Maran (43.68%) and Athira (38.94%) which were relatively tolerant. Similarly, elevated CO_2 reduced the levels of necrosis caused by late blight in potato, and that was linked to increased ß-1,3-glucanase activity, which plays a role in disease resistance under elevated CO_2 (Plessl *et al.,* 2007). Also from the present study it can be proved that ß -1,3-glucanase have a key role to play in defense responses to *P. apanidermatum* because it rapidly accumulated in Athira leading to the degradation of fungal cell walls and inhibiting pathogen growth. Therefore, the antioxidant enzymes like APX, PAL, glucanase can be used in preliminary screening against rhizome rot.

5.2.5 Chlorophyll fluorescence signals

Chlorophyll fluorescence imaging provides a sensitive, non-invasive and non-destructive method that can be used to provide insight into the mechanisms underlying plant pathogen interactions. Biotic stress, through viral, bacterial or fungal pathogen infection has also been shown to affect the photosynthetic electron transport and downstream metabolic reactions (Rolfe and Scholes, 2010).

Fv/Fm (the ratio of variable to maximum fluorescence) is one of the most common fluorescence indicators of plant stress indicting the optimum quantum efficiency values. Environmental stress conditions can reduce the maximum quantum yield of PSII,

and under severe stress conditions an obvious decrease in Fv/Fm has been observed (Inamullah and Isoda, 2005). Therefore, Fv/Fm is used for detecting stress and diagnosing plant stress status. In the present experiment Fv/Fm showed reduction in the *P. aphanidermatum* inoculated leaves compared to healthy leaves. This indicated that inoculation of pathogen had a negative effect on normal functioning of PS II of photosynthetic system. The reduction of Fv/Fm was higher under ambient CO_2 condition probably due to the greater loss of chlorophyll compared to the elevated CO_2. Similar results have been reported in soyabean wherein an increase in Fv/Fm ratio under elevated CO_2 1000 ppm indicated increased efficiency of PS II and also reduced risk of damage caused to PSII by oxidative stress (Wang *et al.,* 2015). Zhao *et al.* (2010) also reported an increase in Fv/Fm ratio in *Betula platyphylla* exposed to 700 ppm CO_2.

Among the varieties highest per cent reduction over control for Fv/Fm was found for Aswathy (45.56%) and least for *Z. zerumbet* (0.12%). Similarly, under elevated CO_2 reduction of Fv/Fm was higher for Aswathy (40.74%) and least for *Z. zerumbet* (1.23%). For unstressed leaves, the value of Fv/Fm is highly consistent, with values of ~0.83, and correlates to the maximum quantum yield of photosynthesis (Björkman, 1987). This range was maintained by *Z. zerumbet* and was least for Aswathy (Table 40). This indicated the tolerance level of *Z. zerumbet* to soft rot.

The Quantum yield of PS II photochemistry (Φ_{PSII}), which gives a measure of proportion of the light absorbed by the chlorophyll associated with PSII that is used in photochemistry (Maxwell and Johnson, 2000), showed drastic reduction under ambient CO_2 compared to elevated CO_2 up on pathogen inoculation. Photosynthetic electron transport (ETR) which drives Rubisco associated CO_2 assimilation also exhibited the same trend as Φ_{PSII}. The damage caused by the ROS in soybean leaves infected with *Soybean mosaic virus* spatially correlated with decreases in Φ_{PSII} (Aldea *et al.,* 2006). Similarly, in the present study the decrease in ΦPSII and ETR can be owed to the greater oxidative stress which was observed higher under ambient CO_2. Since, the ROS are predominantly formed in the reaction centers of both PSI and II in chloroplast thylakoids, photosynthetic pigments such as chlorophylls and carotenoids could also suffer bleaching. This can occur either by the inhibition of chlorophyll or

carotenoids biosynthesis, or by peroxidative destruction of the pigments already formed (Wakabayashi and Böger, 2004). When stress is strong or prolonged enough, the effective Φ_{PSII} and the photochemical quenching decrease, meaning an inhibition of the electron transport chain (Bueno *et al.*, 2019).

Highest reduction for ΦPSII and ETR was found for Aswathy and least for *Z. zerumbet* under both ambient and elevated CO_2 (Table 41 and 42). The higher decrease in Aswathy at both ambient and elevated CO_2 indicates that the photosynthetic apparatus is damaged which also had lower photosynthetic rate and vice versa in *Z. zerumbet.* Similar results were found in the *Bipolaris sorokiniana*–infected leaves of susceptible wheat plants. In this case, there was a progressive decrease of photosynthesis (measured as F_V/F_M and Φ_{PSII}) correlated to the expansion of lesions, as well as to a progressive loss of chlorophyll (Rios *et al.,* 2017).

Similar results were obtained when cashew seedlings were inoculated with two different isolates of *Lasiodiplodia theobromae* displayed significantly lower F_V/F_M values respecting to the controls, as well as decays in Φ_{PSII} and increases in NPQ values before the appearance of any visual symptoms (Muniz *et al.*, 2014).

5.3. Molecular studies on the impact of elevated CO_2 on the response of ginger to *P. aphanidermatum*

Understanding the molecular mechanisms underlying plant–pathogen interactions is of primary importance. This is essential to decipher the intrinsic changes occurring in a plant to confer tolerance/susceptibility, when it is challenged by a pathogen or various environmental conditions. For this purpose, gene expression analysis is massively applied since it is closely linked to its function. In the present programme molecular studies on the impact of elevated CO_2 on the response of ginger to *P. aphanidermatum* were carried out. The varieties were selected based on the results of second experiment wherein the tolerance levels and underlying tolerance mechanisms to *Pythium* infestation were analysed. Among the varieties Aswathy and Athira exhibited highest and lowest susceptibility to *Pythium* and so were subjected further to molecular analyses. The two techniques utilised were Real-time quantitative PCR (RT-qPCR) which is a powerful and

sensitive methodology to analyse the expression of target genes and SDS-PAGE as it is a simple and informative way to analyse the variation at the proteome level.

5.3.1 Expression pattern in *PAL, CAD* and *CHS*.

Expression analysis of genes induced defense response can provide clues to elucidate major defense mechanisms against *P. aphanidermatum* infection in ginger plants. In this context the expression levels of *PAL, CAD, CHS* related to phenylpropanoid pathway were analyzed using RT-qPCR. Phenylalanine ammonia lyase (*PAL*) is a key enzyme of the phenylpropanoid pathway that catalyzes the deamination of phenylalanine to trans-cinnamic acid, a precursor for the lignin and flavonoid biosynthetic pathways (Bagal *et al.,* 2012). The Cinnamyl alcohol dehydrogenase (*CAD*) is a key enzyme in lignin biosynthesis as it catalyzes the final step in the synthesis of monolignols (Barakat *et al.,* 2009). Chalcone synthase (*CHS*) is the first rate-limiting enzyme in the flavonoid biosynthetic pathway (Yin *et al.,* 2020).

The *PAL* gene expression levels were higher up on *P. aphanidermatum* inoculation under elevated CO_2. Between the varieties Athira had higher expression levels of *PAL* gene up on pathogen inoculation. Similar results were found when *Brassica juncea* cv. Pusa Tarak were exposed to elevated CO_2 concentration of 550 ppm. It revealed lower incidence and severity of Alternaria blight caused by *Alternaria brassicae* and downy mildew caused by *Hyaloperonospora brassicae*. Leaves had higher concentration of total phenols and phenylalanine ammonia lyase activity; this might have increased the ability of mustard plants to resist infection (Mathur *et al.,* 2013). PCR (qPCR) analysis revealed higher gene expression in resistant barley plants inoculated with *Pyrenophora teres* causing net blotch with a maximum expression for *PR2* (6.41-fold) and *PAL* (7.89-fold) at 6 days post inoculation. The results suggest *PAL* and *PR2* genes positively regulate resistance in barley plants during disease progress (Arabi *et al.,* 2020). Therefore, the higher expression of *PAL* gene might have consequently imparted tolerance to variety Athira. The genes *PAL* and *CAD2* that are involved in the monolignol biosynthesis in the phenylpropanoid biosynthetic pathway were strongly up-regulated in *Z. zerumbet* following *P. myriotylum* inoculation (Geetha *et al.,* 2019).

In the present study plants were relatively tolerant under elevated CO_2 compared to ambient. This indicated that the fungus had spread through the plants under ambient CO2 unimpeded, but had been relatively arrested under elevated CO_2. The tolerance might be conferred by the expression of *CAD* which was significantly higher under elevated CO_2 compared to ambient CO_2. Similar results were found when forty days old plants of resistant and susceptible genotypes of peanut were inoculated with the fungus *Cercospora arachidicola*, a causative agent of early leaf spot disease. Transcripts for enzymes involved in *CAD* (5.26-fold) were exclusively up-regulated in the resistant genotype (Rathod *et al.,* 2020).

In the present experiment, the expression levels of *CSH* upon pathogen inoculation were higher under elevated CO_2 for both the varieties. Similar results were reported when tea plants were exposed to elevated CO_2 of 550 and 800 μmol mol^{-1}. Elevated CO_2 significantly increased flavonoid concentration by 19.41% and 35.91%, respectively, compared to that of 400 μmol mol^{-1} CO_2 (ambient) (Li *et al.,* 2019). The increase in *CHS* activity is usually followed by an increase in the C/N ratio derived from the enhanced growth rate under elevated CO_2 (Ghasemzadeh *et al.,* 2014). The expression levels of *CSH* were significantly higher for Athira (1.22-fold) compared to Aswathy (0.38-fold) under elevated CO_2. Similar results were evident when Norway spruce clones were infected with the pathogenic fungus *Ceratocystis polonica* (Siem.) C. Moreau. Transcripts of *CHS* were found to be low constitutive levels in unwounded control tissues whereas strongly up-regulated after fungal inoculation in both resistant and less resistant clone. However, transcript levels peaked soon after infection in the more resistant clone than in the susceptible clone (Nagy *et al.,* 2004). The flavonoids are synthesized via phenylpropanoid pathway in plants (Ferreyra *et al.,* 2012). Higher expression of *PAL* under elevated CO_2 upon pathogen inoculation might have concomitantly enhanced the expression of *CSH* for variety Athira to impart tolerance. Also the reduced levels of β-1, 3-glucanases might have allowed the *P. aphanidermatum* to colonise the susceptible variety Aswathy leading to its early death.

5.3.2 Protein profiling

The protein profile showed a large number of polypeptide bands. The intensity of the band was found to vary with respect to the varieties and treatments. The intensity of the band corresponding to the larger subunit of Rubisco reduced to a greater extent under pathogen inoculation for both the varieties. Rubisco is majorly involved in atmospheric CO_2 fixation for synthesis of carbohydrates. It is observed that activity of Rubisco was downregulated and reduced activity/degradation in response to a range of biotic and abiotic stresses (Demirevska *et al.,* 2009). Similar results were obtained when barley plants were inoculated with *Drechslera graminea*. Down regulation of the Rubisco genes were found, inducing loss in its activity and overall concentration in the host cells (Goel and Kumar, 2020).

In the present study, the plants under the ambient CO_2 exhibited greater reduction in the photosynthetic rate and higher disease index. It might be due to the reduction of Rubisco's activity, thus leading to stoppage of photosynthesis, ultimately leading to loss in vigour and death of the plant (Demirevska *et al.,* 2009).

Results show that 25-35 kDa protein decreased in the variety Aswathy under ambient condition and to a greater extent up on pythium inoculation. Also protein conferring to molecular weight of 20-25 kDa protein was less both in control and pathogen inoculated plants for Aswathy under ambient CO_2. These proteins are related to plant chitinases and β-1, 3-glucanases groups of Pathogenesis related (PR) proteins (Sinha *et al.,* 2014).

The proteins that are induced in response to microbial pathogens are often referred to as PR proteins. In fungal cell wall, it is known that β-1, 3-glucanase has the capacity to release elicitors under hydrolysis which are capable of inducing defense actions (Lawrence *et al.,* 2000). It also has the potency to combat diseases and has proven to hinder the germination and spread of fungal pathogens (Momein and Katatny, 2008). This reduced levels of β-1, 3-glucanases might have allowed the *P. aphanidermatum* to colonise the susceptible variety Aswathy leading to its early death.

Summary

6. SUMMARY

The concentration of atmospheric CO_2 has been increasing rapidly since the industrial revolution. Higher concentrations of CO_2 are generally known to stimulate plant photosynthesis, growth and development and eventually the yield of crops. Although much is known regarding the effects of elevated CO_2 on crop plants, the effect of elevated levels of atmospheric CO_2 on the growth of medicinal and spice plants in general and ginger in particular is less investigated. There is necessity of understanding the physiological, biochemical and molecular parameters contributing to the modifications in yield, quality and variations in varietal performances.

The increasing requirement of ginger for its multitude of use in spice, medicine, food flavour, beverages and pharmaceuticals has created a demand for its regular cultivation. But the annual production of ginger is crucially limited by the plant pathogens such as viruses, bacteria, fungi and nematodes (Le *et al*., 2014). As the largest group of plant fungal pathogens, the oomycete genus *Pythium* causes heavy crop losses worldwide. *Pythium* species alone are accountable for losses of multibillion-dollar worldwide (West *et al*., 2003). Furthermore, it is important for the scientific community to be prepared with suitable research outcomes to cope with the effects of elevated atmospheric CO_2 levels. In this scenario, attempts are to be made to evaluate the performance of ginger varieties under increasing CO_2 condition and also, in response to soft rot up on CO_2 enrichment.

In this context, the investigation entitled "Evaluation of CO_2 enrichment on growth, development and soft rot tolerance in ginger (*Zingiber officinale* Rosc.)" was undertaken with the objective to assess the impact of elevated CO_2 on growth, yield, quality and tolerance to soft rot in ginger through morphological, physiological, biochemical and molecular analysis.

In the first experiment three varieties of ginger namely Aswathy, Athira and Maran were utilized to assess the impact of elevated CO_2 on growth, yield and quality of ginger. One set of three month old plants were shifted to OTC and another set was retained under ambient CO_2. The plants were harvested at 4^{th}, 5^{th}, 7^{th} and 8^{th} months after planting

(MAP). Observations on growth, physiological and biochemical parameters were recorded at each harvest. Yield, yield parameters, tissue nutrient status and quality parameters were assessed at 8 MAP.

The results indicated a significant improvement in the parameters like plant height, number of leaves, root weight, shoot weight, stem diameter, number of tillers, total chlorophyll, photosynthetic rate and total sugars in the rhizome by recording significantly higher values upon CO_2 enrichment. Significant reduction in transpiration rate, stomatal conductance and specific leaf area were recorded upon CO_2 enrichment when compared to ambient CO_2. The per cent increase in yield parameters like number of fingers (38.19%), rhizome length (11.32%) thickness (42.72%) and dry weight of rhizomes (31.43%) recorded higher values upon CO_2 enrichment leading to higher yield. The per cent increase in the fresh weight of rhizome recorded higher for variety Aswathy (38.34%), followed by Maran (20.00%) and Athira (12.12%).

Upon CO_2 enrichment the quality parameters of rhizome were modified with increase in fiber (60.26%), gingerol (9.06%), phenol (19.25%), total oleoresin (11.52%), starch (44.84%) and essential oils (31.10%). Significant reduction in protein and nutritional status in the rhizomes were also recorded. Nitrogen, potassium, iron and zinc contents decreased by 15.78%, 3.22%, 11.78% and 18.57% respectively in response to elevated CO_2. Phosphorous and copper showed decrease in trend though not significant. Among the three varieties of ginger, Aswathy was found to be performing better compared to Athira and Maran not only in terms of yield but also for majority of quality parameters assessed.

Second experiment was conducted to assess the impact of elevated CO_2 on tolerance to soft rot in three ginger varieties Aswathy, Athira, Maran and wild ginger congener *Zingiber zerumbet*. One set of three and a half month old plants were shifted to OTC and another set retained at ambient CO_2 and were challenged with *Pythium aphanidermatum* at 4 MAP. The CO_2 concentration of 500 ppm was maintained in the OTC.

In the case of impact of elevated CO_2 on soft rot tolerance there was no variation in the per cent disease incidence. However, lesser number of days was taken for development of symptom. The per cent disease index was reduced by 11.10% upon CO_2 enrichment. Growth responses to elevated CO_2 as affected by pathogen inoculation could not be illustrated appropriately in this experiment because of succumbence of experimental plants to the pathogen before realising significant growth. However, there was an increase in number of leaves and plant height when the plants were allowed to acclimatise for 15 days under elevated CO_2 before inoculation. The reduction in the fresh weight of rhizome upon pathogen inoculation was higher (17.60%) under ambient CO_2 when compared to elevated CO_2 (14.34%).

Upon *P. aphanidermatum* inoculation, the retention of photosynthetic pigments like chlorophyll a (0.90 mg g^{-1} FW) chlorophyll b (0.74 mg g^{-1} FW) total chlorophyll (1.64 mg g^{-1} FW) carotenoids (0.58 mg g^{-1} FW), xanthophyll (0.40 mg g^{-1} FW) and photosynthetic rate (5.92 µmol CO_2 m^{-2} s^{-1}) were higher under elevated CO_2 when compared to ambient CO_2. Higher membrane integrity values and lower malondialdehyde content (0.75 µg g^{-1} FW) in all the selected varieties were evident upon pathogen inoculation under CO_2 enrichment.

Enhanced activity of ascorbate peroxidase (4.86 activity Units mg^{-1} total protein), PAL (Phenylalanine ammonia lyase) (20.91 Units mg^{-1} total protein), and glucanase (4.57 Units mg^{-1} total protein) activity, as well as higher accumulation of phenols (32.40 mg g^{-1} DW), ascorbic acid (7.14 mg 100 g^{-1} FW) were found in the inoculated plants under CO_2 enrichment compared to the ambient CO_2. The catalase activity was found to reduce upon pathogen inoculation and the reduction was higher (0.11 Units mg^{-1} total protein) under ambient CO_2 as compared to elevated CO_2 (0.09 Units mg^{-1} total protein).

Highest levels of hydrogen peroxide, superoxide and cell death were observed upon pathogen inoculation under ambient CO_2 observed through staining techniques and it was highest for variety Aswathy and least for *Z. zerumbet*. After pathogen inoculation maximum values for variable to maximum fluorescence Fv/Fm (0.63), photosynthetic electron transport rate (82.60 micro equiv m^{-2} s^{-1}) and quantum yield of PS II photochemistry (0.29) were recorded under elevated CO_2.

Protein profiling performed in varieties Athira and Aswathy revealed lower expressions of 20-25 kDa and 25-35 kDa protein under pathogen inoculation in variety Aswathy. The expression of 55 kDa (Rubisco Large Sub-Unit) was down regulated to a greater extent under pathogen inoculation in both the varieties. Higher expression levels of the defense responsive genes *Phenylalanine ammonium-lyase* (*PAL*), *Cinnamyl alcohol dehydrogenase* (*CAD*) and *Chalcone synthase* (*CSH*) were also observed upon pathogen inoculation under CO_2 enrichment in both the varieties. Between the varieties, Athira had higher relative fold changes of *PAL, CAD* and *CSH* under both ambient and elevated CO_2 upon pathogen inoculation.

To conclude, the present investigation has shown that CO_2 enrichment can improve the performance of ginger in terms of growth, rhizome development and rhizome characters finally translating in terms of enhanced yield. It also indicates that increased chlorophyll content changes in photosynthetic allocation pattern and phytochemical profile with enhanced contents of oleoresins and essential oils.

Ginger plants showed better tolerance against *P. aphanidermatum* upon CO_2 enrichment. This was evident in the form of reduced disease severity, oxidative stress and yield, by activating multiple mechanisms, including enhanced production of anti-oxidants and upregulation of pathogen resistance proteins. Allocation of photosynthates to defense related metabolites played a crucial role under CO_2 enrichment for slowing down the disease progress. A balance of ROS and anti-oxidative enzymes helped to maintain membrane integrity which is vital for cell survival. This is one of the first studies to comprehend on possible effects of elevated CO_2 on ginger to soft rot.

The study also demonstrated the varietal variations in CO_2 enrichment which will help in selection of suitable varieties for changing climatic scenario. The information generated has paved way for the possibility of enhancing both qualitative and quantitative yield of ginger through low cost CO_2 enrichment technologies. It also provides insights to the tolerant levels and the mechanisms involved to ameliorate the soft rot disease under the ever increasing atmospheric CO_2 which can be utilized in the near future crop improvement programmes.

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Appendices

APPENDIX I

Sodium Phosphate Buffer

Sodium phosphate monobasic	1 M
Sodium phosphate dibasic	1 M

APPENDIX II

Potato Dextrose Agar

Peeled and sliced potatoes	200 g
Dextrose ($C_6H_{12}O_6$)	20 g
Agar-agar	20 g
Distilled water	1000 ml

APPENDIX III

Sodium Borate Buffer (0.1 M)

Boric acid	0.2 M
Borax	0.05 M

APPENDIX IV

Sodium Acetate Buffer (0.1 M)

Na acetate	0.07 M
Acetate	0.02 M

Abstracts

EVALUATION OF CO_2 ENRICHMENT ON GROWTH, DEVELOPMENT AND SOFT ROT TOLERANCE IN GINGER (*Zingiber officinale* Rosc.)

By

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Abstract

The present investigation entitled "Evaluation of CO_2 enrichment on growth, development and soft rot tolerance in ginger (*Zingiber officinale* Rosc.)" was undertaken with the objective to assess the impact of elevated CO_2 on growth, yield, quality and tolerance to soft rot in ginger through morphological, physiological, biochemical and molecular analysis. Two sets of pot culture experiments were conducted during 2017-2019. The technology utilized for CO_2 enrichment was Open Top Chamber (OTC) system established under Department of Plant Physiology, College of Agriculture, Vellayani.

In the first experiment three varieties of ginger Aswathy, Athira and Maran were utilized to assess the impact of elevated CO_2 on growth, yield and quality of ginger. One set of three month old plants were shifted to OTC and another set was retained under ambient CO_2. The plants were harvested at 4^{th}, 5^{th}, 7^{th} and 8^{th} months after planting (MAP). Observations on growth, physiological and biochemical parameters were recorded at each harvest. Yield, yield parameters, tissue nutrient status and quality parameters were assessed at 8 MAP. Second experiment was conducted to assess the impact of elevated CO_2 on tolerance to soft rot in three ginger varieties Aswathy, Athira, Maran and wild ginger congener *Zingiber zerumbet*. One set of three and a half month old plants were shifted to OTC and another set retained at ambient CO_2 and were challenged with *Pythium aphanidermatum* at 4 MAP. The CO_2 concentration of 500 ppm was maintained in the OTC.

The results of the first experiment indicated an improvement in growth parameters like plant height, number of leaves, root weight, shoot weight, stem diameter and number of tillers, physiological and biochemical parameters like total chlorophyll, photosynthetic rate and total sugars in the rhizome by recording significantly higher values upon CO_2 enrichment when compared to ambient CO_2 condition. Significant reduction in transpiration rate, stomatal conductance and specific leaf area were recorded upon CO_2 enrichment.

The per cent increase in yield parameters like number of fingers (38.19%), rhizome spread (11.32%), thickness (42.72%) and dry weight of rhizomes (31.43%) recorded

higher values upon CO_2 enrichment, based on average performance of all the selected varieties, leading to greater yield. The per cent increase in the fresh weight of rhizome recorded higher for variety Aswathy (38.34%), followed by Maran (20.00%) and Athira (12.12%) with a total fresh rhizome yield of 165.35 g plant^{-1} under ambient and 228.75 g plant^{-1} under elevated CO_2 condition in the case of Aswathy.

Upon CO_2 enrichment the quality of rhizome was modified with increase in fiber (60.26%), gingerol (9.06%), phenol (19.25%), total oleoresin (11.52%), starch (44.84%) and essential oils (31.10%). Significant reduction in protein and nutritional status in the rhizomes were also recorded. Nitrogen, potassium, iron and zinc contents decreased by 15.78%, 3.22%, 11.78% and 18.57% respectively. Phosphorous and copper showed decrease in trend though not significant. Among the three varieties of ginger, Aswathy was found to be performing better compared to Athira and Maran not only in terms of yield, also for majority of quality parameters assessed upon exposure to elevated CO_2 condition.

In the case of impact of elevated CO_2 on soft rot development, there was no variation in the per cent disease incidence. However, lesser number of days was taken for development of symptom. The per cent disease index was reduced by 11.10% upon CO_2 enrichment. The reduction in the fresh weight of rhizome upon pathogen inoculation was also less under elevated CO_2 (14.34%) when compared to ambient CO_2 (17.60%) showing that increasing CO_2 levels might bring down the disease severity.

Upon *P. aphanidermatum* inoculation, the retention of photosynthetic pigments like chlorophyll a (0.90 mg g^{-1} FW) chlorophyll b (0.74 mg g^{-1} FW) total chlorophyll (1.64 mg g^{-1} FW) carotenoids (0.58 mg g^{-1} FW), xanthophyll (0.40 mg g^{-1} FW) and photosynthetic rate (5.92 µmol CO_2 m^{-2} s^{-1}) were higher under elevated CO_2 when compared to ambient CO_2. Higher membrane integrity values and lower malondialdehyde (0.75 µg g^{-1} FW) content in all the selected varieties were evident upon pathogen inoculation under CO_2 enrichment.

Enhanced activity of ascorbate peroxidase (4.86 activity Units mg^{-1} total protein), PAL (Phenylalanine ammonia lyase) (20.91 Units mg^{-1} total protein), and glucanase (4.57

Units mg^{-1} total protein) activity, as well as higher accumulation of phenols (32.40 mg g^{-1} DW) and ascorbic acid (7.14 mg 100 g^{-1} FW) were found in the inoculated plants under CO_2 enrichment compared to the ambient CO_2. The catalase activity was found to reduce upon pathogen inoculation and the reduction was higher (0.11 Units mg^{-1} total protein) under ambient CO_2 as compared to elevated CO_2 (0.09 Units mg^{-1} total protein).

Highest levels of hydrogen peroxide, superoxide and cell death were observed upon pathogen inoculation under ambient CO_2 through staining techniques and it was highest for variety Aswathy and least for *Z. zerumbet*. After pathogen inoculation maximum values for variable to maximum fluorescence Fv/Fm (0.63), photosynthetic electron transport rate (82.60 micro equiv m^{-2} s^{-1}) and quantum yield of PS II photochemistry (0.29) were recorded under elevated CO_2. Protein profiling performed in varieties Athira and Aswathy revealed lower expressions of 20-25 kDa and 25-35 kDa protein under pathogen inoculation in variety Aswathy. The expression of 55 kDa (Rubisco Large Sub-Unit) was down regulated to a greater extent under pathogen inoculation in both the varieties. Higher expression levels of the defense responsive genes *Phenylalanine ammonium-lyase* (*PAL*), *Cinnamyl alcohol dehydrogenase* (*CAD*) and *Chalcone synthase* (*CSH*) were also observed upon pathogen inoculation under CO_2 enrichment in both the varieties. Between the varieties, Athira had higher relative fold changes of *PAL*, *CAD* and *CSH* under both ambient and elevated CO_2 upon pathogen inoculation.

The present study revealed that CO_2 enrichment can improve the performance of ginger in terms of growth, yield and quality. It also indicates changes in photosynthetic allocation pattern and phytochemical profiles with enhanced contents of oleoresins and essential oils. Ginger plants showed better tolerance against *P. aphanidermatum* upon CO_2 enrichment. This was apparent in the form of reduced disease severity, oxidative stress level and less reduction in yield upon pathogen infestation under elevated CO_2 condition. This was evidently achieved through the activation of multiple mechanisms, including enhanced production of anti-oxidants and up regulation of pathogen resistance proteins. This is one of the first studies to comprehend on possible effects of elevated CO_2 on the susceptibility of ginger to soft rot. The study also demonstrated the varietal variations existing in the response to CO_2 enrichment which will help in selection of

suitable varieties for changing climatic scenario. The information generated indicates the possibility of enhancing both qualitative and quantitative yield of ginger through low cost CO_2 enrichment technologies. The insights obtained on the tolerance mechanisms involved will help to restructure the soft rot disease management strategies in the coming future.