Expression of *Chalcone synthase* gene in ginger (*Zingiber officinale* Rosc.) as influenced by various management practices

By

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THESIS

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DECLARATION

I, hereby declare that the thesis entitled **"Expression of *Chalcone synthase* gene in ginger (*Zingiber officinale* Rosc.) as influenced by various management practices"** is a bonafide record of research done by me during the course of research and that it has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara

Date: 05/08/2019

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CERTIFICATE

Certified that this thesis entitled **"Expression of *Chalcone synthase* gene in ginger (*Zingiber officinale* Rosc.) as influenced by various management practices"** is a record of research work done independently by **Ms. Archita Unnikrishnan (2017-11-004)** 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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ABBREVIATIONS

%	Percentage
@	At the rate
<	Less than
=	Equal to
>	Greater than
°C	Degree Celsius
ΔCt	Threshold cycle
µg	Microgram
µl	Microlitre
µm	Micrometre
µM	Micromole
t.	Tonnes
ACAA1	Acetyl CoA Acyl Transferase 1
AMF	Arbuscular Mycorrhizal Fungus
bp	Base pair
cDNA	complementary DNA
CHS	Chalcone Synthases
cm	Centimetre
CPBMB	Centre for Plant Biotechnology and Molecular Biology
CNM	Chemical Nutrient Management
CO_2	Carbon dioxide
DAP	Days After Planting

DBT-BIRAC	Department of Biotechnology-Biotechnology Industry Research Assistance Council
DEPC	Diethyl pyrocarbonate
DNA	Deoxyribonucleic Acid
dNTPs	Deoxyribo Nucleoside Triphosphates
DPPH	2, 2- Diphenyl-1-picrylhydrazyl
ds	double stranded
EDTA	Ethylene Diamine Tetra Acetic acid
EST	Expressed Sequence Tag
EtBr	Ethidium Bromide
g	Gram
FYM	Farm Yard Manure
GC-MS	Gas Chromatography-Mass Spectrometry
H_2O	Water
HFD	High Fat Diet
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
KAU	Kerala Agricultural University
kb	Kilobase
L	Litre
M	Molar
MAP	Months After Planting
mg	Milligram
$MgCl_2$	Magnesium Chloride
min	Minutes

ml	Millilitre
mm	Millimetre
mM	Millimole
MoP	Muriate of Potash
mRNA	messenger RNA
ng	Nanogram
nm	Nanometre
NMR	Nuclear Magnetic Resonance
OD	Optical Density
ONM	Organic Nutrient Management
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
pH	Hydrogen ion Concentration
PKS	Polyketide Synthases
PoP	Package of Practices
ppm	parts per million
PVP	Poly Vinyl Pyrrolidone
RNA	Ribonucleic Acid
RNase	Ribonuclease
RP-HPLC	Reverse Phase-High Performance Liquid Chromatography
rpm	Revolutions per minute
rRNA	ribosomal RNA
s	Seconds
ss	Single stranded

SSH	Suppression Subtractive Hybridisation
TAE	Tris Acetate EDTA
Taq	*Thermus aquaticus*
TLC	Thin Layer Chromatography
tRNA	transfer RNA
U	Unit
UV	Ultra Violet
V	Volts
Viz.	Namely

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Introduction

1. INTRODUCTION

Ginger (*Zingiber officinale* Rosc.), a rhizomatous herbaceous perennial belonging to the family Zingiberaceae is one of the most valued spice crops for its medicinal and nutraceutical properties. It finds a wide range of applications in traditional medicine with carminative, stimulant, analgesic, antipyretic, gastroprotective, cardiotonic and antihepatotoxic activities. The anti-oxidant, anti-inflammatory, anti-diabetic and anti-hypercholesterolemic properties of ginger find applications in nutraceutical industries. In India, during the year 2017-18, the area under cultivation of ginger was estimated to be about 1,60,860 ha. with a total production of 10,43,130 t. whereas in the state of Kerala the production was about 20,190 t. from 4,970 ha. area of land. It also fetched Rs. 21,606.55 lakhs during 2017-18 from an export of 22,605 t. of ginger (www.indianspices.com).

The common spice is rich in non-volatile and pungent bioactive phenolic compounds like gingerols, paradols and shogaols. They are derived from "stilbenoid, diarylheptanoid and gingerol biosynthesis" pathways (Ramirez-Ahumada *et al.* 2006). Therapeutic properties are attributed by various bioactive compounds like flavonoids, coumarins and gingerols. The main natural principles of ginger known as gingerols are a homologous series of aldols containing a phenolic group (Baranowski, 1986 and McHale *et.al.*, 1989). Gingerols, particularly 6-gingerol is the most potent bioactive compound, which is now a target for drug development. The synthesis of these metabolites is acted upon by the polyketide synthases enzyme family (Schroder, 1977 and Dennif *et al.*, 1980). The type III polyketide synthase family of enzymes are involved in the synthesis of plant secondary metabolites possessing high pharmacological importance. Chalcone synthase is one such type III polyketide synthase which is involved in the synthesis of 6-gingerol (Schroder, 1977 and Dennif *et al.*, 1980). The same role of chalcone synthase was reported by Ramirez-Ahumada *et al.* (2006), Ghosh and Mandi (2015) and Sreeja (2017) in the production of gingerol. The key enzyme involved in gingerol biosynthesis is chalcone synthase (CHS).

Several investigations revealed the pharmacological importance of the pungent principles not only as a potent chemopreventive but also as a chemotherapeutic agent on various cell lines of cancer and animal models. Lee and Surh (1998) recorded that apoptosis was induced in human leukemia (HL-60) cells by 6-gingerol and 6-paradol. Gingerols are active anti-inflammatory agents suppressing motility, invasion and adhesion in various cancer cell lines (Rhode *et al.*, 2007; Kundu *et al.*, 2009; Dugasani *et al.*, 2010). Ginger leaf is reported to reduce *in vitro* cell viability and apoptosis through ATF3 promoter activation and subsequently increasing ATF3 expression by ERK1/2 activation in human colorectal cancer (Park *et al.* 2014). The inhibition of proteasome and induction of p53 reactivation and apoptotic cell death was reported in cervical cancer cells by 6-gingerol. The cytotoxic effects of cisplatin, a traditional chemotherapeutic agent was also found to have enhanced by 6-gingerol. The results reveal the potential of 6-gingerol to be used as a sole agent or in combination with conventional chemotherapeutic drugs as a promising therapeutic strategy in the treatment of cervical cancers Rastogi *et al.* (2014).

Knowledge in the biosynthesis of gingerols can be well understood using molecular and bioinformatics tools. Radhakrishnan and Soniya (2009) observed the putative chalcone forming and non-chalcone forming members when genome wide studies was carried out in ginger for the PKS superfamily by PCR. Radhakrishnan *et al.* (2009) cloned and characterised the novel Type III PKS and the gene was analysed *in silico*. The amplification of the second exon region comprising of the conserved amino acid stretches of PKS gene in *Alpinia calcarata,* a Zingiberaceous plant was done by PCR (Radhakrishnan *et al.* (2010). The High Performance Liquid Chromatography (HPLC) was carried out by Kizhakkayil and Sasikumar (2012) for the analysis of pungent principles of ginger.

Sreeja (2017) revealed that *Chalcone synthase* gene expression for gingerol production was observed in leaves during the active growth phase of ginger (first four months after planting) and the gene expression was not observed in rhizomes

at seven months after planting. She inferred that leaf is the site of synthesis of gingerols and the metabolite got accumulated in rhizomes in the rhizome formation stage and thereafter it is distributed in the growing rhizomes. She also observed that clonal variations in gingerol content were evident even at sprouting stage of rhizome when she assessed the *Chalcone synthase* gene activity by Real Time PCR assay.

Nutrient management is found to influence the secondary metabolite production. A study conducted at Centre for Plant Biotechnology and Molecular Biology (CPBMB) revealed that integrated nutrient management in ginger was found to yield higher gingerol content than organic nutrient management practices (Shylaja, 2017). Similar result was reported by Srinivasan *et al.* (2019) in which integrated nutrient management showed the highest yield of essential oil as compared to the organic nutrient management and integrated nutrient management practices in ginger under rainfed condition.

The combination of salicylic acid and CO_2 increased anthocyanin and flavonoid production in the ginger leaves (Ghasemzadeh *et al.*, 2012a). The flavonoids and phenolic compounds accumulated more in the leaves and rhizomes of ginger raised under 60 and zero per cent of shade respectively. (Ghazemsadeh and Ghazemsadeh, 2011).

Spraying of elicitors was found to improve secondary metabolite production in several crops like pepper (Elwan and El-Hamahmy, 2009 and Zunun-Perez *et al.*, 2017), saffron (Tajik and Niknam, 2015), *Salvia macrosiphon* (Rowshan *et al.*, 2010), *Achillea millefolium* (Rowshan and Bahmanzadegan, 2013). An increase in the production of total flavonoids was recorded in ginger upon elicitation by salicylic acid (Ghasemzadeh *et al.*, 2012b).

With this background, the present study was conducted at CPBMB, College of Horticulture, Vellanikkara from 2017-2019 to analyse the influence of nutrient

management and spraying of elicitors on *Chalcone synthase* gene expression in ginger under polyhouse and open conditions which in turn will help to develop management practices for high gingerol recovery from ginger rhizomes.

Review of literature

2. REVIEW OF LITERATURE

The present work on "Expression of *Chalcone synthase* gene in ginger (*Zingiber officinale* Rosc.) as influenced by various management practices" was carried out at Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture, Kerala Agricultural University, Thrissur from 2017 to 2019 with the objective to analyse the influence of nutrient management and spraying of elicitors on *Chalcone synthase* gene expression in ginger under polyhouse and open conditions. The chemistry of ginger, varieties released in ginger, ginger as an effective nutraceutical, quality of ginger as influenced by nutrient management practices and spraying of elicitors, influence of growing conditions on quality, quantification of secondary metabolite using HPLC and *CHS* gene expression studies are reviewed in this chapter.

Ginger (2n=22) belongs to the monocotyledonous family, Zingiberaceae and order Zingiberales. The typical character of the species under Zingiberaceae is that they have thickened rhizomes with secretory cells that have the ability to produce essential oil (Mabberley, 1997). Ginger is mostly propagated by vegetative means as most of the cultivars seldom flower or are sterile (Sutarno *et al.*, 1999).

2.1 Chemistry of ginger

Natarajan *et al.* (1972) when analysed the quality of ginger rhizomes of Kerala, observed 1.0 to 2.7 per cent, 3.9 to 9.3 per cent, 4.8 to 9.8 per cent and 40.4 to 59.0 per cent of essential oil, acetone extract, crude fiber and starch respectively.

There are numerous constituents present in ginger which vary based on the place where it originated and whether the rhizomes are fresh or dry. Steam volatile oil, mineral elements, proteins, resins, non-volatile pungent compounds and cellulose are the components present in ginger rhizomes. Starch that constitutes

about 40 to 60 per cent of rhizome on dry weight basis is the most abundant constituent in rhizomes of ginger (Parthasarathy *et al.*, 2008).

A sample of green ginger when analysed was found to contain moisture, protein, fat, carbohydrates, fibre and minerals at 80.9, 2.3, 0.9, 12.3, 2.4 and 1.2 per cent respectively. The minerals such as Ca, P, Fe and vitamins such as thiamine, riboflavin, niacin and ascorbic acid were recorded at 20.0, 60.0, 2.6, 0.06, 0.03, 0.6 and 6.0 mg per 100 g of the rhizome. On a dry weight basis the rhizome was found to contain 7.6 per cent of fructose and meagre quantities of free sugars such as glucose, sucrose and fructose. Non-protein nitrogen constituted about one-third of the 1.6-2.4 per cent of the nitrogen present in the ginger rhizome. Of the extractable proteins albumin, globulin, prolamine and glutelin constituted about 35.6, 16.9, 11.0 and 17.9 per cent respectively and about 18.6 per cent of the proteins could not be extracted. (Govindarajan, 1982) He also reported that dry ginger which is commercially available is reported to contain about 3.5 to 10 per cent and 1.5 to 3.0 per cent of oleoresins and volatile oil respectively.

Ekundayo *et al.* (1988) identified the chemical components of ginger oils obtained from both the fresh and dried rhizomes of ginger from Nigerian origin by Gas Chromatography- Mass Spectroscopy (GC-MS). The ginger oil was mainly constituted of contained mainly mono- and sesqui-terpenoids of which the major constituents were geranial, neral, 1,8 cineole, zingiberene, β-bisabolene and β-sesquiphellandren.

The oleoresin yield and the contents of bioactive principles such as 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol from dried ginger of Jamaican ginger was studied by Bailey-Shaw *et al.* (2008) at different stages of maturity ranging from seven months to nine months. The ginger harvested at eighth month recorded the highest yield of oleoresin. The most abundant principle responsible for pungency was found to be 6-gingerol and was recorded to be the highest in the sample harvested at ninth month.

The secondary metabolites obtained from the ginger rhizomes can be classified broadly as volatile and non-volatile compounds. The non-volatile principles present in ginger are identified as phenolic compounds that confers pharmacological importance to ginger rhizomes (Wohlmuth, 2008).

The term oleoresin in ginger generally refers to the volatile oil, pungent principles and other solvent (acetone or ethanol) extracted compounds. (Connell, 1970 and Govindarajan, 1982).

The pungency of ginger is conferred by the non-volatile phenolic compounds. The major constituents are gingerols which includes 6-gingerol, 8-gingerol and 10-gingerol are derived from gingerdiols. These gingerols when subjected to heat or alkali are dehydrated to yield shogaols and zingerone (Connell and Sutherland, 1969 and Connell, 1970) and the shogaols are more pungent than their corresponding gingerols (Denniff *et al.*, 1980).

The gingerols, shogaols, paradols and zingerone are the pungent principles that produce a ''hot'' sensation in the mouth. The gingerols were identified as the major active components in the fresh rhizomes of ginger (Govindarajan, 1982).

The hot pungent sensation of ginger is attributed by the non-volatile and biologically active principles such as gingerols, shogaols, paradols and zingerone. The phenylalkylketones or vanillyl ketones such as 6-gingerol, 8-gingerol and 10-gingerol, 6- shogaol, 8-shogaol and 10-shogaol and zingerone, 6-paradol, 6- dehydrogingerdione and 10- dehydrogingerdione, 6- gingerdione and 10-gingerdione are the primarily observed pungent principles (Chrubasik *et al.*, 2007).

As per the report of Langner *et al.* (1998) and Evans (2002) over 50 components of the volatile oil were characterized and were identified as monoterpenoids (b-phellandrene, camphene, cineole, geraniol, curcumene, citral,

terpineol, borneol) and sesquiterpenoids (a-zingiberene, b- sesquiphellandrene (, b-bisabolene, a-farnesene, arcurcumene, zingiberol).

Ali *et al.* (2008) reported that the aroma of ginger is the result of volatile compounds present and the yield of volatile oil ranged from 1 to 3 per cent.

The hydrodistillation of ginger rhizomes yielded essential oils from the varieties Gorkha, Sindhupalchowk and Tanahu at 1.9 per cent, 1.8 per cent and 1.1 per cent respectively. The GC-MS analysis revealed the presence of 105, 85 and 88 constituents collected from places such as Sindhupalchowk, Tanahu and Gorkha. The major constituents were monoterpenes and sesquiterpenes derivatives (Bhattarai *et al.*, 2018).

2.2 Varieties released in ginger

2.2.1 Ginger varieties released from Kerala Agricultural University

2.2.1.1 Athira

Athira is single plant selection from somaclones of cultivar Maran, is a high yielding ginger variety, best suited for fresh as well as dry ginger recorded an average yield of 22.6 t $ha^{-1}$with a dry recovery of 22.6 per cent. The volatile oil content was found to be about 3.1 per cent. Of which zingiberene was found to be about 35.76 per cent and citral was about 1.31 per cent. The oleoresin content was found to be 6.8 per cent and gingerols constituted 16.5 per cent of the oleoresin. The rhizomes were bold with less crude fibre content of 3.4 per cent. The variety is tolerant to soft rot and bacterial wilt diseases as compared to the parent cultivar Maran (Shylaja *et al.*, 2010).

2.2.1.2 Karthika

The high yielding and highly pungent variety Karthika is single plant selection from somaclones of cultivar Maran. It is best suitable for oleoresin extraction. The rhizomes are of medium bold nature with high volatile oil and oleoresin recovery. The oleoresin was found to be rich in total gingerols and volatile oil was rich in citral. The crop is suitable as pure as well as intercrop. The variety is tolerant to soft rot and bacterial wilt diseases as compared to the parent cultivar, Maran. The infestation by shoot borer was low in the field crop. The average yield was found to be 19 t $ha^{-1}$with a dry recovery of 21.6 per cent. The quality attributes like volatile oil and oleoresin content was found to be 3.2 per cent and 7.2 per cent respectively. Gingerols constituted 21.3 per cent of the oleoresin. Zingiberene and citral constituted about 22.87 and 4.03 per cent of the volatile oil. The crude fibre content was found to be about 3.7 per cent (Shylaja *et al.*, 2010).

2.2.1.3. Aswathy

Aswathy variety was released from College of Horticulture, Vellanikkara, Kerala Agricultural University in 2013 is single plant selection from somaclones of the cultivar Rio-de-Janeiro. It was the somaclonal selection from the parent cultivar, Rio de Janeiro. The average yield was found to be 23 t ha^{-1}. The rhizomes were bold and reported a dry recovery of 19.7 per cent, volatile oil of 3.32 per cent and oleoresin of 7.45 per cent. The volatile oil constituted of zingiberene (32.46%) and citral (2.127%). The non-volatile oleoresin constituted of gingerols (16.7%) and shogaols (2.10). The crude fibre content was about 3.5 per cent. The variety is ideal for pure crop as well intercrop (Shylaja *et al.*, 2014).

The other varieties released from KAU include KAU Chandra and KAU Chitra. The variety KAU Chandra is a high yielding with fresh yield of 23.51 t ha^{-1} and dry yield of 5.23 t ha^{-1}. This is a selection from the induced

polyploidy of Rio-de-Janeiro. The rhizomes are bold with less fibre content (www.kau.in).

The variety KAU Chitra is a selection from the somaclones of cultivar Himachal Pradesh. This also a high yielding ginger variety with a driage of 23.4 per cent (www.kau.in).

2.2.2 Ginger varieties released from IISR, Calicut

2.2.2.1 IISR Varada

The ginger variety IISR-Mahima is a selection from germplasm and was released in 1996. The variety is adopted for cultivation all over India. The average yield was found to be 22.6 t $ha^{-1}$and the crop was found to take about 200 days to harvest. The quality attributes such as essential oil, dry recovery and fibre content were recorded as 1.75 per cent, 20.7 per cent and 3.29 to 4.5 per cent respectively. The rhizomes were plumpy with flattened fingers with reddish brown scales. This was found to be a high yielding variety with good quality rhizomes. The dry ginger was found to be less susceptible to the infestation by storage pests. The variety was found to contain less fibre and was tolerant to diseases (http://www.spices.res.in).

2.2.2.2 IISR Mahima

The ginger variety released in 2001 is a selection from germplasm. It recorded an average yield of 23.2 t $ha^{-1}$and the crop was found to take about 200 days to harvest. The essential oil, dry recovery and fibre content were recorded as 1.72 per cent, 19.0 per cent and 3.26 per cent respectively. The plant height, leaf length/breadth, number of tillers per clump, number of leaves per tiller were found to be 65.3 cm, 23.9/2.9 cm, 12.8 and 12.5 respectively and the aerial stem was green in colour. The rhizomes were found to be plumpy, round and bold with brown scales. The variety was found to be resistant to root-knot nematode (http://www.spices.res.in).

2.2.2.3 IISR Rejatha

The ginger variety IISR-Rejatha was also released in 2001 and is a selection from germplasm. This is more suitable for cultivation in the state of Kerala. The average yield was found to be 22.4 t $ha^{-1}$and the crop was found to take about 200 days to harvest. The essential oil, dry recovery and fibre content were recorded to be 2.36 per cent, 23.0 per cent and 4.0 per cent respectively. The colour of the aerial shoots was green and the rhizomes were plumpy, round and bold with brown scales. The plant height, leaf length/breadth, number of tillers per clump, number of leaves per tiller were recorded to be 67.7 cm, 23.6/3.0 cm, 8.26 and 13.65 respectively. The rhizomes were found to be three layered compact clumps with low fibre content and rich in essential oil and oleoresin (http://www.spices.res.in).

2.2.3 Ginger varieties released from Odhisha University of Agriculture and Technology

The ginger varieties Suprabha, Suruchi and Suravi were released from High Altitude Research Station, Pottangi, Odhisha University of Agriculture and Technology.

2.2.3.1 Suprabha

The average yield recorded was 16.6 t $ha^{-1}$with a dry recovery of 20.5 per cent and crude fibre content of 4.4 per cent. The time taken for its maturity is 229 days. The quality attributes such as oleoresin and essential oil was found to be 8.9 per cent and 1.9 per cent respectively (www.ouat.nic.in).

2.2.3.2 Suruchi

The Suruchi variety recorded an average yield of 16.6 t ha^{-1}. The time taken for maturity was found to be 218 days. The dry recovery and crude fibre was found

to be 23 per cent and 4 per cent respectively. The oleoresin and essential oil contents were recorded to be 10.2 per cent and 2.1 per cent respectively (www.ouat.nic.in).

2.2.3.3 Suravi

The variety was found to yield about 17.5 t ha^{-1} on an average. The variety requires 225 days to maturity. The dry recovery and crude fibre was found to be 23 per cent and 4 per cent respectively. The oleoresin an essential oil contents were recorded to be 10.2 per cent and 2.1 per cent respectively (www.ouat.nic.in).

2.2.4 Ginger varieties released from other State Agricultural Universities

2.2.4.1 Himgiri

The variety, Himgiri was released from Dr. Y.S. Parmar University of Horticulture and Forestry, Solan. The average yield of the variety was found to be 13-14 t ha^{-1}. The days taken for maturity was recorded to be 230. The oleoresin and essential oil content was found to be 4.29 and 1.63 per cent respectively (www.yspuniversity.ac.in).

2.3 Ginger as an effective nutraceutical

The bioactive compounds such as gingerols, shogaols, diarylheptanoids, and flavonoids in ginger are of immense value to human health because of their, anti-oxidant, anti-inflammatory, anti-obesity, anti-cancerous, anti-aging, cardiovascular pharmacological and anti-hemorrhagic properties.

2.3.1 Anti-oxidant properties of ginger

The gingerols such as 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol, expressed anti-oxidant activities with IC_{50} values between 8.05 to 26.3 μM, 0.85–

4.05 μM and 0.72–4.62 μM for the DPPH radical, superoxide radical and hydroxyl radical respectively (Dugasani *et al.*, 2010).

The antioxidant ability of 6–gingerol was observed to be greater than that of 8-gingerol and 10-gingerol. Thus the short carbon chains of 6-gingerol and 6-shogaol played a significant role in making them more potent antioxidants than the other long carbon chain compounds. This is attributed to the conversion of gingerols to shogaols during oven drying (Du *et al.*, 2014).

2.3.2 Anti-inflammatory properties of ginger

Prasad *et al.* (2012) reviewed and enlisted the molecular targets of 6-gingerol linked to inflammation, cell survival, cell proliferation and angiogenesis. NF-κB, TNF, IL-6, IL-1β and iNOS are the targets for inflammation acted upon by 6-gingerol. Bax, Bcl-2, caspase, p53 and survivin were the cell survival targets on which 6-gingerol acted. CyclinD1, c-Myc, COX-2 are the cell proliferation targets for 6-gingerol whereas VEGF related to angiogenesis served as the target for 6-gingerol to act.

2.3.3 Anti-obesity properties of ginger

Wang *et al.* (2017) reviewed the benefits of ginger on obesity and metabolic syndromes. Ginger extract when orally administered, the body weights and serum lipid levels in high-fat diet (HFD) fed rats significantly reduced. It was also found that HFD-induced obesity was attenuated by ginger extract by fastening skeletal muscle fat catabolism and expenditure of energy. In particular, 6-gingerol attributed to anti-obesity effects by altering the action and expressions of some lipid metabolism marker enzymes, such as fatty acid synthase (FAS), acetylcoA carboxylase, HMG co-A reductase, lecithin choline acyl transferase, and lipoprotein lipase. 6-gingerol also suppressed the activity of amylase and pancreatic lipase, thus reducing plasma and tissue lipids. The anti-obesity effects of ginger

was associated with its anti-inflammatory properties. Ginger extract and 6-gingerol could downregulate mRNA levels of interleukin-6 (IL-6) and tumor necrosis factor α (TNF- α) in adipose tissue of HFD fed male rats significantly.

2.3.4 Anti-cancerous properties of ginger

de Lima *et al.* (2018) made a comprehensive review on the protective and therapeutic effects of ginger extract and 6-gingerol against cancer. The ginger extract and 6-gingerol possessed the ability to act upon various important mediators and cell signaling pathways such as Bax/Bcl2, p38/MAPK, Nrf2, p65/NF-κB, TNF-α, ERK1/2, SAPK/JNK, ROS/NF-κB/COX-2, caspase-3, caspase-9, and p53. This exhibited the relevance of ginger extract and 6-gingerol as an anti-proliferative, anti-tumor, invasive, and anti-inflammatory agent.

de Lima *et al.* (2019) studied the potential of 6-gingerol as an effective pharmaceutical formulation against tumors. They evaluated the toxicogenic effects of 6-gingerol various cells and cell lines. HL-60 cell line and Sarcoma 180 (S-180) ascitic fluid cells and peripheral blood mononuclear cells (PBMC) were treated with 6-gingerol at 5, 10, 20, 40 μg ml^{-1} for 72 hours. The IC50 of [6]-G were 1.14, 5.73 and 11.18 μg mL-1 for HL-60, S-180 and PBMC, respectively thus indicating a possibility of action of 6-gingerol against tumor cell lines.

2.3.5 Anti-aging properties of ginger

Liu *et al.* (2019) investigated the activity of 6-gingerol as an effective stimulant in plasma adiponectin and muscular adiponectin receptor signaling in the naturally ageing rats. Twenty-two-month-old male Sprague Dawley rats when treated with 6-gingerol at 0.2 mg/kg once in a day for 7 weeks showed an attenuation of age-associated high plasma triglyceride, glucose and insulin concentrations under fasting conditions and to have suppressed the increase in the HOMA-IR index and an inhibition in the decrease of muscular p-Akt/Akt protein

in ageing rats. This suggested an improvement of systemic and muscular insulin sensitivity. 6-gingerol increased the plasma and adipose tissue adiponectin concentrations, improved muscular AdipoR1 expression and activated downstream AMPK phosphorylation and upregulated PGC-1α *in vivo* and *in vitro*.

2.3.6 Cardiovascular pharmacological effects of 6-gingerol

Zhang *et al.* (2019) tested 6-gingerol for the attenuation of Ischemia-Reperfusion (I/R) induced cell apoptosis in human AC16 cardiomyocytes. I/R induced the increase in the apoptosis and reactive oxygen species level in AC16 cardiomyocytes. The administration of 6-gingerol decreased cardiomyocyte apoptosis and improved oxidative stress indexes. 6-gingerol also inhibited I/R-induced HMGB2 expression upregulation, JNK activation and reduced the Cleaved Poly (ADP-ribose) polymerases (PARP) and Caspase-3 expression. The treatment with HMGB2 mimicked the effect of I/R-induced cell damage, that was reversed by the treatment with 6-gingerol. The transcriptional activity of NF-κB was reduced in cells treated with 6-gingerol. This indicates that administration of 6-gingerol protected I/R induced cardiomyocytes apoptosis via JNK/NF-κB pathway in the regulation of HMGB2.

2.3.7 Anti-hemorrhagic properties of ginger

Ohnishi *et al.* (2019) investigated the interaction of shogaol and gingerol with Kelch-like ECH-associated protein (Keap)1. Shogaol and gingerol significantly increased heme oxygenase (HO)-1 protein levels in cultured microglia at different concentrations without cytotoxicity. The release of the inflammation marker nitric oxide was suppressed by shogaol. The α, β-unsaturated carbonyl of shogaol interacted with Keap1 and not that of gingerol. The usefulness of α, β-unsaturated carbonyl for intracerebral hemorrhage (ICH) therapy was investigated with shogaol on an *in vivo* mouse ICH model. 0.2 nmol shogaol when administered as an intracerebroventricular injection there was an increase in striatal HO-1 protein

levels and rescued the ICH-induced neuron loss. Thus for compounds, such as shogaol the α, β -unsaturated carbonyl is necessary for the interaction with Keap1.

2.4 Quality of ginger as influenced by nutrient management practices

Guchhait *et al.* (2005) reported that application of Zinc resulted in maximum dehydrogenase, protease, alkaline phosphatase and urease activity in the rhizosphere soil vis-à-vis yield of ginger.

Majumdar *et al.* (2005) recorded that the application of farmyard manure significantly increased the crude protein and oleoresin content of ginger by 12.3 and 6.3 per cent respectively. The interaction effect of potassium and farmyard manure was non- significant for both crude protein and oleoresin content of ginger. Maximum value of crude protein of 8.90 per cent and oleoresin content of 9.65 per cent were recorded with 200 kg K_2O and five tonnes of farmyard manure per hectare application in ginger.

Singh and Dwivedi (2007) recorded that the plant growth as well as rhizome yield of ginger increased upon soil application of zinc sulphate at 10kg/ha and spraying of 0.5 per cent Zinc sulphate or two sprays of one per cent ferrous sulphate at 45^{th} and 55^{th} days after sowing.

Jyotsna *et al.* (2012) analysed the quality of few ginger varieties Manipur Local, Bhaisey, Gorubathan and Nadia that were organically grown under rainfed condition in Manipur. Bhaisey was found to have good quality rhizome with high specific gravity of 1.25 g cc^{-1}, 20.4 per cent of dry matter and 5.12 per cent of oleoresin content.

Seyie *et al.* (2013) conducted an experiment to study the effect of macronutrients nitrogen (N), phosphorus (P) and potassium (K) on the growth, yield and quality of ginger in the cultivars Akya Local and Suprabha. The

treatments included N at 100, 125 kgha^{-1}, P at 40, 60 kgha^{-1}and K at 40 and 60 kgha^{-1}. The highest yield of fresh ginger oil and oleoresin was recorded as 1.52 per cent and 6.2 per cent respectively at 125:60:60 kgha^{-1} of N, P and K respectively.

An experiment to study the effect of organic soilless substrates on 6-gingerol content in ginger was studied by Suhaimi *et al.,* 2018. The various treatments included 100 per cent coir dust, 100 per cent burnt paddy husks, 70 per cent coir dust + 30 per cent burnt paddy husk, 30 per cent coir dust + 70 per cent burnt paddy husks, 50 per cent coir dust + 30 per cent burnt paddy husk and no significant difference between the treatments could be found with respect to 6-gingerol content.

Srinivasan *et al.* (2019) conducted field experiments from 2007 to 2016 with different nutrient management practices in ginger under rainfed condition and analysed the quality of rhizomes. The essential oil content ranged between 1.0 and 1.7 per cent for organic nutrient management (ONM) and chemical nutrient management (CNM). Integrated nutrient management (INM) yielded 1.32 to 4 per cent of essential oil content. Pinene, d-camphene and b-phellandrene contents were present in a higher quantity in CNM, higher amounts of b-citral (neral) and citronellol were present in ONM and a-citral (geranial) was found in higher quantities in INM.

2.5 Spraying of elicitors to improve quality

Spray of methyl jasmonate and salicylic acid was also found to affect the chemical components of ginger and other medicinal plants.

2.5.1 Spraying of elicitors to improve quality of ginger

Ghasemzadeh *et al.* (2012a) affirmed that ginger plants treated with salicylic acid and with CO_2 enrichment exhibited highest CHS enzyme activity

whereas the plants not treated with salicylic acid (400 and 800 µmol mol^{-1}) and kept under ambient CO_2 conditions showed the lowest CHS enzyme activity.

Ghasemzadeh *et al.* (2012a) added that salicylic acid enhanced the CHS enzyme activity in ginger and recorded the highest activity value of 5.77 nkat/mg protein in Halia Bara with the 10^{-5} M salicylic acid treatment.

Ghasemzadeh *et al.* (2012b) added that application of an increased level of salicylic acid from 10^{-5} M to 10^{-3} M, attributed to the increased production of total flavonoids and decreased the synthesis of total phenolics and also induced the production of total soluble carbohydrate content.

2.4.2 Spraying of elicitors to improve quality in other spice crops

Elwan and El-Hamahmy (2009) investigated the effect of salicylic acid as foliar spray at 10^{-6} and 10^{-4} M on pepper and found that those pepper seedlings treated with 10^{-6} M increased the vitamin C content, carotenoid content considerably as compared to the control.

The influence of weekly and the day before harvest foliar sprays of the elicitors hydrogen peroxide, salicylic acid and xyloglucan oligosaccharide on the concentration of capsiate component in the fruits of pepper was studied by Zunun-Perez *et al.* (2017). As compared to the control, hydrogen peroxide at 200 mM displayed the largest increase in capsiate content by 134 per cent followed by 400 mM hydrogen peroxide with an increase of 75 per cent, xyloglucan at 6 ppm exhibited only 22 per cent increase in capsiate content. Salicylic acid showed only a minor increase of 6 per cent in the capsiate content in the day before harvest foliar sprays at 0.1 mM, 1 mM and 1 mM weekly foliar sprays.

The effects of different concentrations of salicylic acid on two main components crocin and safranal obtained from saffron were studied by Tajik *et al.*

(2015).The Saffron corms were treated with salicylic acid at 0.01 mM, 0.1 mM and 1 mM for 12 hours followed by the planting in pots and irrigation with Hoagland nutrient solution twice a week. The flowers were collected daily during the early hours of the day and dried. The dried stigmas were then used for HPLC analysis of carotenoid compounds. Salicylic acid showed a positive effect on the crocin content. The corms treated with 1 mM salicylic acid showed the highest quantity of crocin of 25.27 mg per gram of dry weight. The safranal content was found to be reduced in corms treated with 1 mM salicylic acid as compared to the control.

Manoj *et al.* (2017) studied the influence of spraying various elicitors such as salicylic acid, methyl jasmonic acid, chitosan, ethephon, potassium silicate and potassium phosphate at two concentrations (50 ppm and 100 ppm) at 120, 150 and 180 DAP on turmeric (*Curcuma longa*). The highest curcumin content was recorded in rhizomes obtained from the plants treated with chitosan @ 100 ppm which was on par with the plants treated with salicylic acid @ 100 ppm. Also curcumin content was found to have increased significantly with the application of all the elicitors at both the concentrations (50 ppm and 100 ppm), except for methyl jasmonic acid @ 100 ppm. However all the treatments recorded higher curcumin content as compared to the control.

2.4.3 Spraying of elicitors to improve quality of medicinal plants

Wei *et al.* (2010) affirmed that methyl jasmonate induced and coordinated the suppression of key enzyme, Chalcone synthase in flavonoid biosynthesis in *Scutellaria viscidula.*

The effects of exogenous application of salicylic acid at concentrations 200 and 400 ngl^{-1} was studied in *Salvia macrosiphon* by Rowshan *et al.* (2010). The plants were treated with the elicitor during early flowering stage. When the components of essential oils were studied linalool, hexyl isobutanat was found to have increased to 41.37 and 5.6 per cent respectively in treatment 400 mgl^{-1}

salicylic acid. The ɗ-cadinen was found to have decreased in treatment 400 mgl^{-1} salicylic acid. The yield of essential was about 0.48 per cent (400 mgl^{-1} salicylic acid) and that of the control was only 0.23 per cent.

The essential oil obtained from *Achillea millefolium* Boiss. is extensively used in traditional medicine. The application of salicylic acid at 200, 400 mgl^{-1} in the earlier stages of flower development caused a considerable increase composition of essential oils. The salicylic acid application at 400 mgl^{-1} increased 1,8-cineol (5.9%), β- caryphyllene (2.6%), and spathulionl (6.2%). The salicylic acid application at 200 mgl^{-1} showed increased Ar-curcumene (2.5%). There was a decrease in sabenin, comphore and α-bisabolene contents. The compounds iso-spathulinol, γ-cadinene, β-himachalene, and trans-caryophyllen were found only in salicylic acid treatment (Rowshan and Bahmanzadegan, 2013).

Awasthi *et al.* (2016) analysed the total flavonoid content from the different tissues of *Coleus forskohli,* an important medicinal herb. They treated the elicitors salicylic acid at 1 mM, methyl jasmonate at 100 µM, abscisic acid at 50 µM and 2, 4-dichlorophenoxyacetic acid at 50 µM on four weeks old herb. It was found that the leaves and roots of the plants treated with 100 µM methyl jasmonate showed a significant increase in the total flavonoid content when quantified in a spectrophotometer at 510 nm.

2.5 Influence of growing conditions on quality of ginger

Wilson and Ovid (1993) conducted a field level experiment so as to study how shade and fertilizer application affected the growth of and yield of ginger. The height of the plants and the number of tillers were more in plants under shade but there was no significance of fertilizers on the yield.

The extracts of leaves and rhizomes of the ginger varieties Halia Bara and Halia Bentong raised under zero and 60 per cent shade when examined in HPLC

revealed that the most abundant phenolic acid in ginger was gallic acid and the flavonoids were quercetin and apigenin The accumulation of flavonoids in the leaves was found to be high in the plants raised under 60 per cent shade and the accumulation of phenolics was recorded in rhizomes that was obtained from zero per cent shade (Ghasemzadeh and Ghasemzadeh, 2011).

The ginger varieties Halia Bentong and Halia Bara were found to have increased phenolic compounds and flavonoids when the amount of CO_2 to which the plants were exposed changed from 400 μmol mol^{-1} to 800 μmol mol^{-1}. The flavonoid compounds detected were the kaempferol and fisetin. Gallic acid and vanillic acid were the phenolic compounds present (Ghasemzadeh *et al.*, 2010).

An increased level of carbon di oxide (CO_2) at 800 $\mu mol \cdot mol^{-1}$ caused a significant increase in anthocyanin, rutin, naringenin, myricetin, apigenin, fisetin and morin contents in ginger leaves. The combination of salicylic acid and CO_2 increased anthocyanin and flavonoid production as compared to the effects of individual treatments. The plants exposed to elevated CO_2 level at 800 $\mu mol \cdot mol^{-1}$and salicylic acid showed high anthocyanin content and chalcone synthase (CHS) activity. The plants neither treated with salicylic acid nor exposed to elevated CO_2 conditions showed the lowest activity of CHS (Ghasemzadeh *et al.*, 2012a).

2.5.1 Influence of growing conditions on quality of turmeric

Padmapriya *et al.* (2007) conducted field experiments to study the effects of shade and integrated nutrient management on the quality of turmeric rhizomes. The highest curcumin content of 5.57 per cent and essential oil content of 5.68 per cent was recorded in the treatment 50 per cent farmyard manure + coir compost + *Azospirillum* (10 kg ha^{-1}) + phosphobacteria (10 kg ha^{-1}) + 3 per cent Panchagavya raised under shade.

2.6 Quantification of metabolite using HPLC

Chen *et al.* (1986) isolated shogaols from gingerols of ginger (*Zingiber officinale* Rosc.) by thin layer chromatography (TLC) and fractionated by high performance liquid chromatography (HPLC). This led to the identification of shogaol components such as cis- and trans-, 6-shogaol, 8-shogaol, 10-shogaol, 12-shogaol and syn- and anti- methyl-6-shogaol, methyl-8-shogaol, methyl-10-shogaol. This was identified based on the results of HPLC, fast atom bombardment-mass spectrometry, gas chromatography, gas chromatography-mass spectrometry and Nuclear Magnetic Resonance (NMR) spectroscopy.

The rhizomes of fresh ginger and dry ginger were extracted with acetone and the obtained oleoresin was then subjected to HPLC on a reverse phase column (RP-18). The total gingerol (constituting 6-gingerol, 8-gingerol, 10-gingerol) of fresh ginger rhizomes was found to be between 0.65-0.88 per cent while that of dry ginger was between 1.10-1.56 per cent (Chen *et al.*, 1986).

The pungent principles of West Indian ginger were analysed for quality and quantity using HPLC. The rhizomes of fresh ginger, solar dried ginger and solar dried cum steam distilled ginger were analysed for pungency. The yield of total oleoresin obtained was high in fresh ginger as compared to solar dried ginger and solar dried cum steam distilled ginger. The 6-gingerol content of the rhizomes was also high in fresh ginger for the West Indian ginger as suggested by Balladin *et al.* (1998).

He *et al.* (1998) successfully employed HPLC-UV electrospray MS to identify the pungent principles in ginger extract from the chromatogram. The pungent compounds were identified as 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, 8-shogaol, 10-shogaol and 6-gingediol. A few other minor compounds were also identified as gingerol analogues.

Jiang *et al.* (2005) characterised the gingerol compounds in ginger by HPLC/electrospray ionization MS (LC/ESI-MS). They could identify about 31 gingerol related compounds from the methanolic extracts of fresh ginger rhizomes. Of which three were found to be new compunds. Thus LC/ESI-MS is a powerful online tool for the identification of those thermo-labile gingerol related compounds which cannot be detected by GC/MS.

Rai *et al.* (2006) developed a high performance thin layer chromatography (HPTLC) method to estimate the amount of 6-gingerol from the rhizomes of ginger. The retention factor was 0.40. The 6-gingerol content varied from 57.91 to 62.97 mg per gram of ginger extract. This method also showed better resolving of 6-gingerol from the ginger extract.

Peerapak and Tongchitpakdee (2010) studied the effect of hot air and freeze-drying methods on 6-gingerol content, total phenolic content and antioxidant capacity of ginger. The 6-gingerol content when analyzed using High Performance Thin Layer Chromatography (HPTLC), showed that 6-gingerol content of ginger dried using both methods were not significant. However, the total phenolic content and anti-oxidant capacity was higher in hot air dried ginger rhizomes.

Pawar *et al.* (2011) quantified the 6-gingerol content from the rhizomes of twelve ginger cultivars from various agro-climatic zones of India. Reverse Phase-High Performance Liquid Chromatography (RP- HPLC) was employed to quantify 6-gingerol content. It ranged from 0.1 to 0.2 per cent. The cultivar with high 6-gingerol content showed the strongest free radical scavenging activity.

Zhang *et al.* (2012) developed an HPLC method in order to identify and estimate the amounts of zingerone, 6-gingerol, 8-gingerol, 6-shogaol, 10-gingerol in fresh, dried, roasted, and charry ginger rhizomes. It was found that the 6-gingerol, 8-gingerol and 10-gingerol contents decreased gradually from fresh ginger to roasted ginger, dried ones and charry ginger. The 6-shogaol content

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increased in the same sequence. Zingerone was found in roasted ginger and its content increased in charry ginger.

Rafi *et al.* (2013) developed reversed phase capillary liquid chromatography method for determining the pungent principles 6-gingerol, 8-gingerol, 10- gingerol, and 6-shogaol. The separation of these compounds was done using C30 as stationary phase and 60 per cent acetonitrile as mobile phase. All these principle separated within 25minutes with good resolution.

Mishra *et al.* (2013) estimated the gingerol content from ginger powder under different brand names in the market. The method used for the estimation of gingerol was Simple reversed-phase HPLC-UV method, with gradient elution. The amount of gingerol in the sample S1, S2, S3, S4, S5 and S6 were estimated to be 04.54, 08.01, 06.74, 04.20, 06.74 and 08.54 per cent respectively by HPLC analysis. Among the samples, S6 (ginger cultivated through organic farming) was found to have highest quantity of gingerol than the other market samples.

A total of 27 compounds were identified from four kinds of ginger samples such as fresh ginger, dried ginger, stir-frying ginger and carbonized ginger. Five main constituents (zingerone, 6-gingerol, 8-gingerol, 6-shogaol and 10-gingerol) in these fresh ginger, dried ginger, stir-frying ginger and carbonized ginger sample extracts were simultaneously determined by Ultra-Performance Liquid Chromatography- Photo Diode Array (UPLC-PDA) (Li *et al.*, 2016).

Sreeja (2017) studied the pungency principles in ginger at various growth stages using High Performance Liquid Chromatography (HPLC). The pungency principles accumulated in ginger rhizome at the rhizome formation stage, at five months after planting.

The aim of the study conducted by Mian *et al.* (2019) was to evaluate and standardize the parameters that determine the quality and purity of ginger rhizomes.

Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC), fluorescence analysis were some among the methods used for the standardization. Thin Layer Chromatography of ginger showed five and thirteen spots when exposed to UV short and anisaldehyde-sulphuric acid respectively. A total of 29 peaks were seen in HPLC and the peaks numbers 2, 7 and 5 with retention times 2.66, 3.25 and 3.21 minutes produced an area concentration of 9.64, 10.12 and 15.22 per cent respectively. These three peaks were identified as the major ones.

2.7 *Chalcone synthase* gene expression studies

The expression of genes involved in the pathways responsible for the biosynthesis of major bioactive compounds differed between tissues such as matured ginger rhizomes, young ginger rhizomes and fibrous roots. The pathways for the synthesis of non-volatile oils such as gingerols, and diarylheptanoids are the "terpenoid backbone biosynthesis" and "stilbenoid, diarylheptanoid and gingerol biosynthesis". The *de novo* transcriptome sequencing generated a total of 361,876 unigenes, with an average length of 515.89 bp. Pairwise comparisons of unigenes showed that the unigenes' expression pattern were similar between matured and young ginger rhizomes, but different between ginger rhizomes and fibrous roots. Most of the unigenes mapped in these pathways expressed significantly higher in fibrous roots than in young and matured ginger rhizomes. This concludes that bioactive compounds in ginger is primarily synthesized in fibrous roots and then transported to rhizomes and accumulated (Jiang *et al.*, 2017).

Beritognolo *et al.* (2002) studied the expression of *CHS* gene that was associated with flavonol accumulation in *Juglans nigra* at heartwood formation stage. The flavonol accumulation was found to have correlated with the *CHS* transcript levels. Also the accumulation of *CHS* proteins was found upon western immunoblotting suggesting the accumulation of flavonols in *Juglans* nigra undergoing transformation to heartwood.

Manaf (2013) reviewed the *chalcone synthase* genes isolated and characterized from *Ginkgo biloba*, *Scutellaria viscidula*, and *Paeonia suffruticosa*. The cDNA of the chalcone synthase gene obtained from *Ginkgo biloba*, *Scutellaria viscidula*, and *Paeonia suffruticosa* were 1608, 1649 and 1475 bp respectively. Out of which only 1173, 1170 and 1185 bp was found to be open reading frame (ORF). All the three chalcone synthase genes were designated as *GbCHS*, *SvCHS* and *Ps-CHS1* respectively. They were found to be homologous with *chalcone synthase* gene from other plants. *GbCHS* and *Ps-CHS1* showed expression in different tissues at varying levels.

Ghosh and Mandi (2015) studied *Chalcone synthase* gene expression carried out in landraces of ginger revealed that the landraces with specific amino acids change from aspargine to serine caused low 6-gingerol content as asparagine is one of the critical amino acids of the catalytic triad of *Chalcone synthase* gene.

Feng *et al.* (2015) performed cloning and expression analysis of *CHS* gene in *Lamiophlomis rotata*. The full length *CHS* gene encoding cDNA was cloned from *Lamiophlomis rotata*. It was found that *LrCHS* was 2026 bp with an open reading frame of 1176 bp encoding for 391 amino acids containing protein. The *LrCHS* gene showed higher expression in flowers and leaves at flowering stage. However the total flavonoid content was found to be in agreement with *CHS* gene expression pattern during organ development stage of *Lamiophlomis rotata*. Also the gene expression co-ordinated with the flavonoid content upon methyl jasmonate elicitation between 6 and 18 hours.

Conrad and Mathabatha (2016) isolated the cDNA from the flowers of *Clivia miniata* which showed the presence of three unigenes *CmiCHS* 11996, *CmiCHS* 43839 and *CmiANS* of 933, 951 and 983 bp respectively. The *CHS* gene corresponded to 390 amino acids containing protein and *ANS* gene corresponded to 355 amino acid containg protein. The expression pattern of these genes in different

tissues as well as the tepals (orange and yellow flower) were analysed using the real-time quantitative PCR. The expression of *CmiCHS* and *CmiANS* were higher in tepals than other tissues (leaves, style and stigma and scape). These pattern of gene expression in the tissues corresponded to the of anthocyanin accumulation. Thus the orange and yellow colour pigments certainly related to the regulation of chalcone synthase and anthocyanin synthase.

Sreeja, (2017) studied the *Chalcone synthase* gene expression in the ginger somaclone B3 using the cultivar Maran as the control. The somaclone B3 showed 54 per cent increase in the expression of *Chalcone synthase* gene than that of Maran. She also performed suppression subtractive hybridization (SSH) so as to identify the differentially expressed genes in both somaclone B3 and Maran. A total of 25 and 19 Expressed Sequence Tags (ESTs) could be identified from the rhizome and leaf. Genes involved in the production of gingerol was not present in rhizome ESTs. The leaf ESTs when analysed for differentially expressed genes it was found that *3-ketoacyl CoA thiolase* (ACAA1) gene which is involved in the gingerol biosynthetic pathway was differentially expressed in somaclone B3. The higher expression of ACAA1 gene attributed to the increased gingerol content in somaclone B3.

Singh *et al.* (2019) cloned and sequenced the full length cDNA sequence of the genes *Chalcone synthase* and *Chalcone isomerase* from *Phyllanthus emblica* and were designated as *PeCHS* and *PeCHI* respectively. They were 1514 and 843 base pairs long nucleotides respectively. They were found to contain 390 and 209 amino acids and coded for the enzymes PeCHS and PeCHI respectively. The expression analysis of the genes was studied from the leaf, flower and fruit. *PeCHS* expressed the highest in mature fruit and *PeCHI* expressed the maximum in young leaves. *PeCHI* expression lowered initially and then increased as the fruit matured and *PeCHS* expression gradually increased as the fruits developed.

Zhang *et al.* (2019) isolated and analysed three *Chalcone synthase* genes from *Carya illinoinensis* designated as *CiCHS1*, *CiCHS2* and *CiCHS3.* The genes were found to have expressed at higher levels in various tissues and also during the initial stages of development of the Pecan kernels. The variation in the expression of the genes *CiCHS2* and *CiCHS3* caused changes in the phenolic content.

Hu *et al.* (2019) overexpressed *Chalcone synthase* gene in *Nicotiana tabacum* and found that the transgenic tobacco plants were more tolerant to drought stress compared to the control plants. The levels of expression of genes involved in flavonoid pathway and the flavonoid contents were also high in the transgenic tobacco plants. Malondialdehyde, product of oxidative stress was found in lower concentration upon the over-expression of *Chalcone synthase* gene. This was hence identified as the candidate gene to enhance drought tolerance in plants via genetic engineering.

Materials and Methods

3. Materials and methods

The research work entitled "Expression of *Chalcone synthase* gene in ginger (*Zingiber officinale* Rosc.) as influenced by various management practices" was carried out at Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture, Kerala Agricultural University, Thrissur during 2017 to 2019. The objectives of the study were to analyse the influence of nutrient management and spraying of elicitors on *Chalcone synthase* gene expression in ginger under polyhouse and open conditions.

3.1 MATERIALS

3.1.1 Plant Materials

The KAU released ginger variety Karthika, received from the germplasm maintained at Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture, Kerala Agricultural University was used as the experimental material for the study. The variety was developed from the local cultivar Maran. In India, Karthika and Athira varieties of ginger are the first of its kind to be exploited from somaclonal variation. The variety Karthika was found to yield the highest gingerol content of about 21.3 per cent (Shylaja *et al.*, 2010).

3.1.2 Laboratory chemicals and glasswares

Pure and certified chemicals obtained from firms like Merck India Ltd., HIMEDIA, and Sigma Aldrich Pvt. Ltd. were used in the present study. The chemicals DEPC and Trizol were obtained from Sigma-Aldrich. The primers specific to the genes *Chalcone synthase* and *Actin* were supplied by Sigma Aldrich Pvt. Ltd. The components for PCR *viz.*, *Taq polymerase*, dNTPs, Taq buffer, $MgCl_2$, DNA ladder were obtained from GeNei Pvt. Ltd. RevertAid first strand cDNA synthesis kit was supplied by ThermoScientific and SYBR® Premix Ex

Taq™ (Tli RNaseH Plus) for Real Time PCR was provided by TaKaRa. The plastic wares were procured from Axygen, Tarsons India Ltd. and glass wares used were that of Borosil and Vensil. The solvents such as acetonitrile, orthophosphoric acid and methanol used for HPLC analysis were of HPLC grade and obtained from Merck Scientific Private Limited. The standards used for HPLC analysis were of HPLC grade and it composed of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shagaol which were supplied by Sigma Aldrich Pvt. Ltd.

3.1.3 Laboratory equipment and facilities

The study was carried out using the high tech polyhouse, molecular biology laboratory and metabolomics laboratory facilities available at CPBMB, College of Horticulture. The instruments such as electronic balance (Shimadzu), pH meter (EuTech Instruments PC 510), micropipettes (Eppendorf), icematic (F100 compact), high speed refrigerated centrifuge (KUBOTA 6500), thermocycler (ProFlex PCR System by Life Technologies) were used for the research work. Nanodrop spectrophotometer (NanoDrop ND-1000) was used for the quantity analysis of RNA. The quality of RNA was analysed using gel electrophoresis unit (BioRad) and the agarose gels were visualized using Gel documentation unit (BioRad Gel DocTM XR+). The quantitative gene expression analysis was carried out by Real-Time PCR system (Applied Biosystems 7300). High performance liquid chromatography (Shimadzu Prominence *i*) was used for the estimation of gingerol.

3.2 METHODS

3.2.1 Raising of ginger and imposing treatments

3.2.1.1 Raising of ginger

The study was carried out at the Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture, Vellanikkara from 2017 to 2019. The experiment was carried under out in completely randomized design.

A. Plants at 210 days after planting inside polyhouse
B. Plants at 120 days after planting inside polyhouse

Plate 1. General view of the experiment inside polyhouse

The plants were raised under two situations, in high tech polyhouse available at CPBMB and in open conditions. The following treatments were imposed.

T1- Nutrient management as per KAU package of practices (control)

T2- Soil test based nutrient management

T3- Adhoc organic package of practices (KAU)

T4- PoP nutrient management (KAU) + salicylic acid foliar spray (100 µM)

T5- PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

The experiment was carried out in completely randomized design with six growbags per treatment and there were four replications. There was a total of 120 growbags each under the polyhouse and open conditions.

3.2.1.2 Seed rhizomes

The seed rhizomes of ginger were obtained from CPBMB. The healthy and disease free rhizomes were treated with 2 ml l^{-1} and 3 g l^{-1} of quinolphos and zineb respectively for 30 minutes, shade dried and stored. These rhizomes were used as planting material for the experiment. The fresh and healthy rhizomes were treated with *Pseudomonas fluorescens* at 20 g l^{-1} for 30 minutes and shade dried and used for imposing organic management practices as per Adhoc organic PoP. Seed bits of 20 g with at least two viable buds were selected as planting material and planted in each growbag.

3.2.1.3 Preparation of potting mixture

The potting mixture was prepared using sand, soil, cow dung and coir pith in the ratio 1:1:1:1. About 500 g of the potting mixture was given for nutrient analysis at Radio Tracer Lab, College of Horticulture. The potting mixture was filled in the growbags of size 40 cm x 24 cm x 24 cm @ 13 kg per growbag.

3.2.1.4 Preparation of abiotic elicitors

3.2.1.4.1 Preparation of control spray

Double autoclaved dissolved water and tween 20 (0.1 per cent) was used as control spray solution (Elwan and El-Hamahmy, 2009).

3.2.1.4.2 Preparation of salicylic acid elicitor spray

Salicylic acid was dissolved as reported by Elwan and El-Hamahmy (2009) in few drops of dimethyl sulfoxide. The volume was made up using distilled water and the pH was set at 7.0 using 1.0 N KOH. Tween 20 was used as a sticking agent @ 0.1 per cent. The volume was made up to 500 ml using required amount of double autoclaved distilled water.

3.2.1.4.3 Preparation of methyl jasmonate elicitor spray

Methyl jasmonate was dissolved in 0.8 per cent ethanol as per Boughton *et al.* (2005) and the volume was made up using double autoclaved distilled water. Tween 20 was used as a surfactant at 0.1 per cent (Elwan and El-Hamahmy, 2009).

3.2.1.5 Nutrient management as per PoP (KAU)

The N, P, K requirement for ginger as per PoP is 75:50:50 kg/ha. Urea, Rajphos and Muriate of potash (MoP) were used as the nitrogenous, phosphatic and potassic fertilizers respectively. The full dose of P and 50 per cent of K was applied as basal on the day of sowing at the rate of 3.13 g of Rajphos and 0.52 g of MoP per growbag (13 kg of potting mixture) respectively. The first dose of 50 per cent of N was applied at 45 Days After Planting (DAP) at the rate of 1.019 g of urea per growbag and the remaining 50 per cent each of N and K was applied at 90 DAP at

the rate of 1.02 g of urea and 0.52 g of MoP per growbag respectively (Kerala Agricultural University, 2016). The control sprays were given as foliar sprays in the early hours of the day as followed by Kazemi (2014). The surface of the leaves was ensured to be free of water at the time of spraying. This nutrient management as per PoP (KAU) served as the control treatment (T1).

3.2.1.6 Soil test based nutrient management

Rajphos was applied at 0.40 g per plant as basal dose. Urea was applied at 0.24 g per growbag each at 45 DAP and 90 DAP. MoP was applied at 0.06 g per plant at basal and 90 DAP. As boron was found deficient, borax spray (0.1%) was given as foliar spray from one MAP up to four MAP at weekly intervals. Soil test based nutrient management served as T2.

3.2.1.7 Nutrient management as per Adhoc organic PoP (KAU)

Farmyard manure (FYM), neem cake and *Trichoderma viride* mixture was applied to growbags @ 100 g per growbag at the time of planting. Arbuscular mycorrhizal fungus (AMF) was applied @ 1 g per growbag at the time of planting and *Azospirillum* was applied @ 1.5 g per growbag as basal and at 90 DAP. The organic nutrient management recommended in Adhoc organic PoP of KAU (2017) was followed as treatment T3.

3.2.1.8 Nutrient management as per PoP (KAU) with salicylic acid foliar spray

For the treatment (T4), PoP recommendations of KAU (T1) was followed for nutrient management and salicylic acid (100 µM) was given as foliar spray at weekly intervals from one Month After Planting (MAP) up to four MAP. The elicitor foliar sprays were given in the early hours of the day as followed by Kazemi (2014). The surface of the leaves was ensured to be free of water at the time of spraying.

3.2.1.9 Nutrient management as per PoP (KAU) with methyl jasmonate foliar spray

For the treatment T5 PoP recommendations of KAU (T1) was followed for nutrient management and methyl jasmonate (100 µM) was given as foliar spray at weekly intervals from one Month After Planting (MAP) up to four MAP. The elicitor foliar sprays were given in the early hours of the day as followed by Kazemi (2014). The surface of the leaves was ensured to be free of water at the time of spraying.

3.3 Growth performance of ginger in various treatments

Morphological characters of the plants were recorded at 60, 90 and 120 Days After Planting (DAP). The morphological characters studied included length of the pseudostem, number of tillers per plant, number of leaves per tiller, length, width and area of the last fully opened leaf. The length of the pseudostem, length and width of last fully opened leaf was measured using a scale and recorded in cm. The leaf area was recorded as cm^2.

3.4 Recording of micro-meteorological observations

Micro-meteorological observations such as the maximum temperature, minimum temperature and relative humidity were recorded at 8.30 am and 2.30 pm daily both in open condition and in the polyhouse using a whirling psychrometer from one Month After Planting (MAP) up to harvest. Five readings were recorded each for maximum and minimum temperature both in polyhouse and open conditions and daily mean and monthly mean were worked out.

3.5 Gene expression studies of *Chalcone synthase* using Real Time PCR

The expression of *Chalcone synthase* gene in leaves of different treatments was studied using quantitative real time PCR at the active growth phase of the crop (4 MAP).

3.5.1 Isolation and analysis of total RNA

Total RNA was isolated from the plants of different treatments at four months after planting.

3.5.1.1 Collection of leaves for RNA isolation

The young and tender leaves were collected after 24 hours of elicitation in early morning hours before 8 am. The leaves were cleaned and wiped with RNaseZap and flash frozen. These flash frozen leaf samples were stored at -80^0C deep freezer until isolation of RNA.

3.5.1.1.1 Isolation of total RNA

The procedure given by Sreeja (2017) was followed for the isolation of total RNA from the leaves of ginger plants of various treatments with modifications. The modifications carried out were the addition of polyvinylpyrrolidone (PVP) to the homogenized samples prior to the addition of Trizol (Step 2), reduction of incubation time with Trizol from five minutes to three minutes (Step 3), omission of first and the second incubations of the sample with chloroform, decrease in the duration of centrifuging while washing the pellet with 75 per cent DEPC ethanol at 7500 x g at 4°C from five minutes to three minutes (Step 10), decrease in the

duration of drying the pellet from 30 minutes to about two to three minutes until the region just above the pellet becomes free of water (Step 11).

Reagents

- Liquid nitrogen
- TRI reagent (Sigma-Aldrich)
- Chloroform
- Isopropanol
- 3 M sodium acetate
- DEPC ethanol (75%)
- Nuclease free water

Procedure

1. Hundred milligrams of leaf tissue was ground to a fine powder in a pestle and mortar using liquid nitrogen.
2. A pinch of PVP was added to the powdered sample and mixed thoroughly.
3. One milliliter of Trizol was immediately added to the sample after mixing thoroughly with PVP. The whole sample was mixed with the reagent properly and was transferred to a 1.5 ml microcentrifuge tube. The homogenate was then vortexed thoroughly for about 15 seconds and incubated at room temperature for three minutes. (The tube was incubated by laying it horizontally so as to enhance the surface area.)
4. Two hundred microliters of chloroform was added to tube and the contents were mixed by inversion for 15 seconds.
5. The tube containing the sample was then centrifuged at 12,000 x g for 10 minutes at 4°C.
6. The supernatant was carefully pipetted and transferred to a new 1.5 ml microcentrifuge tube. To this two hundred microliters of chloroform was added and the contents were mixed by inversion for 15 seconds.

7. The contents were then centrifuged at 12,000 x g for 15 minutes at 4°C.
8. The supernatant was then transferred to a 1.5 ml microcentrifuge tube. To this $1/10^{th}$ volume of 3M sodium acetate and equal volume of ice cold isopropanol was added and incubated in freezer for one hour.
9. The contents were centrifuged at 12,000 x g for 10 minutes at 4°C.
10. The supernatant was discarded and the pellet was washed with one milliliter of ice cold 75 per cent DEPC ethanol at 7500 x g for three minutes at 4°C.
11. The ethanol was removed using a pipette and the pellet was dried at room temperature for about two to three minutes until the region just above the pellet becomes free of water.
12. The RNA pellet was then resuspended in nuclease free water of 20 microlitre and stored in -80^0C for prolonged use.

3.5.1.1.2 Quantity analysis of isolated total RNA

The quantity of the isolated total RNA was spectrophotometrically analysed using NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies Inc., USA). One microliter of nuclease free water was used to set the blank to zero before the sample readings were taken. One microliter of the sample was pipetted to quantify and the readings were measured in ng/µl. The absorbance of nucleic acids was measured at wavelengths of 260 nm and 280 nm. The A_{260}/A_{280} ratio of 1.8-2.3 and A_{260}/A_{230} ratio above one indicated the purity of RNA samples.

3.5.1.1.3 Quality analysis of total RNA by agarose gel electrophoresis

The quality of the isolated total RNA was analysed by agarose gel electrophoresis.

Materials required

- Agarose (HIMEDIA)

- 50X TAE buffer (double autoclaved DEPC water)
- Double autoclaved distilled water
- 6X Loading/Tracking dye
- Ethidium bromide solution (0.5 µg/ml)
- Electrophoresis unit (BioRad)
- Power pack (BioRad)
- Casting tray and comb (BioRad)
- Gel documentation and analysis system (BioRad/UV transilluminator)

Procedure

1. One litre of 1X TAE buffer was prepared by diluting 50X TAE stock solution with double autoclaved distilled water. The TAE buffer was used to fill the electrophoresis tank and to prepare the gel.
2. The casting dam, casting tray and comb was cleaned with 70 per cent ethanol and RNaseZap. The casting tray was mounted horizontally over the casting dam such that the free ends were tightened and the comb was placed on the gel tray at one end of the tray at about one centimeter from the ends of the tray.
3. One per cent of agarose gel (1 g in 100 ml) was prepared in a conical flask with 100 ml 1X TAE buffer by boiling in a microwave oven to dissolve the agarose completely.
4. The solution was then cooled to lukewarm temperature, ethidium bromide was added and the solution was mixed thoroughly.
5. The solution was then poured on the gel casting tray along with the comb. The gel was left undisturbed so as to solidify.
6. The gel tray containing the gel and the comb was removed from the casting dam once the gel got solidified.
7. The gel was then placed in the electrophoresis tank containing 1X TAE buffer (with the wells at the cathode end) such that the wells are completely submerged in the buffer.

8. To the wells, around 3-4 µl of RNA (total concentration of 3 µg) samples were mixed with 1 µl 6X loading dye and then loaded into the wells with the help of a micropipette.
9. The cathode and the anode ends were connected to the power pack and the gel was run at a steady voltage of 50 V.
10. The power was turned off when then the tracking dye reached about 2/3rd of the gel.
11. The gel was placed on a gel documentation unit. The bands in the gel were then visualized under UV light and documented.

3.5.2 Reverse Transcription Polymerase Chain Reaction for the synthesis of cDNA

Total RNA consists of rRNA, mRNA, and tRNA. The effective isolation of non-degraded mRNA is very crucial for the first strand synthesis and subsequent amplifications by PCR. The total RNA was reverse transcribed using the First Strand cDNA Synthesis Kit (Thermoscientific), as per the guidelines of the manufacturer. The total RNA was incubated with reverse transcriptase enzyme and oligo dT primer along with RevertAid.

3.5.2.1 First strand cDNA synthesis

The total RNA from the ginger leaves of different treatments was isolated and used for first strand cDNA synthesis. The synthesized single stranded cDNA was used for quantitative Real Time PCR.

Materials required

- Total RNA sample
- Oligo $(dT)_{18}$ primer (100 µM)
- Nuclease free water
- 5X reaction buffer
- RiboLock RNase inhibitor (20 U/µl)

- 10 mM dNTP mix
- RevertAid M-MuLV RT (200 U/µl)

Procedure

A 0.2 ml microcentrifuge tube was taken and placed on ice. To the tube the following reagents were added in the indicated order as per manufacturer's guidelines.

Preparation of first strand cDNA synthesis incubation mix

Component	Volume per reaction (µl)
Total RNA (2 µg/µl)	1.0
Oligo $(dT)_{18}$ primer	1.0
Nuclease free water	up to 12.0

1. The contents were gently mixed, briefly centrifuged and incubated at 65°C for five minutes.
2. The mixture was chilled on ice immediately, spun down in a spinner and again kept on ice.
3. The following components were added to the same tube in the order as mentioned

Preparation of first strand cDNA synthesis reaction mix

Component	Volume per reaction (µl)
5 X Reaction buffer	4.0
RiboLock RNase Inhibitor (20U/ µl)	1.0
10 mM dNTPMix	2.0
RevertAid M-MuL RT (200U/µl)	1.0
Total volume	**20.0**

4. The tube along with the contents were mixed gently and briefly centrifuged in a spinner.
5. The reaction tube was incubated in a thermocycler machine (ProFlex PCR System by Life Technologies) for 60 minutes at 42°C and the reaction was then terminated by heating at 70° C for five minutes.

3.5.3 Confirmation of the synthesis of first strand cDNA

The first strand cDNA was amplified by normal PCR using endogenous gene (*Actin*) and the test gene (*Chalcone synthase*) specific primers.

Details of endogenous control *Actin* gene primers

Orientation	Name of primers	Sequence (5' to 3')	No. of bases
Forward	*Zo Actin* forward	GTGTGAGCCACACTGTGCCTAT	22
Reverse	*Zo Actin* reverse	CAGCAGTGGTGGTGAACGAAT	21

Details of *Chalcone synthase* gene specific primers

Orientation	Name of primers	Sequence (5' to 3')	No. of bases
Forward	*Zo CHS* forward	GGGACACCTGAGGGAAATCG	20
Reverse	*Zo CHS* reverse	CCACCCAAAACACGTCGTTC	20

Procedure

The Master Mix for the PCR reaction was prepared as mentioned below. The reagents were gently spun and properly mixed. The cDNA was added

separately and aliquots of the PCR reaction mix were added to 0.2 ml PCR tubes and the reaction mix was gently spun for proper mixing.

Preparation of PCR Master Mix for first strand cDNA amplification

Components	Volume per reaction (µl)
cDNA	2.0 (2 µg/µl)
10X PCR buffer	5.0
10 mM dNTP Mix	1.0
25 mM $MgCl_2$	2.0
Forward primer	1.0
Reverse primer	1.0
Taq DNA polymerase (3U/µl)	1.0
Nuclease free water	37.0
Total volume	**50.0**

The PCR reaction was carried out with the following thermal profile

Thermal profile for first strand cDNA amplification

Steps	Temperature	Time	No. of cycles
Initial denaturation	95°C	5 minutes	1
Denaturation	95°C	30 seconds	35
Annealing	60°C	30 seconds	
Extension	72°C	30 seconds	
Final extension	72°C	10 minutes	1

The PCR products were stored at -20°C. 20 µl of the PCR product was loaded on 1.8 per cent agarose gel.

3.5.3.1 Quality analysis of the amplified cDNA by agarose gel electrophoresis

Materials required

- Agarose (HIMEDIA)
- 50x TAE buffer (pH 8.0)
- 6X Loading/Tracking dye
- Ethidium bromide solution (0.5 µg/ml)
- Electrophoresis unit (BioRad)
- Power pack (BioRad)
- Casting tray and comb (BioRad)
- Gel documentation and analysis system (BioRad/UV transilluminator)

Procedure

1. One litre of 1X TAE buffer was prepared by diluting 50X TAE stock solution with double autoclaved distilled water. The TAE buffer was used to fill the electrophoresis tank and to prepare the gel.
2. The casting dam, casting tray and comb were cleaned with 70 per cent ethanol. The casting tray was mounted horizontally over the casting dam such that the free ends were tightened and the comb was placed on the gel tray at one end of the tray at about one centimeter from the ends of the tray.
3. 1.8 per cent of agarose gel (1.8 g in 100 ml) was prepared in a conical flask with 100 ml 1X TAE buffer by boiling in a microwave oven to dissolve the agarose completely.
4. The solution was then cooled to lukewarm temperature, ethidium bromide was added and the solution was mixed thoroughly.
5. The solution was then poured on the gel casting tray along with the comb. The gel was left undisturbed so as to solidify.
6. The gel tray containing the gel and the comb was removed from the casting dam once the gel got solidified.

7. The gel was then placed in the electrophoresis tank containing 1X TAE buffer (with the wells at the cathode end) such that the wells are completely submerged in the buffer.
8. To the wells, around 20 µl of the PCR product mixed with 2 µl 6X loading dye and then loaded into the wells with the help of a micropipette.
9. The cathode and the anode ends were connected to the power pack and the gel was run at a steady voltage of 80 V.
10. The power was turned off when the tracking dye reached about 2/3rd of the gel.
11. The gel was placed on a gel documentation unit. The bands in the gel were then visualized under UV light and documented.

3.5.4 Real time PCR assay for *Chalcone synthase* gene expression analysis

The *Chalcone synthase* (*CHS)* gene expression analysis (test gene) was performed using Real-Time PCR. *Actin* gene was used as endogenous (control) gene.

Materials required

- SYBR® Premix Ex Taq^{TM} (Tli RNaseH Plus) (2X)
- ROX Reference Dye (50X)
- cDNA template
- Forward and reverse primers
- Nuclease-Free water
- 96 well plates and the seals for the plate

3.5.4.1 General precautions followed when performing Real-Time PCR assay

- The contents of SYBR® Premix Ex Taq^{TM} (Tli RNaseH Plus) 2X and ROX Reference Dye (50X) was briefly centrifuged before use to ensure adequate reactivity

- The cDNA samples and primers were gradually thawed down and vortexed gently for proper mixing
- Exposure of SYBR Green Master Mix to direct light was avoided as it is sensitive to light
- In order to avoid contamination between the samples fresh disposable tips were used

3.5.4.2 Baseline and threshold value setting

The initial cycle of the PCR reaction which shows a slight change in the fluorescent signal is generally regarded as the baseline. A numerical value was assigned for each run. This corresponds to a statistically significant point over the calculated baseline and the value is considered as the threshold value. The point of measurement at the baseline and threshold level should be exactly determined as this corresponds to the amount of the target gene with in a reaction. By default the Applied Biosystems 7300 sequence detection software system was set from three to fifteen (3-15) cycles.

3.5.4.3 Real Time PCR reaction mix and temperature profile for the reaction

The Real Time PCR reactions for the test gene (*Chalcone synthase*) and the endogenous housekeeping gene (*Actin*) were carried out using the first strand cDNA obtained from the RNAs isolated from the leaves of various treatments. The master mix was prepared as follows and spun down properly to ensure proper mixing of the components. The cDNA samples were loaded carefully on a 96 well plate and the master mix was added separately and mixed gently by pipetting.

Preparation of PCR Master Mix for Real Time PCR

Components	Volume (µl)
SYBR® Premix Ex Taq™ (2X)	10.0
PCR Forward Primer (10µM)	0.4
PCR Reverse Primer (10µM)	0.4
ROX Reference Dye (50X)	0.4
Template (<100 ng)	2.0
Sterile distilled water	6.8
Total volume	**20.0**

Thermal profile setting for Real Time PCR to study *Chalcone synthase* gene expression

S.No.	Stage	Repeats
1.	**Initial denaturation** 95^0C for 30 seconds	1
2.	**PCR** 95^0C for 15 seconds 60^0C for 31 seconds	40
3.	**Initial denaturation** 95^0C for 15 seconds 60^0C for 60 seconds 95^0C for 15 seconds	1

3.5.4.3.1 Relative quantification analysis

The most commonly used methods for the analysis of Real Time PCR data are absolute quantification and relative quantification. In absolute quantification, the C_T of the unknown samples is generally compared against a standard curve and with known copy numbers. However in relative quantification the target is compared either by using the standard curve or by comparative C_T method to an internal standard. The fluorescence signals were recorded at each cycle in order to monitor the amplified product generated. The difference in efficiency of amplification was taken into account for the proper calculation of initial target levels. To calculate the fold change expression, the relative quantification with PCR efficiency correction was adopted. The relative expression ratio of the gene was determined by using comparative C_T method as stated by Livak and Schmittgen (2001).

Relative quantification method was employed in this experiment to compare the expression levels of *CHS* gene in various treatments and *Actin* was used as the endogenous house-keeping gene.

3.6 Harvesting of ginger rhizomes and gingerol estimation

Ginger rhizomes from various treatments both in polyhouse and open conditions were harvested at eight months after planting. Fresh weight of rhizomes per plant per treatment and rhizome characters were recorded.

3.6.1 Fresh weight

Fresh weight of the rhizomes was recorded after removing the roots and the mud particles adhering to the rhizomes and expressed as grams.

3.6.2 Rhizome characters

The rhizome characters of ginger rhizomes harvested from various treatment were recorded at the time of harvest. The rhizome characters such as number length, girth and inter-nodal length of primary, secondary and tertiary fingers, thickness of inner core of rhizome, colour of flesh, plumpiness of rhizomes were also recorded.

3.6.3 Preparation of dry ginger

Dry ginger was prepared from rhizomes harvested from various treatments under polyhouse and open conditions. A known weight of the freshly harvested ginger rhizomes were soaked in water overnight. On the following day, the rhizomes were carefully peeled using a bamboo splinter and kept under sunlight for drying in brown paper covers. The rhizomes were dried until two consecutive weights remained the same.

3.6.4 Extraction of oleoresins

The dry ginger was finely powdered. Five grams of the sample was carefully packed in a thimble and oleoresin extraction was done with acetone. About 250 ml of acetone was used per extraction. The extraction was carried out in a soxhlet apparatus till the solvent has turned colourless. The extract was then transferred into a pre-weighed beaker. The solvent was then vacuum evaporated and the final weight of the beaker was noted (AOAC, 1980). The oleoresin recovery percentage was worked out based on the weight of oleoresin recovered from a known quantity of the dry ginger powder.

3.6.5 Estimation of gingerol by High Performance Liquid Chromatography (HPLC)

The quantity of gingerols in rhizomes of various treatments was estimated using HPLC (Shimadzu Prominence *i*) available in the metabolomics laboratory of CPBMB.

3.6.5.1 Reagents for HPLC analysis

The mobile phase composed of acetonitrile, orthophosphoric acid (0.1%) and methanol in the ratio 55:44:1. Methanol: water in the ratio 50:50 was used as the washing buffer. The standards used for comparison of the sample were of HPLC grade and it composed of 6-gingerol (purity=95%), 8-gingerol (purity=95%), 10-gingerol (purity=94%) and 6-shagaol (purity=90%).

3.6.5.2 Sample preparation

The concentration of the oleoresin was made to 5 mg/ml by dissolving in HPLC grade methanol. The samples were transferred to vials by syringe filtration (Manikesh, 2015 and Sreeja, 2017).

3.6.5.3 HPLC conditions

The mobile phase composed of acetonitrile, orthophosphoric acid (0.1%) and methanol in the ratio 55:44:1 respectively. These solutions were membrane filtered and degassed with the help of a sonicator. The experiment was carried out in an isocratic mode. The flow rate of the mobile phase was set at 1.3 ml per minute and the injection volume of the sample was 10 µl. The ambient temperature was set for the column (5 µm C18 silica column of diameter 4.6 x 250 nm) and the Photo Diode Array (PDA) detector at 282 nm was used for the detection.

3.6.5.4 Gingerol estimation

The total gingerol content was calculated from the peak areas corresponding to samples of different treatments and standards of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol.

Results

4. RESULTS

The research work entitled "Expression of *Chalcone synthase* gene in ginger (*Zingiber officinale* Rosc.) as influenced by various management practices" was carried out at Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture, Kerala Agricultural University, Thrissur, from 2017-2019. The results of the study are presented in this chapter.

4.1 Growth performance of ginger in various treatments

Morphological characters such as length of the pseudostem, number of tillers per plant, number of leaves per tiller, length, width and area of the last fully opened leaf of the plants were recorded for all the twenty four plants in each treatment at 60, 90 and 120 DAP both under polyhouse and open conditions and the results are presented in Tables 1, 2 and 3 respectively.

4.1.1 Growth performance of ginger in various treatments at 60 days after planting (Table 1)

4.1.1.1 Length of the pseudostem

At 60 DAP, the length of the pseudostem in different treatments ranged from 39.27 cm to 63.02 cm under polyhouse condition. The treatment T3 (Adhoc PoP organic - KAU) recorded the highest length of pseudostem (63.02 cm) and the treatment was found significantly superior to all other treatments. The treatments T1 (Nutrient management as per PoP- KAU), T2 (PoP Soil test based nutrient management) and T4 (PoP nutrient management, KAU + salicylic acid foliar spray -100 µM) were found on par. The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray -100 µM) recorded the lowest length of pseudostem.

In open condition the length of the pseudostem ranged from 31.0 cm to 36.79 cm at 60 DAP. The treatment T3 (Adhoc PoP organic- KAU)) recorded the

highest length of pseudostem and was on par with the treatments T1 (Nutrient management as per PoP - KAU), T2 (PoP Soil test based nutrient management) and T4 (PoP nutrient management (KAU) + salicylic acid foliar spray - 100 µM). The treatment T5 (PoP nutrient management (KAU) + methyl jasmonate foliar spray- 100 µM) recorded the lowest length of pseudostem.

When polyhouse and open conditions were compared the length of the pseudostem was more in plants of polyhouse in all the treatments studied. Irrespective of the conditions the treatment T3 (Adhoc PoP organic, KAU) was found to be superior to the other treatments studied.

The interaction effects between treatments and the condition (polyhouse and open) were not significant.

4.1.1.2 Number of tillers per plant

The number of tillers per plant ranged from 5.0 to 7.17 in polyhouse condition and 10.67 to 13.42 in open condition at 60 DAP. In polyhouse condition the treatment T1 (Nutrient management as per PoP, KAU) recorded the highest number of tillers per plant and was on par with the treatments T2 (PoP Soil test based nutrient management), T3 (Adhoc PoP organic, KAU) and T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray- 100 µM). The treatment T4 (PoP nutrient management, KAU + salicylic acid foliar spray- 100 µM) recorded the lowest number of tillers per plant.

In open condition, the treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the highest number of tillers per plant and was on par with the treatments T1 (Nutrient management as per PoP, KAU), T2 (PoP Soil test based nutrient management) and T3 (Adhoc PoP organic, KAU). The treatment T4 (PoP nutrient management, KAU + Salicylic acid foliar spray 100 µM) recorded the lowest number of tillers per plant.

Table 1. Morphological parameters recorded for different treatments in polyhouse and open conditions (60 DAP)

Treatment	Length of the pseudostem (cm)			No. of tillers per plant			No. of leaves per tiller			Leaf area (cm^2)		
	Polyhouse	Open	Mean	Polyhouse	Open	Mean	Polyhouse	Open	Mean	Polyhouse	Open	Mean
T1	49.03	32.68	40.86	7.17	12.75	9.96	6.47	7.46	6.965	49.87	54.49	52.18
T2	52.63	35.08	43.86	5.75	13.03	9.39	6.53	5.81	6.17	50.96	57.85	54.41
T3	63.02	36.79	49.91	6.46	12.00	9.23	6.43	6.09	6.26	60.87	60.51	60.69
T4	49.87	34.36	42.12	5.00	10.67	7.84	6.53	6.07	6.30	50.28	56.24	53.26
T5	39.27	31.00	35.14	6.38	13.42	9.90	4.77	5.31	5.04	44.60	51.42	48.01
Mean	50.76	33.98		6.15	12.37		6.15	6.15		51.32	56.10	
Factors	C.D.	SE (m)		C.D.	SE (m)		C.D.	SE (m)		C.D.	SE (m)	
Factor A	5.72	1.97		1.50	0.52		0.90	0.31		7.49	2.58	
Factor B	3.62	1.25		0.95	0.33		0.57	0.20		4.74	1.63	
Factor A X B	N.S.	2.79		N.S.	0.73		N.S.	0.44		N.S.	3.65	

Factor (A) – Treatment

Factor (B) – Condition (Polyhouse and Open)

Factor (A X B) – (Treatment X Condition)

T1- Nutrient management as per PoP (KAU) (control)

T2- PoP Soil test based nutrient management

T3- Adhoc PoP organic (KAU)

T4- PoP nutrient management (KAU) + Salicylic acid foliar spray (100 µM)

T5- PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

But the tiller production was found to be higher in open condition as compared to the polyhouse condition in all the treatments.

The interaction effects between the treatments and the conditions (polyhouse and open) did not show any significant difference with respect to the number of tillers per plant at 60 DAP.

4.1.1.3 Number of leaves per tiller

In polyhouse condition, the number of leaves per tiller was found to vary from 4.77 to 6.53 at 60 DAP. The treatments T2 (PoP Soil test based nutrient management) and T4 (PoP nutrient management, KAU + salicylic acid foliar spray- 100 µM) recorded the highest number of leaves per tiller and was on par with the treatments T1 (Nutrient management as per PoP, KAU) and T3 (Adhoc PoP organic, KAU). The treatment T5 (PoP nutrient management KAU + methyl jasmonate foliar spray - 100 µM) recorded the least number of leaves per tiller.

At 60 DAP, in open condition the number of tillers ranged from 5.31 to 7.46. The treatment T1 (Nutrient management as per PoP, KAU) recorded the highest number of leaves per tiller and was found to be significantly superior to all the treatments studied. The lowest number of leaves per tiller was recorded in the treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) and was on par with the treatments T2 (PoP Soil test based nutrient management), T3 (Adhoc PoP organic, KAU) and T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM).

Irrespective of the conditions, there was no significant difference between the treatments with respect to number of leaves per tiller.

The interaction effects between the treatments and the conditions (polyhouse and open) were not significant with respect to number of leaves per tiller at 60 DAP.

4.1.1.4 Leaf area

The leaf area was found to range from 44.60 cm^2 to 60.87 cm^2 and 51.20 cm^2 to 60.51 cm^2 in polyhouse and open conditions respectively. In polyhouse condition the treatment T3 (Adhoc PoP organic, KAU) recorded the highest leaf area and was significantly superior to all other treatments studied. The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the least leaf area and was on par with the treatments T1 (Nutrient management as per PoP, KAU), T2 (PoP Soil test based nutrient management) and T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM).

In open condition, the treatment T3 (Adhoc PoP organic, KAU) recorded the highest leaf area and was on par with the treatments T1 (Nutrient management as per PoP, KAU), T2 (PoP Soil test based nutrient management) and T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM). The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the least leaf area. Irrespective of the conditions, there was no significant difference between the treatments with regard to leaf area.

4.1.2 Growth performance of ginger in various treatments at 90 days after planting (Table 2)

4.1.2.1 Length of the pseudostem

At 90 DAP, the length of pseudostem ranged from 31.71 cm to 101.79 cm in polyhouse condition. The treatment T3 (Adhoc PoP organic, KAU) recorded the highest length of pseudostem and the treatment was significantly superior to all the

Table 2. Morphological parameters recorded for different treatments in polyhouse and open conditions (90 DAP)

Treatment	Length of the pseudostem (cm)			No. of tillers per plant			No. of leaves per tiller			Leaf area (cm^2)		
	Polyhouse	Open	Mean	Polyhouse	Open	Mean	Polyhouse	Open	Mean	Polyhouse	Open	Mean
T1	84.21	59.52	71.87	11.38	19.38	15.38	9.51	8.34	8.93	83.77	81.29	82.53
T2	88.25	64.94	76.60	8.75	17.79	13.27	8.92	9.78	9.35	82.15	81.69	81.92
T3	101.79	48.83	75.31	9.17	19.04	14.11	10.92	11.90	11.41	91.89	37.59	64.74
T4	63.17	44.82	53.99	9.83	16.59	13.21	8.30	12.09	10.20	57.63	37.71	47.67
T5	31.71	37.94	34.83	8.21	22.02	15.12	4.46	9.10	6.78	25.81	36.61	31.21
Mean	73.83	51.21		9.47	18.96		8.42	10.24		68.25	54.98	
Factors	C.D.	SE (m)		C.D.	SE (m)		C.D.	SE (m)		C.D.	SE (m)	
Factor A	6.25	2.15		N.S.	0.88		1.27	0.44		7.63	2.63	
Factor B	3.95	1.36		1.62	0.56		0.81	0.28		4.82	1.66	
Factor A X B	8.84	3.05		N.S.	1.25		1.80	0.62		10.79	3.72	

Factor (A) – Treatment

Factor (B) – Condition (Polyhouse and Open)

Factor (A X B) – (Treatment X Condition)

T1- Nutrient management as per PoP (KAU) (control)

T2- PoP Soil test based nutrient management

T3- Adhoc PoP organic (KAU)

T4- PoP nutrient management (KAU) + Salicylic acid foliar spray (100 µM)

T5- PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

other treatments studied. The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the lowest length of pseudostem (31.71 cm).

In open condition, at 90 DAP the length of pseudostem ranged from 37.94 cm to 64.94 cm. The treatment T2 (PoP Soil test based nutrient management) recorded the highest length of pseudostem and was on par with the treatment T1 (Nutrient management as per PoP, KAU). The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the lowest length of pseudostem.

The treatments T1 (Nutrient management as per PoP, KAU), T2 (PoP Soil test based nutrient management) and T3 (Adhoc PoP organic, KAU) were on par with respect to length of the pseudostem irrespective of the growing conditions at 90 DAP (37.94 cm).

A significant interaction effect was observed between treatments in polyhouse and open condition. Higher length of pseudostem was recorded in polyhouse for all the treatments except T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM).

4.1.2.2 Number of tillers per plant

There was no significant difference between the treatments with respect to number of tillers per plant at 90 DAP in both the polyhouse and open conditions. The number of tillers per plant ranged from 8.21 to 11.38 and 16.59 to 22.02 in polyhouse and open conditions respectively. The tiller production was higher in the plants raised in open condition.

No significant difference could be observed in the interaction effects between treatments and conditions (polyhouse and open) with respect to number of tillers per plant at 90 DAP.

4.1.2.3 Number of leaves per tiller

At 90 DAP, the number of leaves per tiller ranged from 4.46 to 10.92 in polyhouse condition and 8.34 to 12.09 in open condition. In polyhouse condition the treatment T3 (Adhoc PoP organic, KAU) recorded the highest number of leaves per tiller and the treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the lowest number of leaves per tiller.

In open condition the treatment T4 (PoP nutrient management, KAU + Salicylic acid foliar spray - 100 µM) recorded the highest number of leaves per tiller and was on par with the treatment T3 (Adhoc PoP organic, KAU).

The plants raised in the polyhouse and open conditions were on par with respect to number of leaves per tiller.

The treatments T3 (Adhoc PoP organic, KAU) and T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM) were on par and superior to the other treatments studied irrespective of the conditions.

4.1.2.4 Leaf area

The leaf area was found to range from 25.81 cm^2 to 91.89 cm^2 in polyhouse condition at 90 DAP. The treatment T3 (Adhoc PoP organic, KAU) recorded the highest leaf area and the treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the lowest leaf area.

Table 3. Morphological parameters recorded for different treatments in polyhouse and open conditions (120 DAP)

Treatment	Length of the pseudostem (cm)			No. of tillers per plant			No. of leaves per tiller			Leaf area (cm^2)		
	Polyhouse	Open	Mean	Polyhouse	Open	Mean	Polyhouse	Open	Mean	Polyhouse	Open	Mean
T1	95.81	66.25	81.03	13.25	22.67	17.96	11.27	11.21	11.24	95.43	99.69	97.56
T2	98.08	69.90	83.99	11.17	20.96	16.07	11.21	10.25	10.73	98.68	101.29	99.99
T3	111.15	52.84	82.00	11.46	22.08	16.62	14.34	12.85	13.60	114.27	52.20	83.24
T4	71.28	48.67	59.98	12.26	19.13	15.70	14.62	11.28	12.95	80.09	48.74	64.42
T5	35.16	39.77	37.47	9.80	23.21	16.51	5.72	9.70	7.71	37.30	41.53	39.42
Mean	82.30	55.49		11.59	21.61		11.43	11.06		85.15	68.69	
Factors	C.D.	SE (m)		C.D.	SE (m)		C.D.	SE (m)		C.D.	SE (m)	
Factor A	7.03	2.42		N.S.	0.91		1.36	0.47		9.96	3.43	
Factor B	4.44	1.53		4.67	0.57		N.S.	0.60		6.30	2.17	
Factor A X B	9.94	3.42		N.S.	1.28		1.92	0.66		14.09	4.86	

Factor (A) – Treatment

Factor (B) – Condition (Polyhouse and Open)

Factor (A X B) – (Treatment X Condition)

T1- Nutrient management as per PoP (KAU) (control)

T2- PoP Soil test based nutrient management

T3- Adhoc PoP organic (KAU)

T4- PoP nutrient management (KAU) + Salicylic acid foliar spray (100 µM)

T5- PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

In open condition at 90 DAP, the leaf area ranged from 36.61 cm^2 to 81.69 cm^2. The treatment T2 (PoP Soil test based nutrient management) recorded the largest leaf area and was on par with the treatment T1 (Nutrient management as per PoP, KAU). The lowest leaf area was recorded in the treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) which was on par with the treatments T3 (Adhoc PoP organic, KAU) and T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM). The plants raised in polyhouse recorded the highest leaf area for all the treatments except T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) as compared to the open condition.

The interaction effects were significant with respect to the parameter studied. The treatments T1 (Nutrient management as per PoP, KAU) and T2 (PoP Soil test based nutrient management) were on par with respect to leaf area irrespective of the conditions.

4.1.3 Growth performance of ginger in various treatments at 120 DAP (Table 3)

4.1.3.1 Length of the pseudostem

At 120 DAP, the length of pseudostem ranged from 35.16 cm to 111.15 cm in polyhouse condition. The treatment T3 (Adhoc PoP organic, KAU) recorded the highest length of pseudostem and was significantly superior to all the other treatments studied. The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the lowest length of pseudostem.

In open condition, the length of pseudostem ranged from 39.77 cm to 69.90 cm at 120 DAP. The treatment T2 (PoP Soil test based nutrient management)

recorded the highest length of pseudostem and was on par with the treatment T1 (Nutrient management as per PoP, KAU). The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 μM) recorded the lowest length of pseudostem.

Higher length of the pseudostem was recorded for all the treatments in polyhouse except the treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 μM) as compared to the plants in open condition.

Irrespective of the conditions the treatments T1 (Nutrient management as per PoP, KAU), T2 (PoP Soil test based nutrient management) and T3 (Adhoc PoP organic, KAU) were on par with respect to length of the pseudostem at 120 DAP.

4.1.3.2 Number of tillers per plant

No significant difference was found between the treatments studied with respect to number of tillers per plant at 120 DAP both in polyhouse and open conditions.

The number of tillers per plant ranged from 9.80 to 13.25 in polyhouse condition and 19.13 to 23.21 in open condition. The tiller production was found to be more in the plants raised in open condition.

No significant interaction effects was observed between the treatments and conditions (polyhouse and open) with respect to number of tillers per plant at 120 DAP.

4.1.3.3 Number of leaves per tiller

At 120 DAP, the number of leaves per tiller ranged from 5.72 to 14.62 in polyhouse condition. The treatment T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 μM) recorded the highest number of leaves per tiller and was

on par with the treatment T3 (Adhoc PoP organic, KAU). The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the least number of leaves per tiller.

In open condition the number of leaves per tiller ranged from 9.7 to 12.85 at 120 DAP. The treatment T3 (Adhoc PoP organic, KAU) recorded the largest number of leaves per tiller and was significantly superior to all the other treatments studied with respect to number of leaves per tiller. The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the least number of leaves per tiller.

The interaction effects between the treatments and the conditions (polyhouse and open) was significant with respect to the number of leaves per tiller. The treatments T3 (Adhoc PoP organic, KAU) was superior to other treatments studied and was on par with the treatment T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM), irrespective of the conditions with respect to number of leaves per tiller at 120 DAP.

4.1.3.4 Leaf area

The leaf area recorded at 120 DAP was found to range from 37.30 cm^2 to 114.27 cm^2 in polyhouse condition. The treatment T3 (Adhoc PoP organic, KAU) recorded the highest leaf area and was significantly superior to all the other treatments studied with respect to leaf area. The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the least leaf area.

In open condition the leaf area was found to range from 41.53 cm^2 to 101.29 cm^2 at 120 DAP. The treatment T2 (PoP Soil test based nutrient management) recorded the highest leaf area and was on par with the treatment T1 (Nutrient management as per PoP, KAU). The treatment T5 (PoP nutrient

management, KAU + methyl jasmonate foliar spray - 100 μM) recorded the least leaf area.

The treatment T2 (PoP Soil test based nutrient management) was superior and was on par with the treatment T1 (Nutrient management as per PoP- KAU) with respect to leaf area at 120 DAP irrespective of the conditions.

4.2 Micro-meteorological observations

The maximum temperature, minimum temperature and relative humidity recorded inside the polyhouse and open condition did not show much difference. The micro-meteorological observations recorded during crop growth period and micrometeorological observations during different stages of crop growth are represented in Table 4a and 4b.

4.3 Expression analysis of *Chalcone synthase* gene in ginger

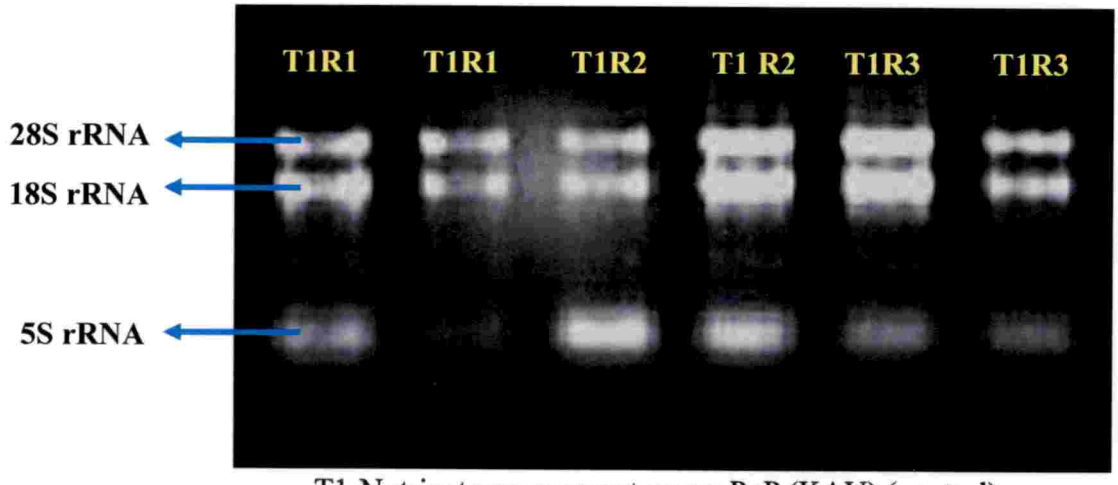
The *Chalcone synthase* gene expression was studied using quantitative real time PCR. The housekeeping gene used was *Actin*.

4.3.1 Isolation and analysis of total RNA

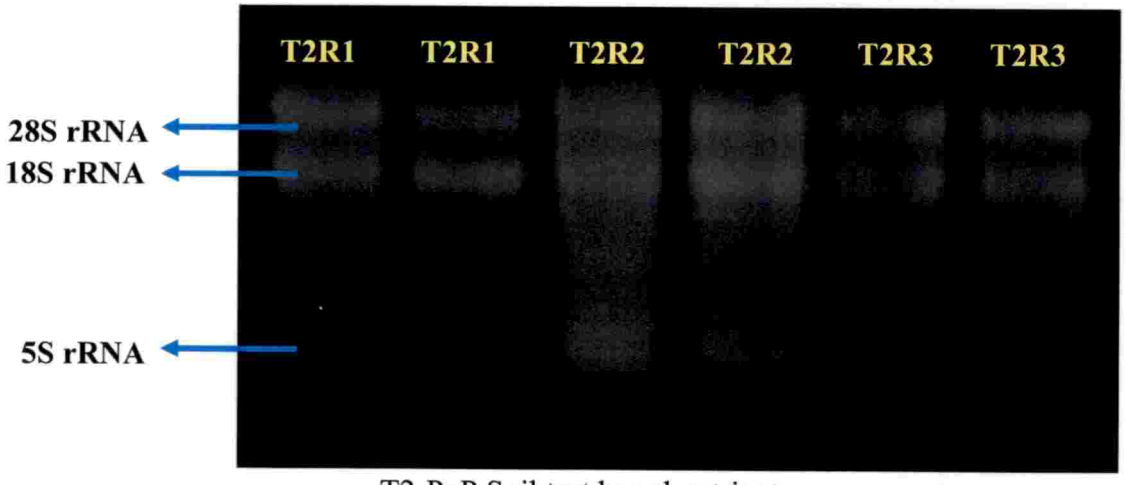
The total RNA was isolated from the leaves of the plant both in polyhouse and open conditions as mentioned in 3.5.1.1.1 and was analysed for quality and quantity of the isolated total RNA.

4.3.1.1 Quality and quantity analysis of isolated total RNA

The total RNA when run on one per cent agarose gel in 1X TAE buffer showed three intact bands each corresponding to 28S, 18S and 5SrRNA respectively confirming to be of good quality (Plates 2 and 3). The total RNA was quantified using NanoDrop® ND-1000 spectrophotometer (NanoDrop



T1-Nutrient management as per PoP (KAU) (control)



T2-PoP Soil test based nutrient management

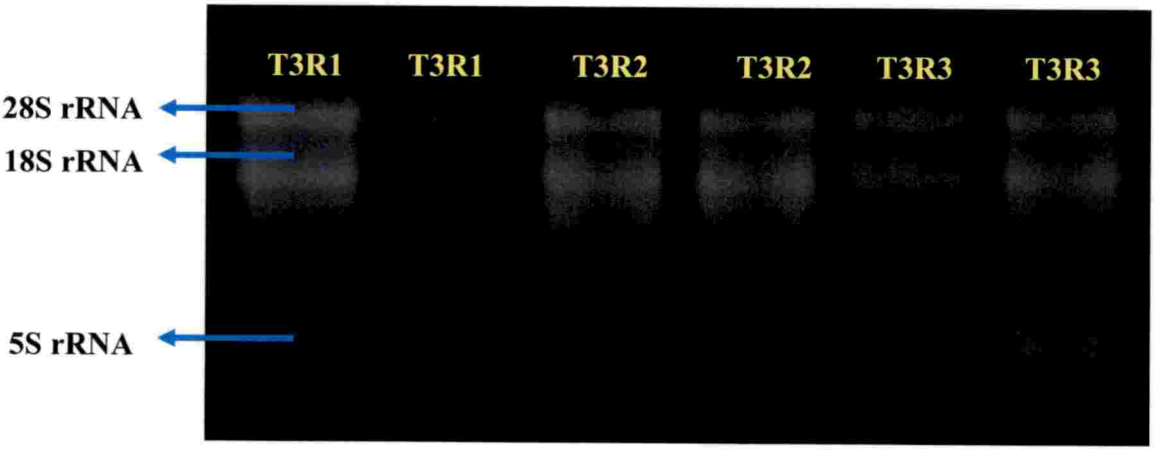
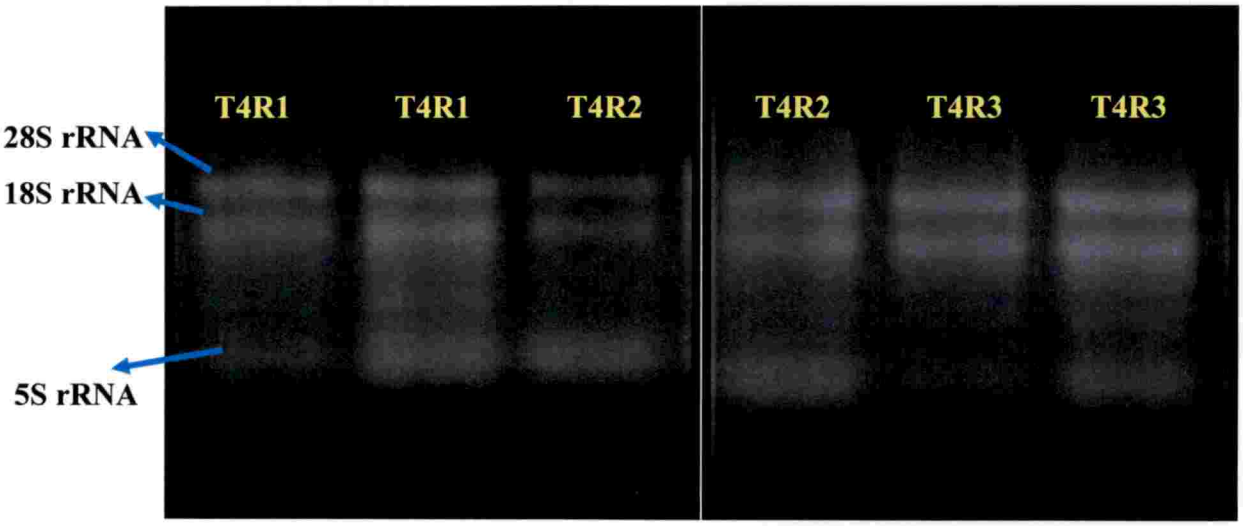
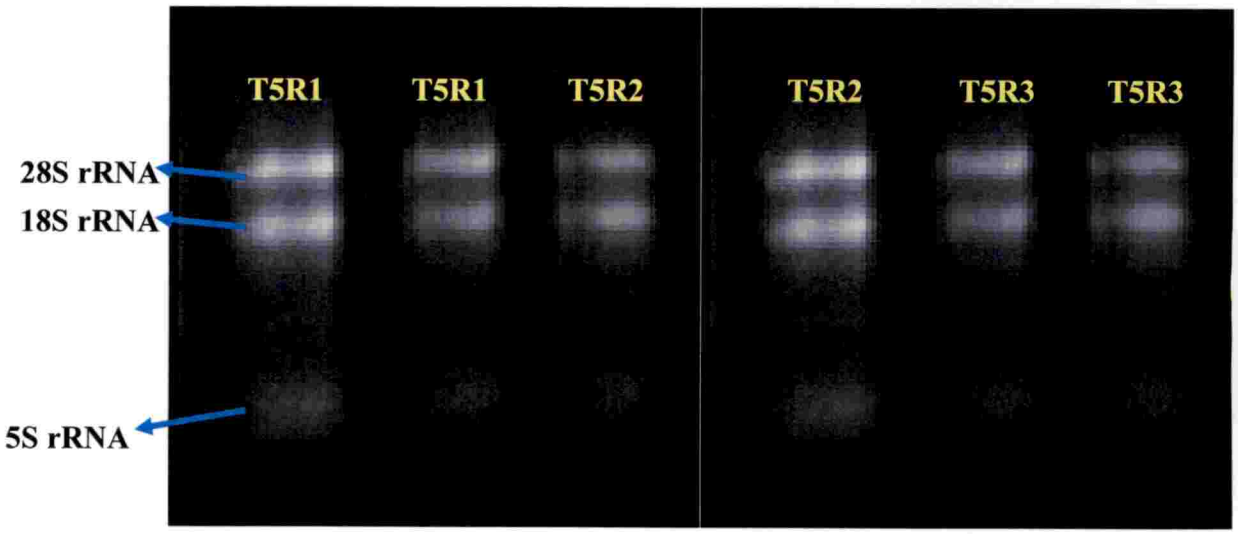


Plate 2. Quality analyses of the isolated total RNA from different treatments in polyhouse condition

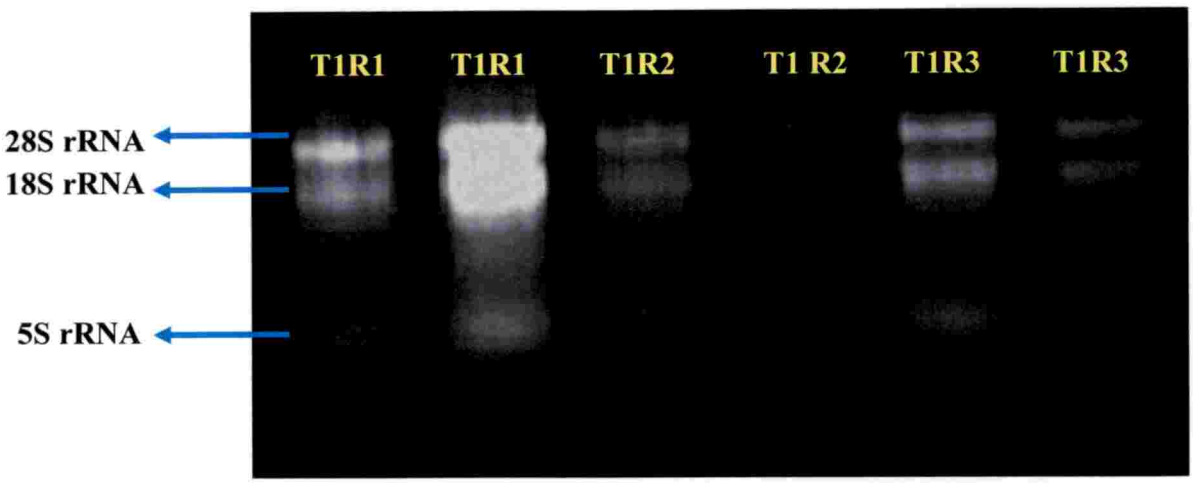


T4- PoP nutrient management (KAU) + salicylic acid foliar spray (100 µM)

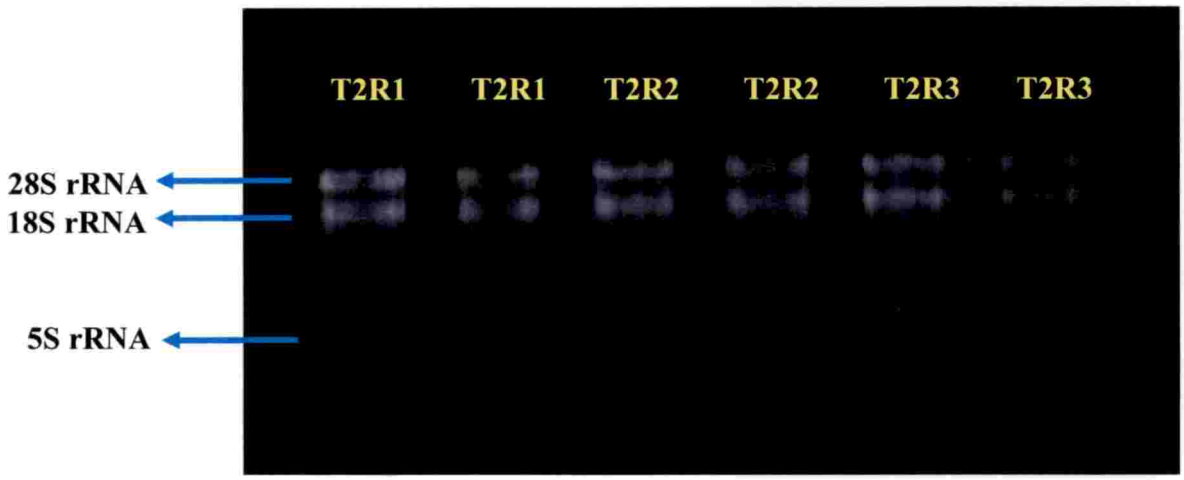


T5- PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

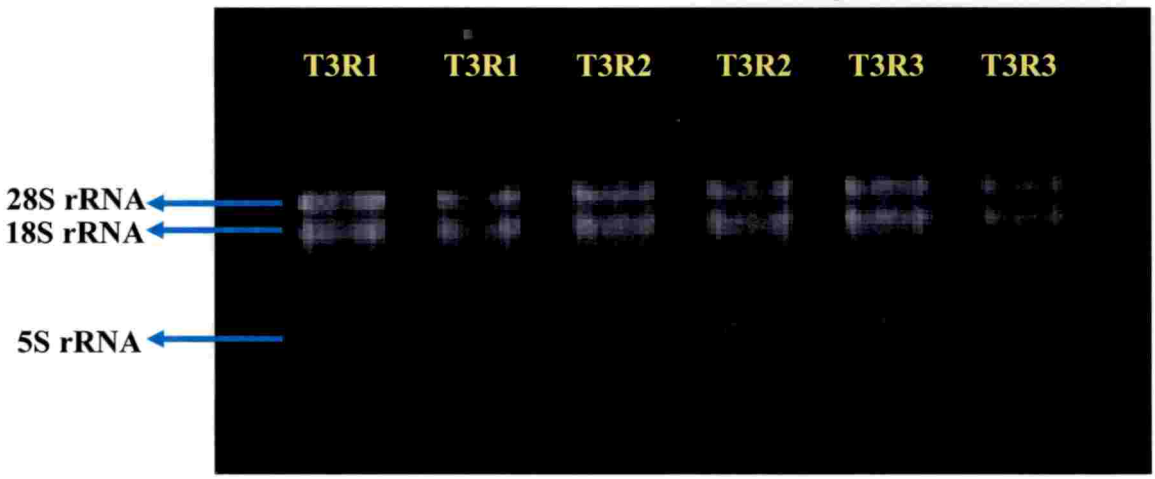
Plate 2. Quality analyses of the isolated total RNA from different treatments in polyhouse condition



T1- Nutrient management as per PoP (KAU) (control)

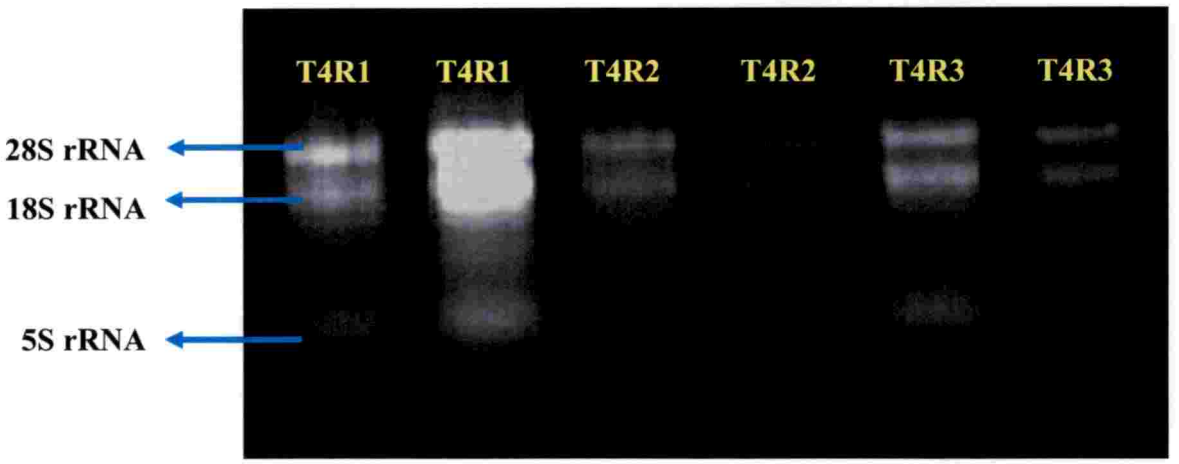


T2- PoP Soil test based nutrient management

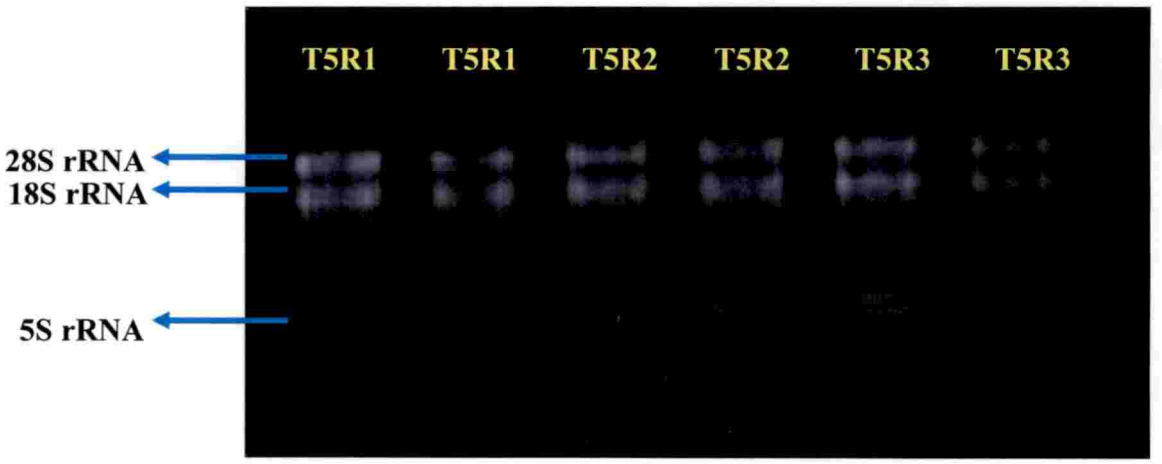


T3- Adhoc PoP organic (KAU)

Plate 3. Quality analyses of the isolated total RNA from different treatments in open condition



T4- PoP nutrient management (KAU) + salicylic acid foliar spray (100 µM)



T5- PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

Plate 3. Quality analyses of the isolated total RNA from different treatments in open condition

Technologies Inc., USA) by determining absorbance of nucleic acids at wavelengths of 260 nm and 280 nm. The A_{260}/A_{280} ratio was observed between 1.8 and 2.3 and A_{260}/A_{230} ratio was observed above one for the isolated RNA samples which indicated the purity of RNA samples. The A_{260}/A_{280}, A_{260}/A_{230} and concentration of the total RNA isolated from various treatments are presented in Table 5.

4.3.2 First strand cDNA synthesis

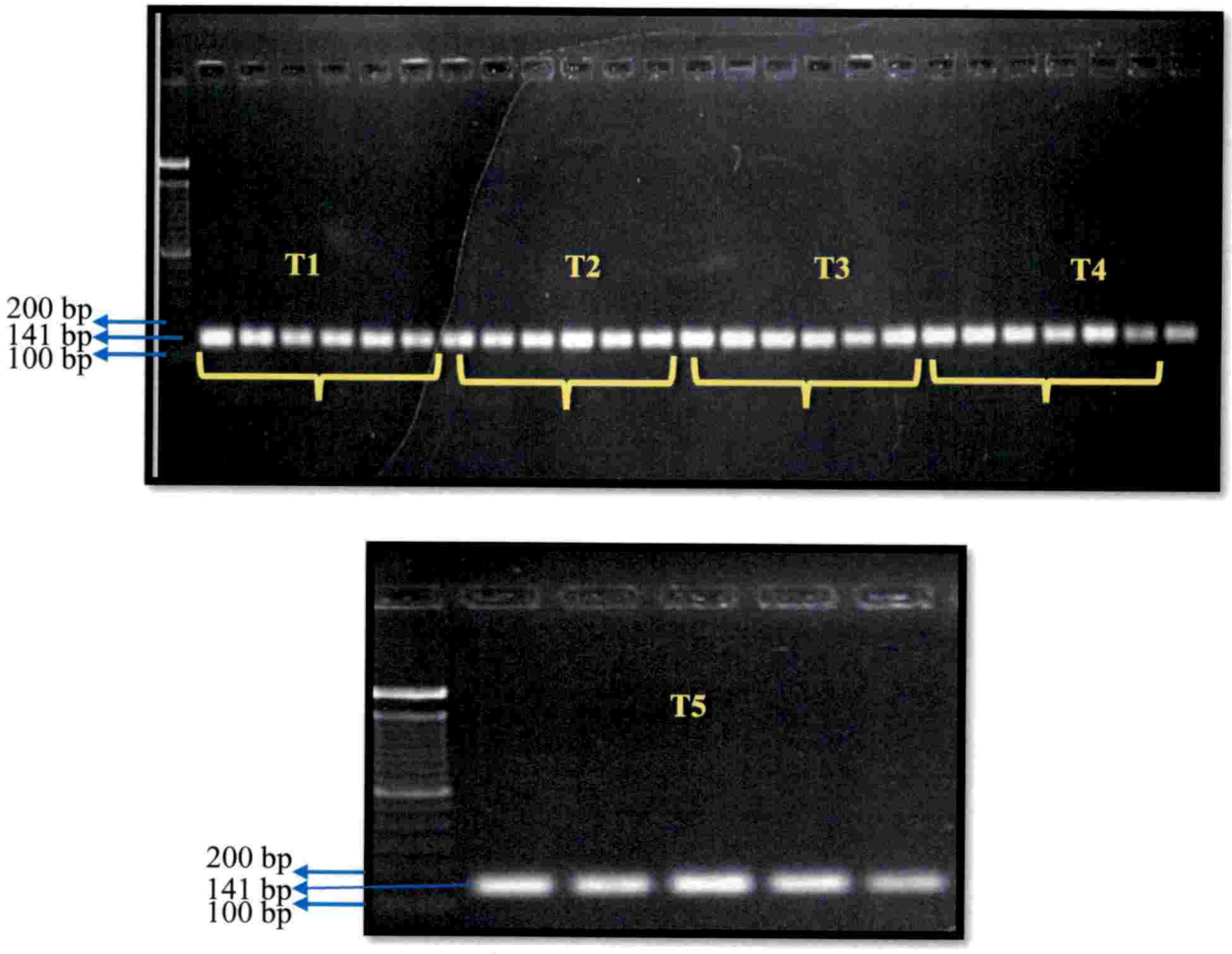
The total RNA was reverse transcribed using the First Strand cDNA Synthesis Kit (Thermoscientific), as per the guidelines of the manufacturer. The total RNA was incubated with reverse transcriptase enzyme and oligo dT primer along with RevertAid.

4.3.2.1 Confirmation of the synthesis of first strand cDNA using primers

The cDNA synthesis was confirmed by the amplification with *Chalcone synthase* and *Actin* gene specific primers. The PCR product when run on 1.8 per cent agarose gel gave the expected band sizes of 141 bp and 138 bp for *CHS* and *Actin* gene specific primers respectively confirming the synthesis of cDNA (Plates 4, 5, 6 and 7).

4.3.3 Real time PCR assay for *Chalcone synthase* gene expression analysis

The quantification of *Chalcone synthase* (*CHS*) gene expression was performed using Real-Time PCR. The gene used as endogenous (control) was the *Actin* gene. The relative gene expression of *CHS* was determined from the C_T values by comparative C_T method.



T1- Nutrient management as per PoP (KAU) (control)

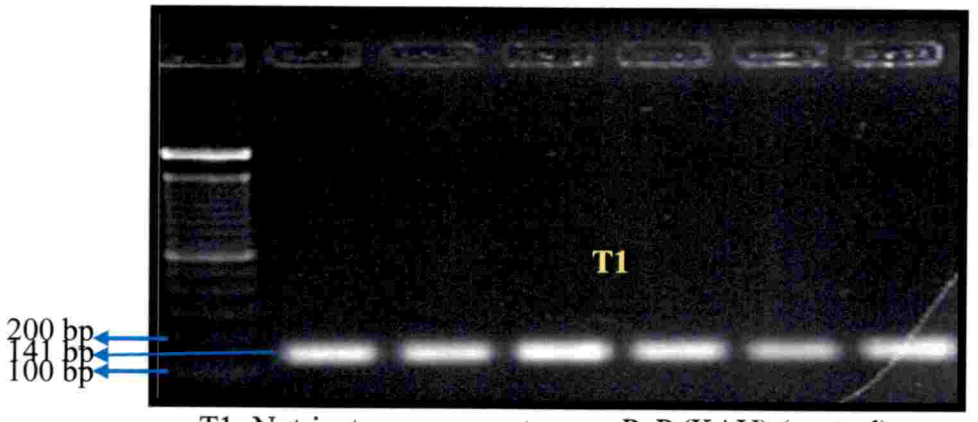
T2- PoP Soil test based nutrient management

T3- Adhoc PoP organic (KAU)

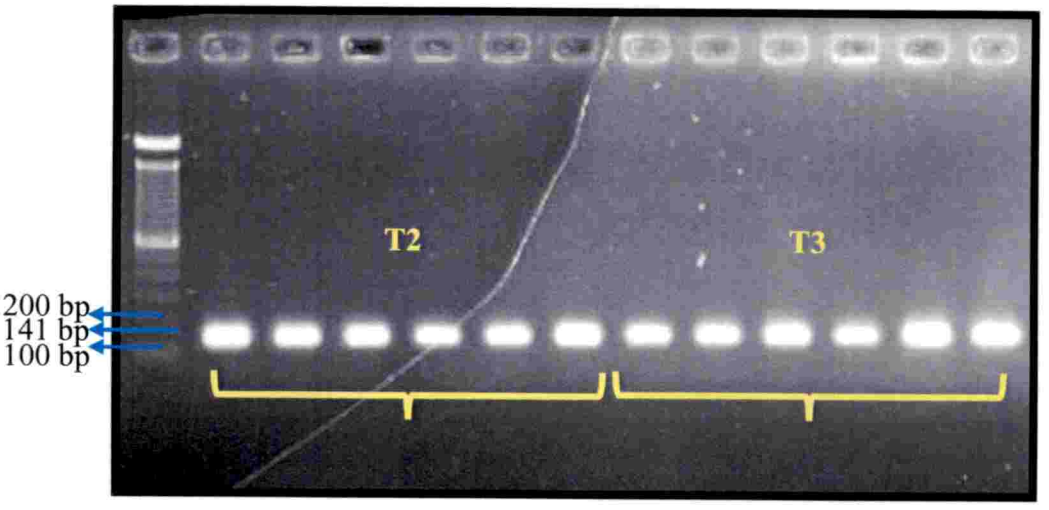
T4- PoP nutrient management (KAU) + salicylic acid foliar spray (100 µM)

T5- PoP nutrient management (KAU) +methyl jasmonate foliar spray (100 µM)

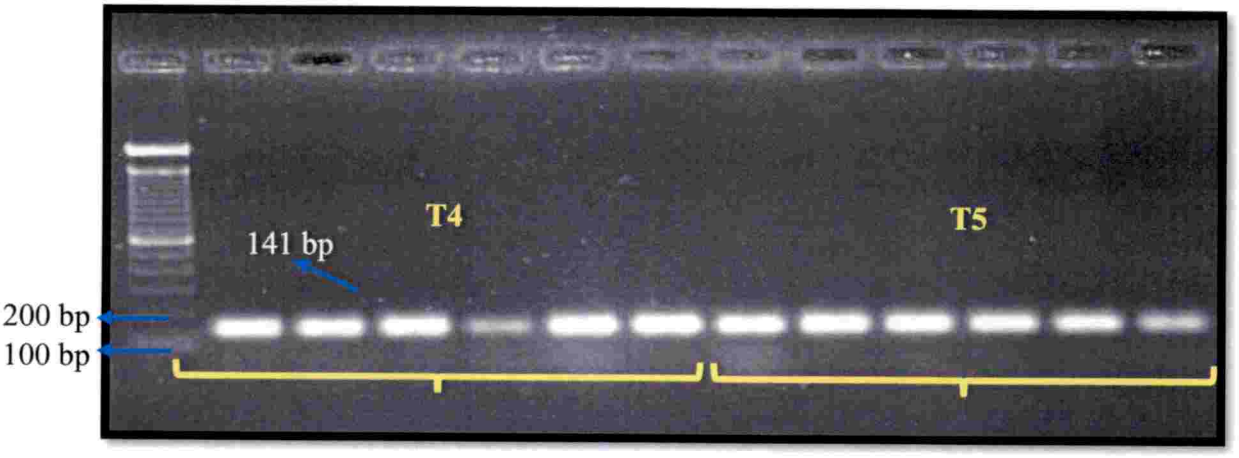
Plate 4. Amplified first strand cDNA from total RNA isolated from the ginger leaves in polyhouse condition using *Actin* gene specific primers



T1- Nutrient management as per PoP (KAU) (control)

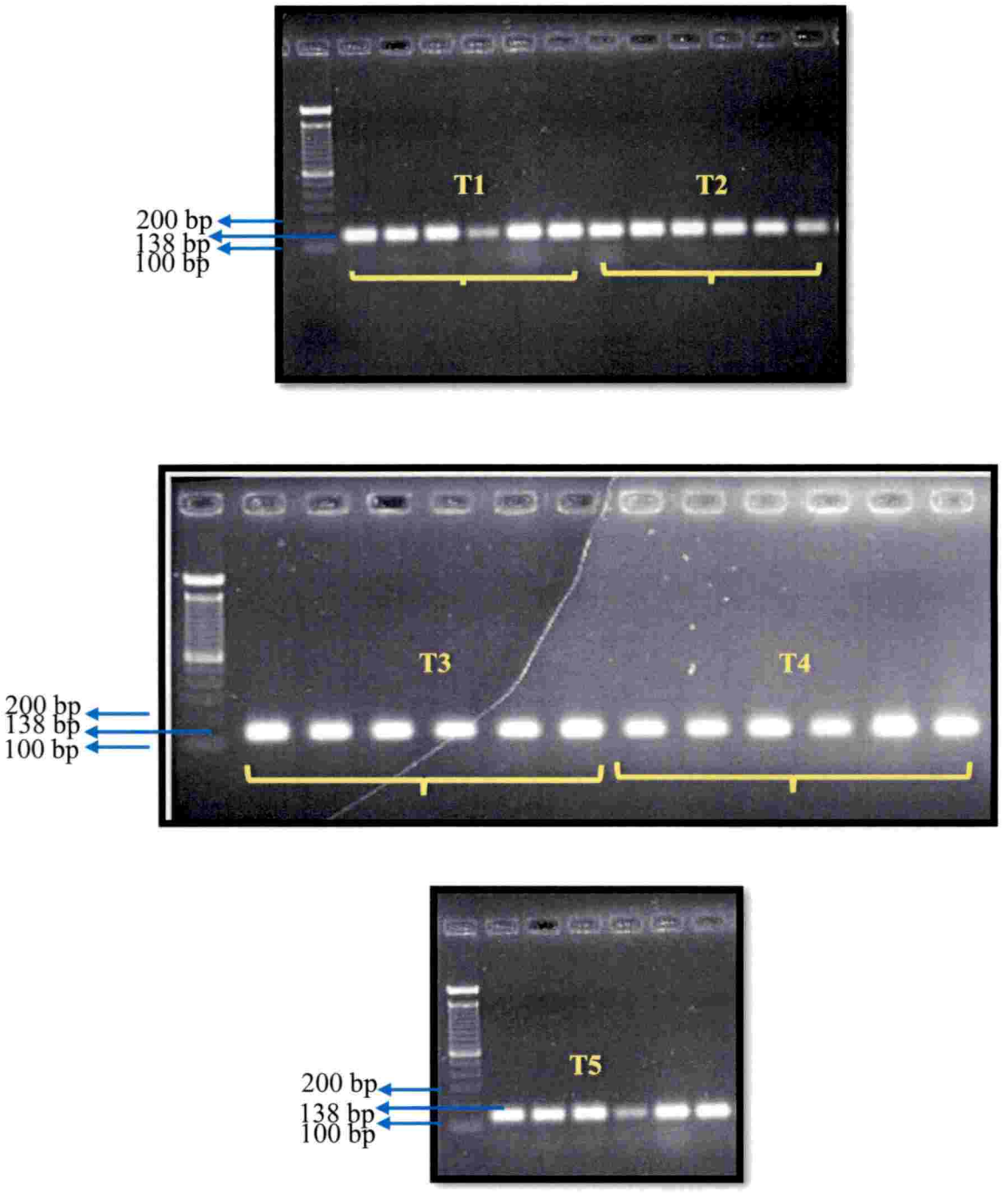


T2- PoP Soil test based nutrient management
T3- Adhoc PoP organic (KAU)



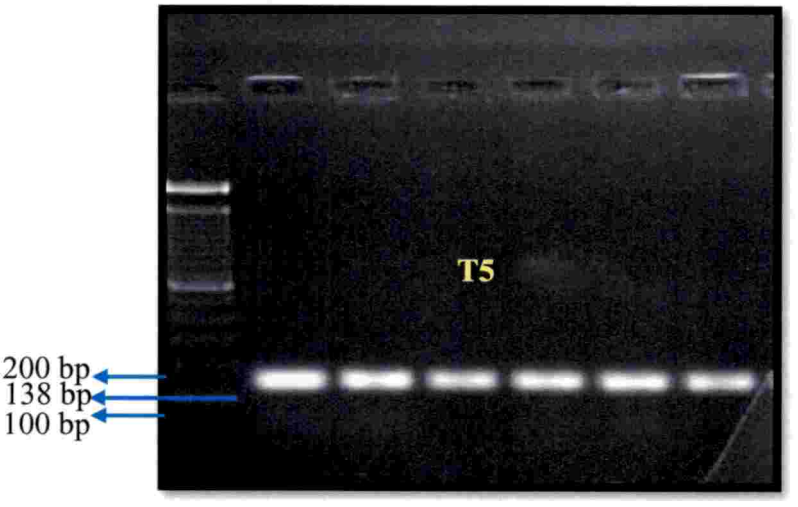
T4- PoP nutrient management (KAU) + salicylic acid foliar spray (100 µM)
T5- PoP nutrient management (KAU) +methyl jasmonate foliar spray (100 µM)

Plate 5. Amplified first strand cDNA from total RNA isolated from the ginger leaves in open condition using *Actin* gene specific primers



T1- Nutrient management as per PoP (KAU) (control)
T2- PoP Soil test based nutrient management
T3- Adhoc PoP organic (KAU)
T4- PoP nutrient management (KAU) + salicylic acid foliar spray (100 µM)
T5- PoP nutrient management (KAU) +methyl jasmonate foliar spray (100 µM)

Plate 6. Amplified first strand cDNA from total RNA isolated from the ginger leaves in polyhouse condition using *Chalcone synthase* gene specific primers



T1- Nutrient management as per PoP (KAU) (control)

T2- PoP Soil test based nutrient management

T3- Adhoc PoP organic (KAU)

T4- PoP nutrient management (KAU) + salicylic acid foliar spray (100 µM)

T5- PoP nutrient management (KAU) +methyl jasmonate foliar spray (100 µM)

Plate 7. Amplified first strand cDNA from total RNA isolated from the ginger leaves in open condition using *Chalcone synthase* gene specific primers

Table 4a. Micro- meteorological observations in polyhouse and open condition

Month	Maximum temperature (°C)		Minimum temperature (°C)		Relative Humidity (%)	
	Polyhouse	Open	Polyhouse	Open	Polyhouse	Open
June, 2018	27.47	27.44	26.92	27.25	81.65	83.33
July, 2018	28.05	27.50	25.58	25.41	81.73	84.48
August, 2018	28.97	28.06	25.99	25.84	78.30	82.85
September, 2018	31.20	30.20	26.20	25.66	67.34	68.71
October, 2018	29.60	28.49	26.09	25.37	76.02	77.42
November, 2018	28.92	29.94	26.14	25.70	78.66	83.41
December, 2018	29.83	29.24	26.50	26.07	75.44	76.93
January, 2019	30.15	30.14	27.00	26.14	73.37	74.34

Table 4b. Micro- meteorological observations in polyhouse and open condition

Growth phase	Maximum temperature (°C)		Minimum temperature (°C)		Relative Humidity (%)	
	Polyhouse	Open	Polyhouse	Open	Polyhouse	Open
Active growth phase	28.92	28.30	26.17	26.04	77.86	79.84
Slow growth phase	29.26	29.22	26.12	25.54	77.34	80.41
Senescence phase	29.99	29.69	26.75	26.11	74.40	75.64

Month	Growth phase
June to September, 2018	Active growth phase
October and November, 2018	Slow growth phase
December, 2018 and January 2019	Senescence phase

Table 5. Quantitative analysis of total RNA isolated from the leaves of various treatments in polyhouse and open condition

Treatment	A 260/280		A 260/230		Conc. ng/µl	
	Polyhouse	Open	Polyhouse	Open	Polyhouse	Open
T1	1.91	1.90	1.77	1.13	1045.00	1009.20
T2	1.91	1.80	1.30	2.04	1165.10	686.53
T3	1.94	1.93	1.02	1.30	1481.48	824.33
T4	2.19	2.10	1.34	1.13	866.48	756.47
T5	2.26	1.96	1.58	1.48	741.88	531.88

Table 6. *Chalcone synthase* gene expression in various treatments of polyhouse and open conditions

Treatment	Average $2^{-\Delta\Delta Ct}$ (fold)		
	Polyhouse	Open	Mean
T_1	1.000	1.000	1.000
T_2	1.346	1.166	1.256
T_3	1.016	1.024	1.020
T_4	1.210	1.049	1.130
T_5	1.001	1.010	1.006
Mean	1.115	1.050	
Factors	C.D.(0.05)	SE (m)	
Factor A	N.S.	0.225	
Factor B	N.S.	0.142	
Factor A X B	N.S.	0.318	

Factor (A) – Treatment

Factor (B) – Condition (Polyhouse and Open)

Factor (A X B) – (Treatment X Condition)

T1- Nutrient management as per PoP (KAU) (control)

T2- PoP Soil test based nutrient management

T3- Adhoc PoP organic (KAU)

T4- PoP nutrient management (KAU) + Salicylic acid foliar spray (100 µM)

T5- PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

4.3.3.1 Threshold cycle values

The real-time PCR data are represented as the cycle number necessary to achieve a threshold cycle value. The reference gene *Actin* was used for expression analysis. The represented C_T value for *Actin* gene was the mean of three biological replicates. In both the conditions, there was no significant fold change in the *CHS* gene expression as compared to the control. The treatment T2 (PoP Soil test based nutrient management) was found with the highest gene expression both in polyhouse and open conditions. The trend of expression of the *CHS* gene was the same in both the conditions for different treatments.

4.3.3.2 Amplification plots

The results of Real-Time PCR were visualized in amplification plot with the fluorescence corresponding to the Y-axis (Rn) and the number of PCR cycles corresponding to the X-axis. The point of interaction between the amplification curve and the threshold line is termed as the threshold cycle.

4.3.3.3 Relative quantification analysis

The relative gene expression of *CHS* was estimated as per the comparative C_T method as stated by Livak and Schmittgen (2001). The relative expression of *CHS* gene ranged from 1.0 fold to 1.166 fold in polyhouse condition and 1.0 fold to 1.346 fold in open condition when normalized with the endogenous gene *Actin.* The highest expression of *CHS* gene was recorded in the treatment T2 (PoP Soil test based nutrient management) both in polyhouse and open conditions. The relative *Chalcone synthase* gene expression in various treatments of polyhouse and open conditions is represented as average $2^{-\Delta\Delta Ct}$ values in Table 6.

4.4 Harvesting of ginger rhizomes and gingerol estimation

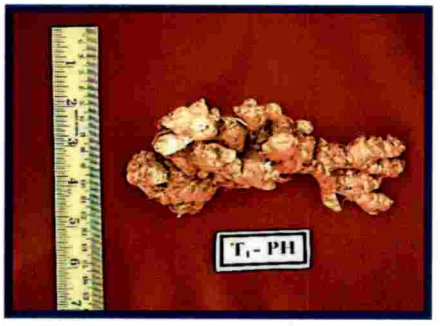
Ginger rhizomes from various treatments were harvested at 240 days after planting from both polyhouse (Plate 8) and open (Plate 9) conditions. Fresh yield of rhizomes per plant per treatment was recorded. The rhizome characters such as number length, girth and inter-nodal length of primary, secondary and tertiary fingers, thickness of inner core of rhizome, colour of flesh, plumpiness of rhizomes were also recorded.

4.4.1. Fresh yield of rhizomes

The fresh yield of rhizomes was recorded both in polyhouse and open conditions for various treatments and data are presented in Table 7.

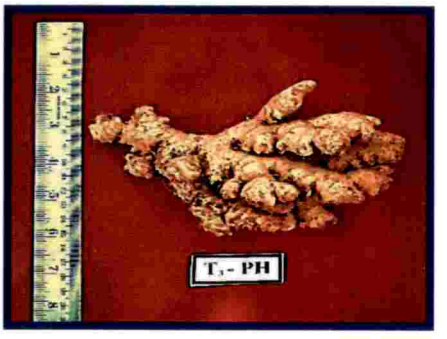
The fresh yield of rhizomes ranged from 82.61 g to 275.30 g in polyhouse condition. The treatment T1 (Nutrient management as per PoP, KAU) recorded the highest fresh yield of rhizomes and was on par with the treatment T3 (Adhoc PoP organic- KAU). The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the least fresh yield of rhizomes.

The fresh yield of rhizomes varied from 36.77 g to 91.52 g in the various treatments of open condition. The highest fresh yield of rhizomes was recorded in the treatment T1 (Nutrient management as per PoP, KAU) and was on par with the fresh yields obtained from the treatments T2 (PoP Soil test based nutrient management) and T3 (Adhoc PoP organic, KAU). The treatment T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM) recorded the lowest fresh yield and was on par with the fresh yield of rhizomes obtained from the treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM). Higher fresh yields of rhizomes was recorded in polyhouse for all the treatments.

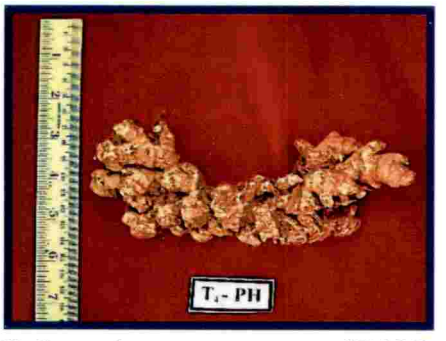


T1-Nutrient management as per PoP (KAU) (control)

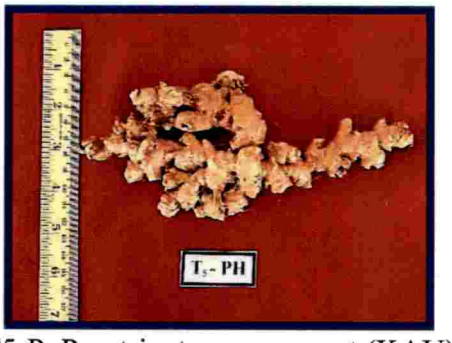
T2-PoP Soil test based nutrient management



T3-Adhoc PoP organic (KAU)



T4-PoP nutrient management (KAU) + salicylic acid foliar spray (100 µM)

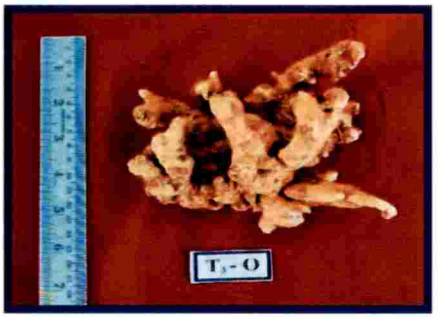


T5-PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

Plate 8. Harvested rhizomes from various treatments in polyhouse

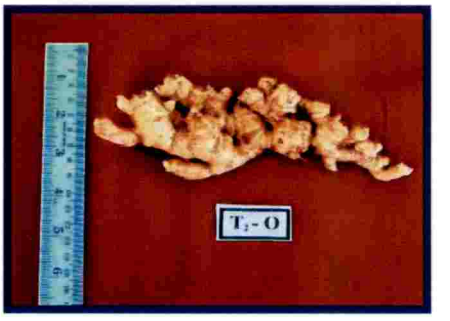
T1- Nutrient management as per PoP (KAU) (control)

T2- PoP soil test based nutrient management



T3-Adhoc PoP organic (KAU)

T4- PoP nutrient management (KAU) + salicylic acid foliar spray (100 µM)



T5- PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

Plate 9. Harvested rhizomes from various treatments in open condition

Table 7. Fresh yield of rhizomes from different treatments in polyhouse and open conditions

Treatment	Fresh yield (g per plant)		
	Polyhouse	Open	Mean
T1	275.30	91.52	183.41
T2	236.03	75.01	155.52
T3	259.50	73.49	166.50
T4	222.50	36.77	129.64
T5	82.61	44.67	63.64
Mean	215.19	64.29	
Factors	C.D.(0.05)	SE (m)	
Factor A	33.60	11.58	
Factor B	21.25	7.32	
Factor A X B	47.52	16.37	

Factor (A) – Treatment

Factor (B) – Condition (Polyhouse and Open)

Factor (A X B) – (Treatment X Condition)

T1- Nutrient management as per PoP (KAU) (control)

T2- PoP Soil test based nutrient management

T3- Adhoc PoP organic (KAU)

T4- PoP nutrient management (KAU) + Salicylic acid foliar spray (100 µM)

T5- PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

Table 8. Rhizome characters recorded from various treatments in polyhouse and open conditions

Treatment	Number of primary fingers		Number of secondary fingers		Number of tertiary fingers		Length of primary fingers (cm)		Length of secondary fingers (cm)		Length of tertiary fingers (cm)		Girth of primary fingers (cm)		Girth of secondary fingers (cm)		Girth of tertiary fingers (cm)		Thickness of the inner core (cm)	
	Polyhouse	Open	Polyhouse	Open	Polyhouse	Open	Polyhouse	Open	Polyhouse	Open	Polyhouse	Open	Polyhouse	Open	Polyhouse	Open	Polyhouse	Open	Polyhouse	Open
T1	8.75	5.25	8.75	6.25	10.75	11.50	4.50	2.51	2.90	1.91	2.73	2.37	5.68	5.14	5.12	3.91	3.42	1.88	1.80	1.00
T2	7.00	3.75	8.50	8.00	9.00	13.75	4.90	1.88	3.06	2.71	2.80	2.50	6.16	4.05	4.89	4.25	3.14	3.65	2.00	1.62
T3	10.25	4.25	9.75	4.50	9.50	8.50	5.11	1.86	3.48	2.12	3.10	1.89	6.27	2.02	5.56	3.58	4.09	1.85	3.07	2.02
T4	5.00	4.50	5.00	5.75	5.50	10.25	2.3	1.96	2.45	2.47	3.00	2.92	3.92	4.06	3.46	3.06	3.87	3.63	1.49	1.36
T5	3.25	4.50	3.75	6.00	4.50	10.50	2.54	2.67	3.09	2.96	1.94	1.18	2.90	5.19	3.00	4.22	1.93	2.25	0.75	0.84
Factors	**C.D.**	**SE (m)**	**C.D.**	**SE (m)**	**C.D.**	**SE (m)**	**C.D.**	**SE (m)**	**C.D.**	**SE (m)**	**C.D.**	**SE (m)**	**C.D.**	**SE (m)**	**C.D.**	**SE (m)**	**C.D.**	**SE (m)**	**C.D.**	**SE (m)**
Factor A	0.76	0.26	0.87	0.30	1.68	0.58	0.27	0.09	0.19	0.07	0.20	0.07	0.30	0.10	0.27	0.10	0.24	0.08	0.17	0.06
Factor B	0.48	0.17	0.55	0.19	1.06	0.37	0.17	0.06	0.12	0.04	0.13	0.04	0.19	0.06	0.17	0.06	0.15	0.05	0.11	0.04
Factor A X B	1.08	0.37	1.24	0.43	2.37	0.82	0.38	0.13	0.27	0.09	0.29	0.10	0.42	0.14	0.39	0.13	0.34	0.12	0.23	0.08

Factor (A) – Treatment

Factor (B) – Condition (Polyhouse and Open)

Factor (A X B) – (Treatment X Condition)

T1- Nutrient management as per PoP (KAU) (control)

T2- PoP Soil test based nutrient management

T3- Adhoc PoP organic (KAU)

T4- PoP nutrient management (KAU) + Salicylic acid foliar spray (100 µM)

T5- PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

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There was a significant interaction effect between the factors, treatments and conditions (polyhouse and open) with regards to fresh yield of rhizomes. Irrespective of the conditions, the treatments T1 (Nutrient management as per PoP, KAU), T2 (PoP Soil test based nutrient management) and T3 (Adhoc PoP organic, KAU) recorded on par fresh yield of rhizomes.

4.4.2 Rhizome characters

The rhizome characters such as the number of primary fingers, number of secondary fingers, number of tertiary fingers, length of primary fingers, length of secondary fingers, length of tertiary fingers, girth of primary fingers, girth of secondary fingers, girth of tertiary fingers, thickness of the inner core, colour and plumpiness of the rhizomes were recorded soon after harvesting and presented in the Table 8.

4.4.2.1 Number of primary fingers

The number of primary fingers ranged from 3.25 to 10.25 in polyhouse conditions. The treatment T3 (Adhoc PoP organic, KAU) recorded the highest number of primary fingers and the treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the lowest number of primary fingers.

In open condition, the number of primary fingers ranged from 3.75 to 5.25. The treatment T1 (Nutrient management as per PoP, KAU) recorded the highest number of primary fingers. The treatment T2 (PoP Soil test based nutrient management) recorded the lowest number of primary fingers and was on par with the treatment T3 (Adhoc PoP organic, KAU).

The plants raised in polyhouse condition recorded higher number of primary fingers as compared to the plants in open condition. The interaction effects between

the treatments and conditions were significantly different Irrespective of the conditions, the treatments T1 (Nutrient management as per PoP, KAU) and T3 (Adhoc PoP organic - KAU) were on par with respect to number of primary fingers.

4.4.2.2 Number of secondary fingers

The number of secondary fingers in polyhouse condition ranged from 3.75 to 9.75. The treatment T3 (Adhoc PoP organic - KAU) recorded the highest number of secondary fingers. The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the lowest number of secondary fingers.

The number of secondary fingers in open condition ranged from 4.50 to 8. The treatment T2 (PoP Soil test based nutrient management) recorded the highest number of secondary fingers and the treatment T3 (Adhoc PoP organic, KAU) recorded the lowest number of secondary fingers.

The interaction effects between the treatments and conditions (polyhouse and open) was significant with respect to number of secondary fingers. The treatments T1 (Nutrient management as per PoP, KAU), T2 (PoP Soil test based nutrient management) and T3 (Adhoc PoP organic, KAU) were on par irrespective of the conditions.

4.4.2.3 Number of tertiary fingers

The number of tertiary fingers ranged from 4.5 to 10.75 in polyhouse condition. The treatment T1 (Nutrient management as per PoP, KAU) recorded the highest number of tertiary fingers and was on par with the treatments T2 (PoP Soil test based nutrient management) and T3 (Adhoc PoP organic, KAU). The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the lowest number of tertiary fingers and was on par with the treatment T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM.

In open condition the number of tertiary fingers ranged from 8.5 to 13.75. The treatment T2 (PoP Soil test based nutrient management) recorded the highest number of tertiary fingers. The treatment T3 (Adhoc PoP organic, KAU) recorded the lowest number of tertiary fingers.

With respect to number of tertiary fingers, a significant interaction was observed between the factors, treatments and conditions (polyhouse and open). All the treatments were superior in polyhouse except T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 μM) as compared to open condition with respect to number of tertiary fingers.

The interaction effects between the factors, treatments and conditions showed a significant difference with respect to number of tertiary fingers and the treatments T1 (Nutrient management as per PoP, KAU), T2 (PoP Soil test based nutrient management) and T3 (Adhoc PoP organic, KAU) were on par irrespective of the conditions.

4.4.2.4 Length of primary fingers

The length of primary fingers ranged from 2.13 cm to 5.11 cm in polyhouse condition. The treatment T3 (Adhoc PoP organic, KAU) recorded the highest length of primary fingers and was on par with the treatment T2 (PoP Soil test based nutrient management). The treatment T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 μM) recorded the lowest length of primary fingers and was on par with the treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 μM).

In open condition, length of primary fingers ranged from 1.86 cm to 2.67 cm. The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 μM) recorded the highest length of primary fingers and was on par with the treatment T1 (Nutrient management as per PoP, KAU). The treatment T3

(Adhoc PoP organic, KAU) recorded the lowest length of primary fingers and was on par with the treatments T2 (PoP Soil test based nutrient management) and T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM).

A significant interaction was found between treatments and conditions (polyhouse and open) and the treatments T1 (Nutrient management as per PoP, KAU), T2 (PoP Soil test based nutrient management) and T3 (Adhoc PoP organic KAU) were on par irrespective of the conditions.

4.4.2.5 Length of secondary fingers

The length of secondary fingers ranged from 2.45 cm to 3.48 cm in polyhouse condition. The treatment T3 (Adhoc PoP organic, KAU) recorded the highest length of secondary fingers and was on par with the treatment T2 (PoP Soil test based nutrient management). The treatment T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM) recorded the lowest length of secondary fingers.

In open condition, the length of secondary fingers ranged from 1.91 cm to 2.96 cm. The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the highest length of secondary fingers and the treatment T1 (Nutrient management as per PoP, KAU) recorded the lowest length of secondary fingers.

A significant interaction was observed between the treatments and the conditions (polyhouse and open) with respect to length of secondary fingers and the treatments T2 (PoP Soil test based nutrient management) and T3 (Adhoc PoP organic, KAU) were on par irrespective of the conditions.

4.4.2.6 Length of tertiary fingers

The length of tertiary fingers ranged from 1.94 cm to 3.1 cm in polyhouse condition. The treatment T3 (Adhoc PoP organic, KAU) recorded the highest length of tertiary fingers and was on par with the treatment T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM). The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the lowest length of tertiary fingers.

In open condition the length of tertiary fingers ranged from 1.18 cm to 2.92 cm. The treatment T4 (PoP nutrient management, KAU + salicylic acid foliar spray- 100 µM) recorded the highest length of tertiary fingers and the treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the lowest length of tertiary fingers.

Higher length of tertiary fingers was observed in the rhizomes obtained from polyhouse condition than the open condition.

The interaction effects between treatments and conditions (polyhouse and open) was significantly different with respect to length of tertiary fingers and the treatment T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM) was significantly superior irrespective of the conditions.

4.4.2.7 Girth of primary fingers

The girth of primary fingers ranged from 2.90 cm to 6.27 cm in polyhouse condition. The treatment T3 (Adhoc PoP organic, KAU) recorded the highest girth of primary fingers and was on par with the treatment T2 (PoP Soil test based nutrient management). The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray, 100 µM) recorded the lowest girth of primary fingers.

The girth of primary fingers in open condition ranged from 2.02 cm to 5.19 cm. The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the highest girth of primary fingers and was on par with the treatment T1 (Nutrient management as per PoP, KAU). The treatment T3 (Adhoc PoP organic, KAU) recorded the lowest girth of primary fingers.

The interaction effects between treatments and conditions (polyhouse and open) were significantly different with respect to girth of primary fingers and the treatments T1 (Nutrient management as per PoP, KAU) and T2 (PoP Soil test based nutrient management) were on par irrespective of the conditions.

4.4.2.8 Girth of secondary fingers

The girth of secondary fingers ranged from 3 cm to 5.56 cm in polyhouse condition. The treatment T3 (Adhoc PoP organic, KAU) recorded the highest girth of secondary fingers. The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the lowest girth of secondary fingers.

In open condition the girth of secondary fingers ranged from 3.06 cm to 4.25 cm. The treatment T2 (PoP Soil test based nutrient management) recorded the highest girth of secondary fingers. The treatment T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM) recorded the lowest girth of secondary fingers.

The interaction effects between treatments and conditions (polyhouse and open) was significant with respect to girth of primary fingers and the treatments T1 (Nutrient management as per PoP, KAU), T2 (PoP Soil test based nutrient management) and T3 (Adhoc PoP organic, KAU) were on par irrespective of the conditions.

4.4.2.9 Girth of tertiary fingers

The girth of tertiary fingers ranged from 1.93 cm to 4.09 cm in polyhouse condition. The treatment T3 (Adhoc PoP organic (KAU)) recorded the highest girth of tertiary fingers and was on par with the treatment T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM). The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the lowest girth of tertiary fingers.

In open condition the girth of tertiary fingers ranged from 1.85 cm to 3.65 cm. The treatment T2 (PoP Soil test based nutrient management) recorded the highest girth of tertiary fingers and was on par with the treatment T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM). The treatment T3 (Adhoc PoP organic, KAU) recorded the lowest girth of tertiary fingers.

Higher girth of tertiary fingers was recorded in the polyhouse condition as compared to open conditions in all the treatments except T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM).

The interaction effects between treatments and conditions (polyhouse and open) was significant with respect to girth of tertiary fingers and irrespective of the conditions, the treatment T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM)) was significantly superior.

4.4.2.10 Thickness of the inner core

The thickness of inner core ranged from 0.75 cm to 3.07 cm in polyhouse condition. The treatment T3 (Adhoc PoP organic, KAU) recorded the highest thickness of inner core and the treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the lowest thickness of inner core.

In open condition the thickness of inner core ranged from 0.93 cm to 1.23 cm. The treatments T2 (PoP Soil test based nutrient management) and T4 (PoP nutrient management, KAU + salicylic acid foliar spray, 100 μM) recorded the highest thickness of inner core and the treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 μM) recorded the lowest thickness of inner core which was on par with the treatments T1 (Nutrient management as per PoP, KAU) and T3 (Adhoc PoP organic, KAU).

With respect to thickness of inner core, there was a significant difference between polyhouse and open conditions. As compared to open condition, all the treatments were superior in polyhouse except T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 μM).

The interaction effects between treatments and conditions (polyhouse and open) was significant and the treatment T3 (Adhoc PoP organic, KAU) was significantly superior to all the other treatments irrespective of the conditions in regards to thickness of the inner core.

4.4.2.11 Colour of the flesh of the rhizomes

The colour of flesh of the rhizomes obtained from all the treatments of polyhouse and open conditions was observed as pale yellow.

4.4.2.12 Plumpiness of rhizomes

The rhizomes obtained from all the treatments in polyhouse and open conditions were found to be of medium boldness

Table 9. Driage of rhizomes from various treatments in polyhouse and open conditions

Treatment	Driage (%)		
	Polyhouse	Open	Mean
T1	22.25	20.56	21.41
T2	22.05	22.74	22.40
T3	22.35	21.76	22.06
T4	22.15	22.22	22.19
T5	22.28	21.71	22.00
Mean	22.22	21.80	
Factors	C.D.		SE (m)
Factor A	N.S		0.35
Factor B	N.S.		0.22
Factor A X B	N.S.		0.49

Table 10. Yield of oleoresin from ginger rhizomes of various treatments

Treatment	Oleoresin yield (%)		
	Polyhouse	Open	Mean
T1	4.08	5.79	4.93
T2	5.39	9.91	7.65
T3	5.03	6.94	5.98
T4	5.17	7.95	6.56
T5	4.51	6.11	5.31
Mean	4.83	7.34	
Factors	C.D.		SE (m)
Factor A	0.53		0.17
Factor B	0.33		0.11
Factor A X B	0.75		0.23

Factor (A) – Treatment

Factor (B) – Condition (Polyhouse and Open)

Factor (A X B) – (Treatment X Condition)

T1- Nutrient management as per PoP (KAU) (control)

T2- PoP Soil test based nutrient management

T3- Adhoc PoP organic (KAU)

T4- PoP nutrient management (KAU) + Salicylic acid foliar spray (100 µM)

T5- PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

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4.5 Dry ginger recovery in different treatments

The dry ginger recovery was calculated and presented in Table 9. The dry ginger recovery of the rhizomes from various treatments ranged from 21.94 per cent to 22.35 per cent in polyhouse condition and 20.78 per cent to 22.69 per cent in open condition. There was no significant difference between the treatments with respect to driage of rhizomes both in polyhouse and open conditions. The dry ginger from rhizomes of various treatments in polyhouse and open conditions are presented in the Plates 10 and 11 respectively.

4.6 Extraction of oleoresin

Oleoresin was extracted from various treatments and the yield of oleoresin is presented in Table 10.

4.6.1 Yield of oleoresin

In polyhouse condition, the oleoresin yield ranged from 4.08 per cent to 5.39 per cent. The highest yield of oleoresin was recorded in the treatment T2 (PoP Soil test based nutrient management) and was on par with the treatments T3 (Adhoc PoP organic, KAU) and T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM). The lowest yield of oleoresin was recorded in the treatment T1 (Nutrient management as per PoP, KAU).

In open condition, the yield of oleoresin ranged from 5.79 per cent to 9.91 per cent. The treatment T2 (PoP Soil test based nutrient management) recorded the highest yield of oleoresin and was significantly superior to all the other treatments studied. The lowest yield of oleoresin was recorded in the treatment T1 (Nutrient management as per PoP, KAU).

T1-Nutrient management as per PoP (KAU) (control)

T2-PoP Soil test based nutrient management

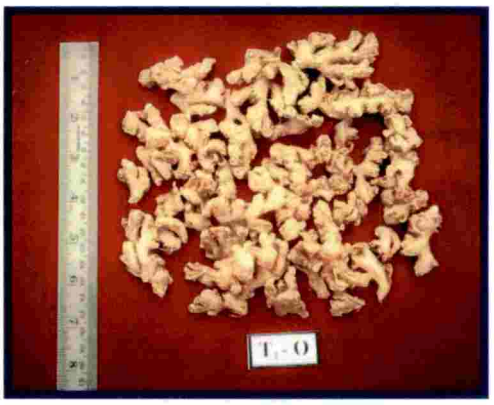
T3-Adhoc PoP organic (KAU)

T4-PoP nutrient management (KAU) + salicylic acid foliar spray (100 µM)

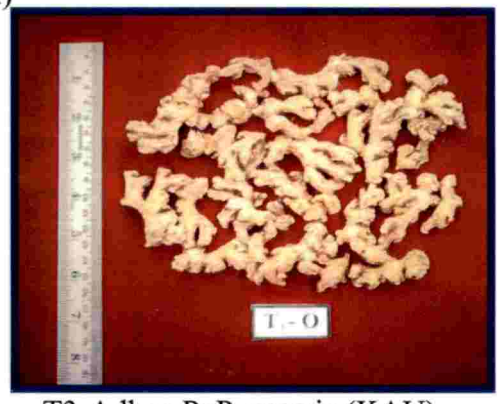
T5-PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

Plate 10. Dry ginger from rhizomes of various treatments in polyhouse

T1-Nutrient management as per PoP (KAU) (control)

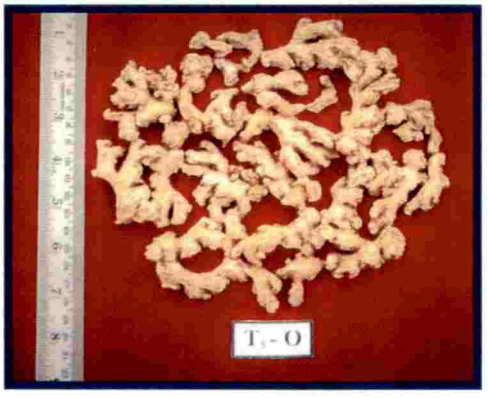


T2-PoP Soil test based nutrient management



T3-Adhoc PoP organic (KAU)

T4-PoP nutrient management (KAU) + salicylic acid foliar spray (100 µM)



T5-PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

Plate 11. Dry ginger from rhizomes of various treatments in open condition

Higher yield of oleoresin was recorded in open condition for all the treatments as compared to the polyhouse condition.

A significant interaction effect was observed between the treatments and the conditions (polyhouse and open) with respect to oleoresin yield. Irrespective of the conditions the treatment T2 (PoP Soil test based nutrient management) recorded the highest yield of oleoresin.

4.7 Estimation of gingerol by High Performance Liquid Chromatography (HPLC)

The quantity of gingerols responsible for the pungency was determined from the extracted oleoresins. The various gingerol components such as 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol were determined in percentage from the chromatogram for the rhizomes obtained from polyhouse and open conditions (Table 11). The chromatograms are presented in the Figures 1 and 2.

4.7.1 Estimation of 6-gingerol content

In polyhouse condition, the 6-gingerol content ranged from 14.24 per cent to 16.28 per cent. The treatment T2 (PoP Soil test based nutrient management) recorded the highest 6-gingerol content and was found to be significantly superior to all the other treatments studied. The treatment T1 (Nutrient management as per PoP, KAU) recorded the lowest 6-gingerol content.

The 6-gingerol content varied from 12.96 per cent to 16.61 per cent in open condition. The treatment T2 (PoP Soil test based nutrient management) recorded the highest 6-gingerol content and was found to be significantly superior to all the other treatments studied. The treatment T1 (Nutrient management as per PoP, KAU) recorded the lowest 6-gingerol content.

The 6-gingerol content did not show significant interaction effects between treatments and conditions (polyhouse and open).

4.7.2 Estimation of 8-gingerol content

In polyhouse condition the 8-gingerol content ranged from 0.96 per cent to 1.17 per cent. The treatment T3 (Adhoc PoP organic, KAU) recorded the highest 8-gingerol content and was on par with the treatments T2 (PoP Soil test based nutrient management) and T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM). The lowest content of 8-gingerol was recorded in the treatment T1 (Nutrient management as per PoP, KAU) and was on par with the treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM).

In open condition 8-gingerol content varied from 0.87 per cent to 1.19 per cent. The treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) recorded the highest 8-gingerol content and was on par with the treatments T2 (PoP Soil test based nutrient management) and T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM) and the treatment T1 (Nutrient management as per PoP, KAU) recorded the lowest 8-gingerol content and was on par with the treatment T3 (Adhoc PoP organic, KAU).

There was significant interaction effect between the treatments and the condition with respect to 8-gingetol content. Irrespective of the conditions, the treatments T2 (PoP Soil test based nutrient management), T3 (Adhoc PoP organic, KAU) and T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM) were on par with respect to 8-gingerol content.

4.7.3 Estimation of 10-gingerol content

The 10-gingerol content ranged from 1.25 per cent to 2.0 per cent in polyhouse condition. The treatment T4 (PoP nutrient management, KAU + Salicylic acid foliar spray - 100 µM) recorded the highest 10-gingerol content and was on par with the treatment T3 (Adhoc PoP organic, KAU). The treatment T1 (Nutrient management as per PoP, KAU) recorded the lowest 10-gingerol content in polyhouse.

In open condition, the 10-gingerol content ranged from 1.16 per cent to 1.99 per cent. The treatment T2 (PoP Soil test based nutrient management) recorded the highest 10-gingerol content and was on par with the treatments T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM) and T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM). The treatment T1 (Nutrient management as per PoP, KAU) recorded the lowest 10-gingerol content in polyhouse condition.

There was no significant interaction between polyhouse and open condition with respect to 10-gingerol content.

4.7.4 Estimation of total gingerol content

The total gingerol content ranged from 16.43 per cent to 18.94 per cent in polyhouse condition. The treatment T2 (PoP Soil test based nutrient management) recorded the highest total gingerol content. The treatment T1 (Nutrient management as per PoP, KAU) recorded the lowest total gingerol content.

In open condition the total gingerol content ranged from 14.98 per cent to 19.77 per cent. The treatment T2 (PoP Soil test based nutrient management) recorded the highest total gingerol content and was found to be significantly

superior to all other treatments studied. The treatment T1 (Nutrient management as per PoP, KAU) recorded the lowest total gingerol content.

There was no significant difference in the interaction effects between the treatments and conditions (polyhouse and open condition) with respect to total gingerol content.

4.7.5 Estimation of 6-shogaol content

The 6-shogaol content ranged from 0.60 per cent to 1.25 per cent in polyhouse condition and 1.03 per cent to 2.83 per cent in open condition. There was no significant difference between the treatments with respect to 6-shogaol content both in polyhouse and open conditions.

There was no significant difference in the interaction effects between treatments and the conditions (polyhouse and open) with respect to 6-shogaol content.

4.8 The expression of *Chalcone synthase* gene versus gingerol content

The *Chalcone synthase* gene expression versus gingerol content was also studied for the treatments in polyhouse and open conditions (Fig. 3). The *CHS* gene expression and gingerol content followed the same trend both in polyhouse and open condition (Table 12). The treatment T2 (PoP Soil test based nutrient management) showed the highest *CHS* gene expression and the highest gingerol content followed by T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM) then followed by T3 (Adhoc PoP organic, KAU) and then by T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM). All the treatments showed increased gene expressions and gingerol content than the T1 (Nutrient management as per PoP, KAU) (control) both in polyhouse and open conditions.

Table 11. The content of gingerols and shogaols in ginger rhizomes of various treatments in polyhouse and open conditions

Treatment	6-gingerol (%)			8-gingerol (%)			10-gingerol (%)			Total gingerol (%)			6-shogaol (%)		
	Polyhouse	Open	Mean	Polyhouse	Open	Mean	Polyhouse	Open	Mean	Polyhouse	Open	Mean	Polyhouse	Open	Mean
T1	14.24	12.96	13.60	0.96	0.87	0.91	1.25	1.16	1.20	16.43	14.98	15.71	1.16	1.03	1.10
T2	16.28	16.61	16.44	1.07	1.18	1.12	1.59	1.99	1.79	18.94	19.77	19.35	1.11	1.80	1.45
T3	14.96	15.24	15.10	1.17	0.97	1.07	1.73	1.50	1.61	17.85	17.71	17.78	1.05	2.83	1.94
T4	14.77	15.51	15.13	1.16	1.13	1.14	2.00	1.80	1.90	17.93	18.43	18.18	0.60	1.26	0.93
T5	15.07	14.27	14.67	0.99	1.19	1.09	1.54	1.82	1.68	17.60	17.28	17.44	1.25	1.15	1.20
Mean	15.06	14.92		1.07	1.07		1.62	1.65		17.75	17.63		1.03	1.61	
Factors	C.D.	SE (m)		C.D.	SE (m)		C.D.	SE (m)		C.D.	SE (m)		C.D.	SE (m)	
Factor A	0.74	0.23		0.10	0.03		0.22	0.07		0.78	0.24		N.S.	0.32	
Factor B	N.S.	0.15		N.S.	0.02		N.S.	0.04		N.S.	0.15		N.S.	0.20	
Factor A X B	N.S.	0.33		0.15	0.05		0.31	0.10		N.S.	0.35		N.S.	0.45	

Factor (A) – Treatment

Factor (B) – Condition (Polyhouse and Open)

Factor (A X B) – (Treatment X Condition)

Table 12. The expression of *Chalcone synthase* gene versus gingerol content

Treatment	*CHS* gene expression (fold)			Total gingerol content (%)		
	Polyhouse	Open	Mean	Polyhouse	Open	Mean
T1	1.000	1.000	1.000	16.43	14.98	15.71
T2	1.346	1.166	1.256	18.94	19.77	19.35
T3	1.016	1.024	1.020	17.85	17.71	17.78
T4	1.210	1.049	1.130	17.93	18.43	18.18
T5	1.001	1.010	1.006	17.60	17.28	17.44

T1- Nutrient management as per PoP (KAU) (control)
T2- PoP Soil test based nutrient management
T3- Adhoc PoP organic (KAU)
T4- PoP nutrient management (KAU) + salicylic acid foliar spray (100 µM)
T5- PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

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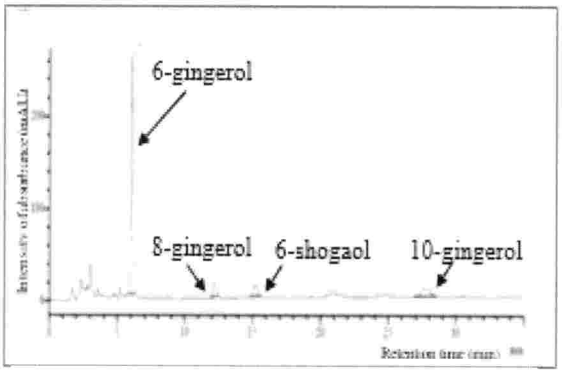
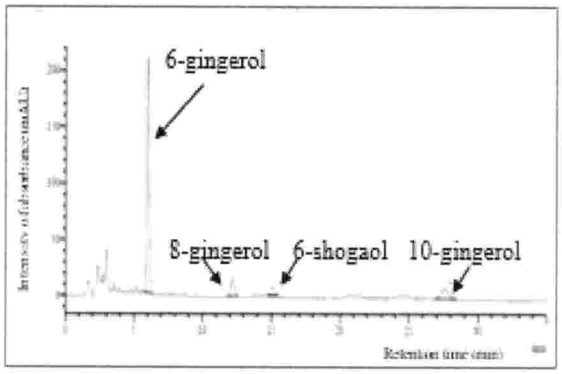
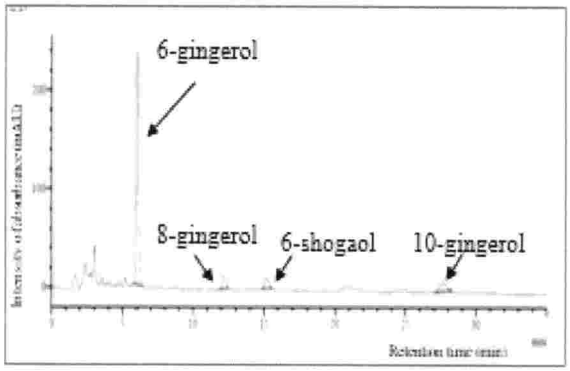


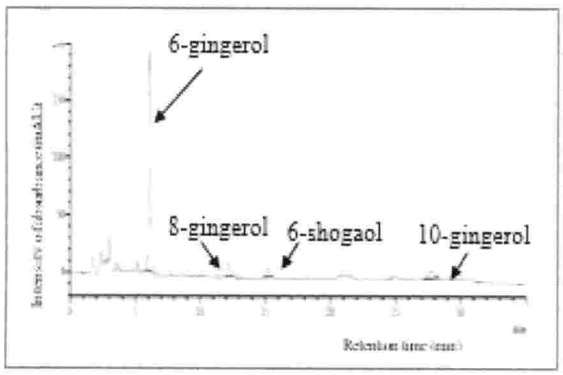
T1-Nutrient management as per PoP (KAU) (control)



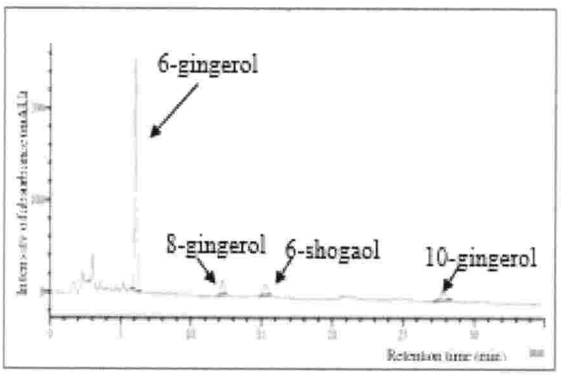
T2-PoP Soil test based nutrient management



T3-Adhoc PoP organic (KAU)

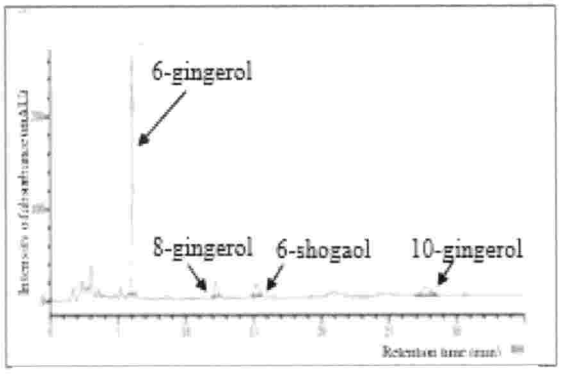


T4-PoP nutrient management (KAU) + salicylic acid foliar spray (100 µM)

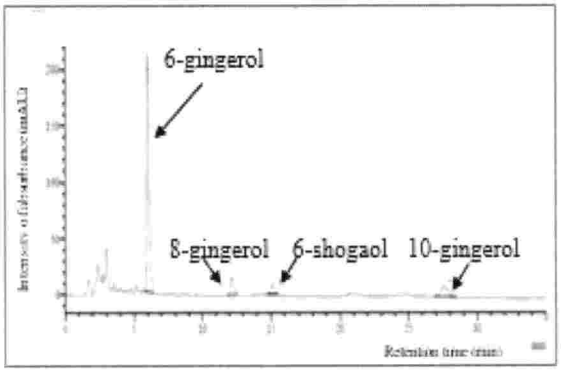


T5-PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

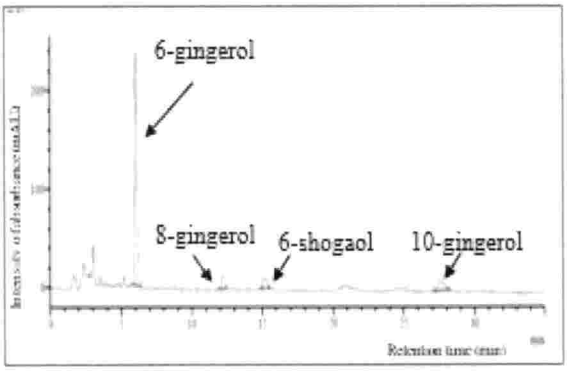
Fig 1. Chromatograms of gingerols and shogaol content in various treatments under polyhouse condition



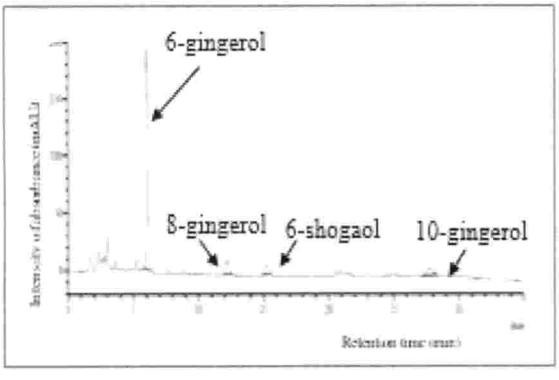
T1-Nutrient management as per PoP (KAU) (control)



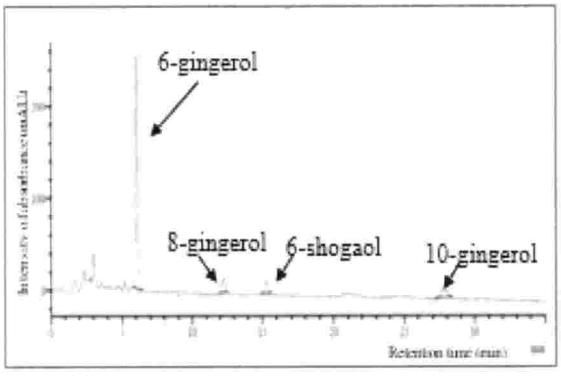
T2-PoP Soil test based nutrient management



T3-Adhoc PoP organic (KAU)

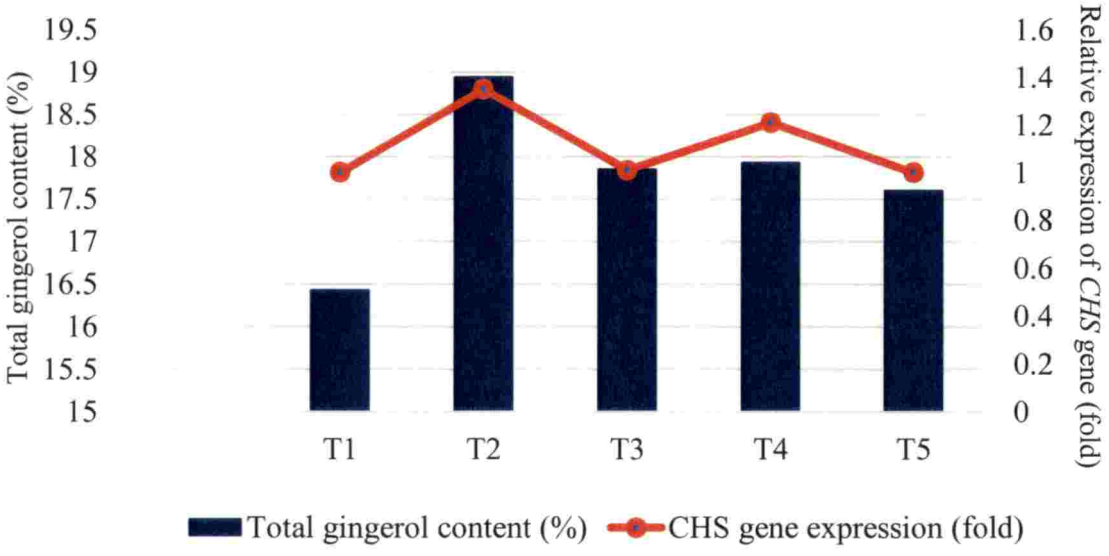


T4-PoP nutrient management (KAU) + salicylic acid foliar spray (100 µM)

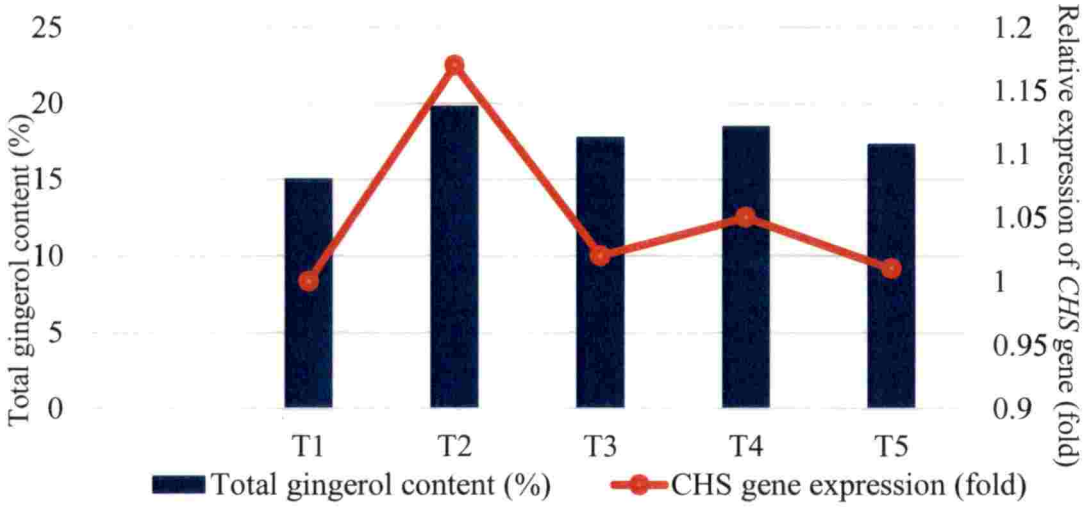


T5-PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

Fig 2. Chromatograms of gingerols and shogaol content in various treatments under open condition



***CHS* gene expression vs. total gingerol content under polyhouse condition**



***CHS* gene expression vs. total gingerol content under open condition**

T1- Nutrient management as per PoP (KAU) (control)

T2- PoP Soil test based nutrient management

T3- Adhoc PoP organic (KAU)

T4- PoP nutrient management (KAU) + Salicylic acid foliar spray (100 µM)

T5- PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

Fig 3. The expression of *Chalcone synthase* gene versus gingerol content

In polyhouse the treatment T2 (PoP Soil test based nutrient management) showed the highest *CHS* gene expression (1.35 fold) and total gingerol content of 18.94 per cent whereas in open condition the treatment T2 (PoP Soil test based nutrient management) showed the highest *CHS* gene expression (1.17 fold) and total gingerol content of 19.77 per cent.

Discussion

5. DISCUSSION

The investigations entitled "Expression of *Chalcone synthase* gene in ginger (*Zingiber officinale* Rosc.) as influenced by various management practices were carried out at Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture, Kerala Agricultural University, Thrissur from 2017 to 2019 to analyse the influence of nutrient management and spraying of elicitors on *Chalcone synthase* gene expression in ginger under polyhouse and open conditions. The results obtained for various treatments are discussed in this chapter.

5.1 Growth performance of ginger in various treatments

The morphological characters such as height of the pseudostem, number of tillers per plant, number of leaves per tiller, length, width and area of the last fully opened leaf of the plants were recorded for twenty four plants in each treatment at 60, 90 and 120 DAP both under polyhouse and open conditions.

5.1.1 Length of the pseudostem

The length of pseudostem recorded at 60, 90 and 120 DAP in polyhouse and open conditions was graphically represented in Figure 4. In polyhouse, length of the pseudostem increased as the age of the crop advanced from 60 to 90 DAP in the treatments T1 (Nutrient management as per PoP (KAU)), T2 (PoP Soil test based nutrient management), T3 (Adhoc PoP organic (KAU)) and T4 (PoP nutrient management (KAU) + Salicylic acid foliar spray (100 µM)). In the treatment T5 (PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)) the length of the pseudostem was found less as compared to other treatments. The mechanism leading to the inhibition of plant growth by methyl jasmonate is not well understood but Swiatek *et al.* (2002, 2004) suggested that jasmonate can inhibit the cell division cycle. However in a recent study, Kim *et al.* (2015) suggested that methyl jasmonate can reduce plant growth as it triggers plant

senescence when applied exogenously. As in the present study, the plant height was reduced in soybean, sunflower and tomato even with the lowest concentration of 0.1 mM methyl jasmonate (Li *et al.*, 2018). Similar reduction in plant height was recorded by Heijari *et al.* (2005) in Scots pine seedlings, Gould *et al.* (2009) in *Pinus radiata*, Sampedro *et al.* (2011) in *Pinus pinaster* and Moreira *et al.* (2012) in young conifer trees upon the application of elevated levels of methyl jasmonate.

In open condition also the length of pseudostem was increased with the advancement of crop age from 60 to 120 DAP.

At all the three stages of observations 60, 90 and 120 DAP, the plants raised under polyhouse condition recorded higher pseudostem length than the plants raised in open condition. The effect of Indole Acetic Acid (IAA) oxidase and other isozymes which acted as peroxidase and catalyzed the degradation of auxins which enhanced the availability of auxin to plants which might have contributed for the increase in length of the pseudostem of the plants in polyhouse (Kawaguchi *et al.*, 1993). Similar observation on increase in height of pseudostem under polyhouse condition was reported by Padmapriya and Chezhiyan (2009).

5.1.2 Number of tillers per plant

The number of tillers per plant was recorded at 60, 90 and 120 DAP in polyhouse and open conditions and the same was graphically represented in Figure 5. The number of tillers per plant was found to have increased progressively irrespective of the treatments studied in polyhouse and open conditions. The tiller production was found more in the plants raised in open condition than in polyhouse condition. The enhanced synthesis of growth promoting hormones would have induced apical dominance thus reducing the tiller production in polyhouse (Padmapriya and Chezhiyan, 2009). The result of the current study is in agreement with the findings of Latha *et al.* (1995) and Padmapriya and Chezhiyan (2009) in turmeric.

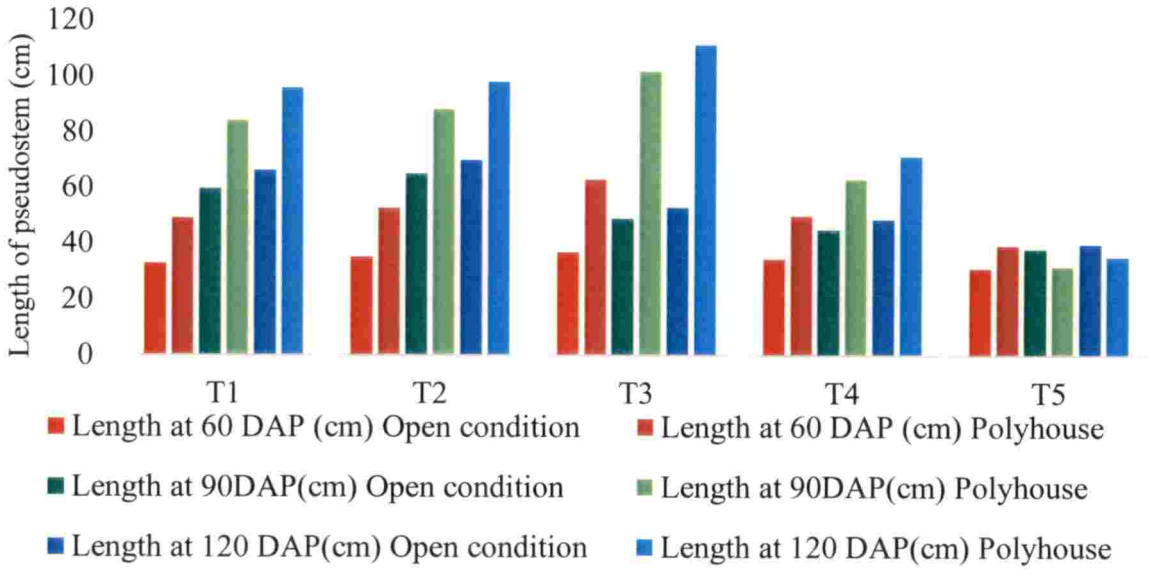


Fig. 4 Length of pseudostem at different growth stages of the crop

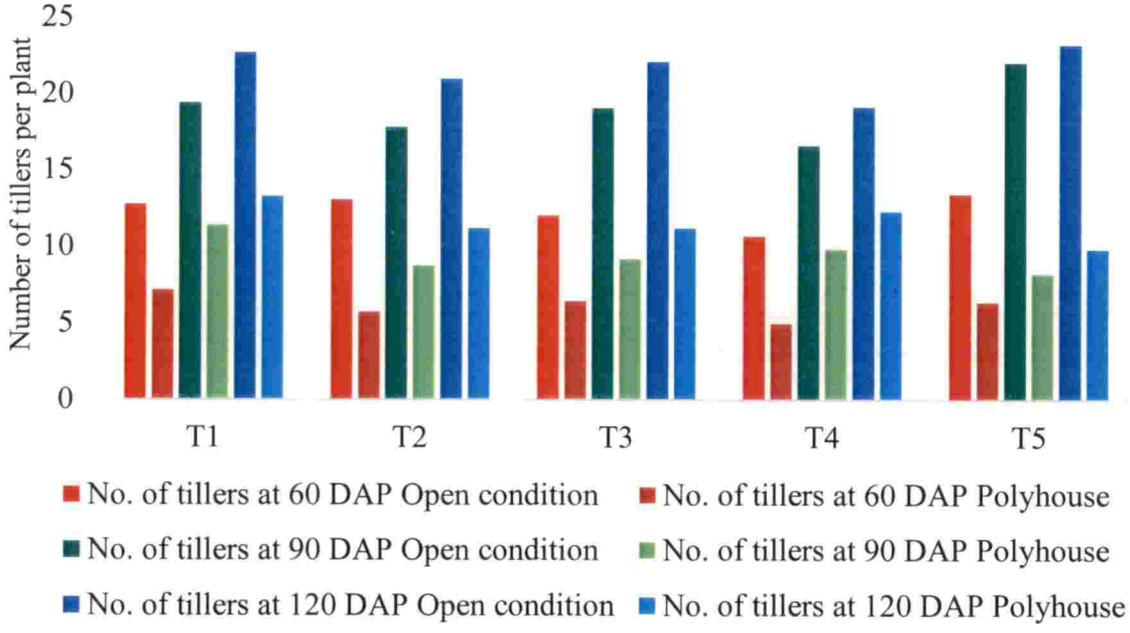


Fig. 5 Number of tillers at different growth stages of the crop

T1- Nutrient management as per PoP (KAU) (control)

T2- PoP Soil test based nutrient management

T3- Adhoc PoP organic (KAU)

T4- PoP nutrient management (KAU) + salicylic acid foliar spray (100 µM)

T5- PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

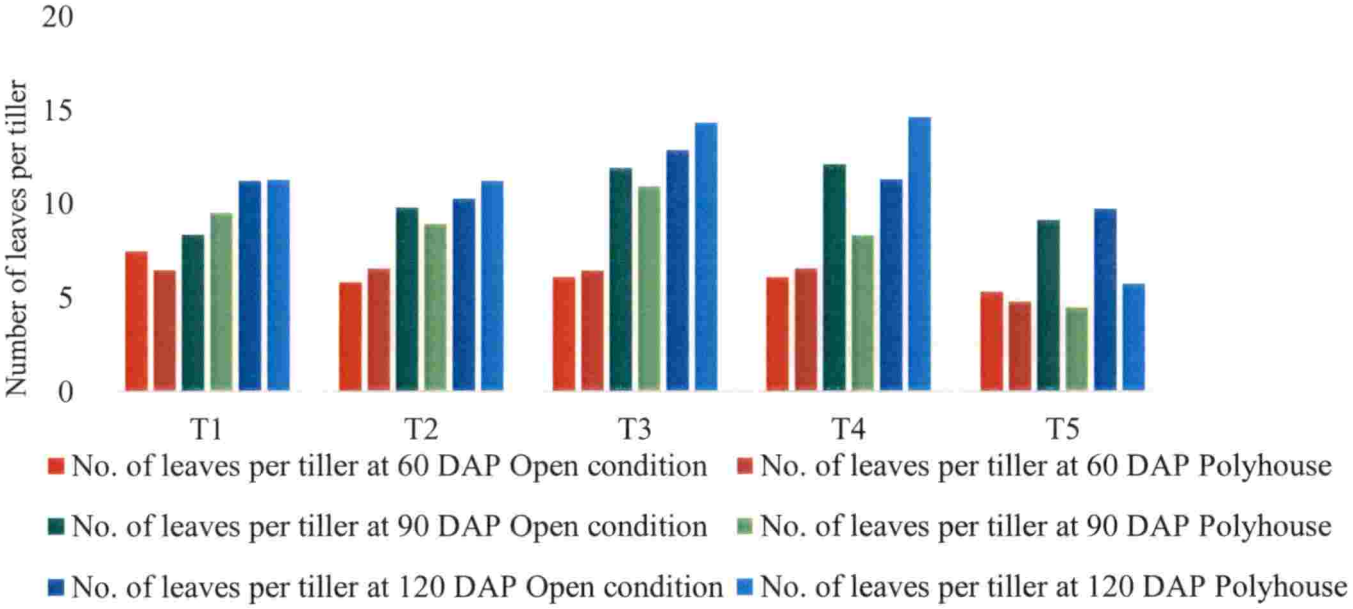


Fig. 6 Number of leaves per tiller at different growth stages of the crop

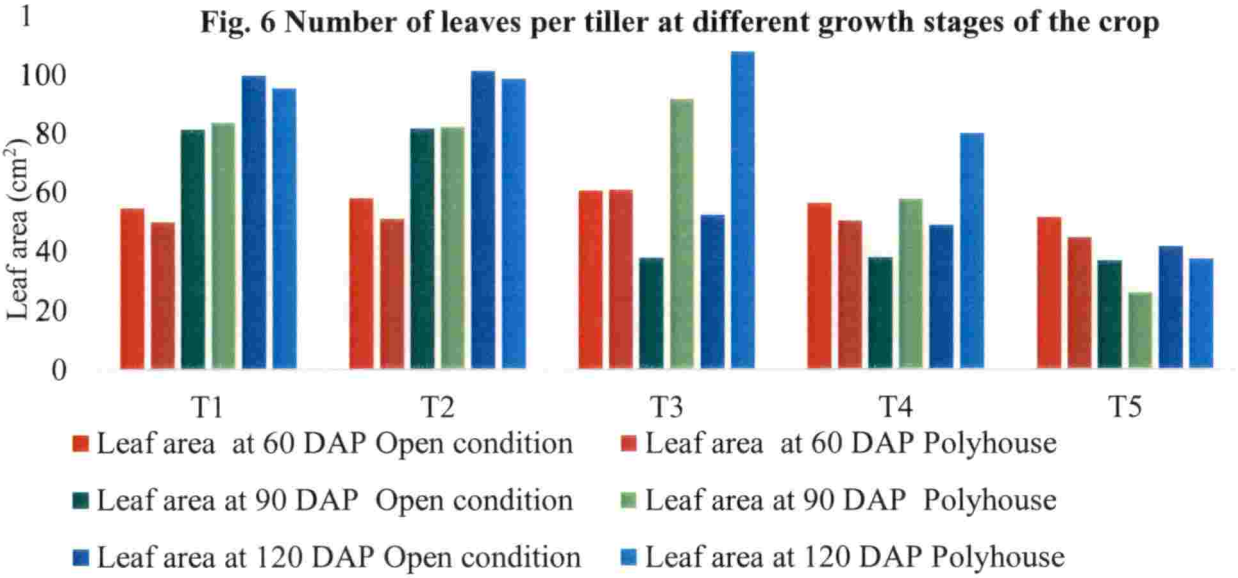


Fig. 7 Leaf area at different growth stages of the crop

T1- Nutrient management as per PoP (KAU) (control)

T2- PoP Soil test based nutrient management

T3- Adhoc PoP organic (KAU)

T4- PoP nutrient management (KAU) + salicylic acid foliar spray (100 µM)

T5- PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)

5.1.3 Number of leaves per tiller

The number of leaves per tiller was also recorded at 60, 90 and 120 DAP and are graphically represented in Figure 6. In polyhouse the plants of all the treatments except the plants of the treatment T5 (PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)) recorded an increase in the number of leaves per tiller. In open condition also the number of leaves per tiller was less in the treatment T5 (PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)). Kim *et al.* (2015) suggested that methyl jasmonate can induce plant senescence upon exogenous application. Similar observation was reported by Li *et al.* (2018) with the exogenous application of methyl jasmonate even at low concentration.

The plants in polyhouse was found superior to the plants in open condition with respect to number of leaves per tiller. Pahalwan *et al.* (2004) opined that under shade conditions, the plants would produce sufficient number of leaves as the light energy harnessed can be efficiently used by the leaves for the synthesis of photosynthates that contribute towards the increased number of leaves to tiller. George *et al.* (1997) and Padmapriya and Chezhiyan (2009) recorded an increased production of leaves in turmeric raised under shaded condition.

5.1.4 Leaf area

The leaf area was found higher in treatments T1 (Nutrient management as per PoP (KAU)), T2 (PoP Soil test based nutrient management) and T3 (Adhoc PoP organic (KAU)) in all the growth stages in the two conditions (polyhouse and open). The treatments T4 (PoP nutrient management (KAU) + Salicylic acid foliar spray (100 µM)) and T5 (PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)) recorded the lowest leaf area at different growth stages in the two growing conditions. Salicylic acid at higher concentration is often considered

inhibitory according to Haroun *et al.* (1998) in lupine, Singh & Usha (2003) in wheat, Abdel-Wahed *et al.* (2006) in maize, Kord and Hathout (1992) in tomato.

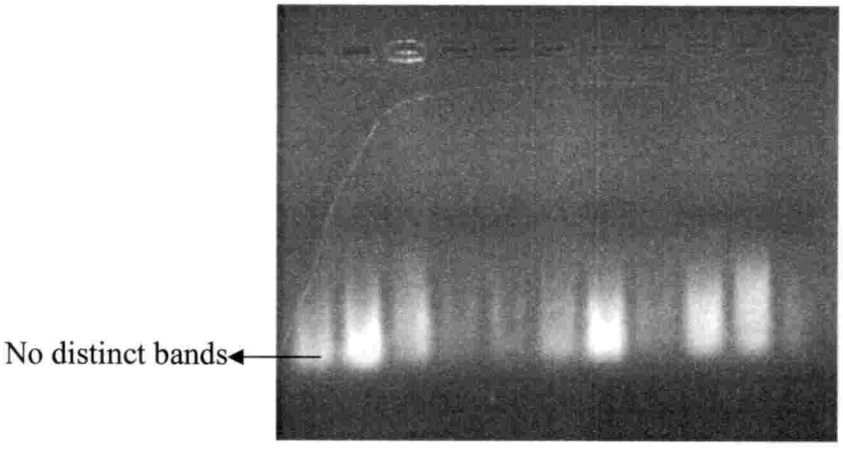
The loss of photosynthetic pigments as suggested by Kazemi *et al.* (2014) in tomato due to exogenous application of methyl jasmonate that can affect the vegetative growth of the plants. Also an increased osmotic stress can impair the vegetative growth of the plants treated with methyl jasmonate.

5.2 Expression analysis of *Chalcone synthase* gene in ginger using quantitative Real Time PCR

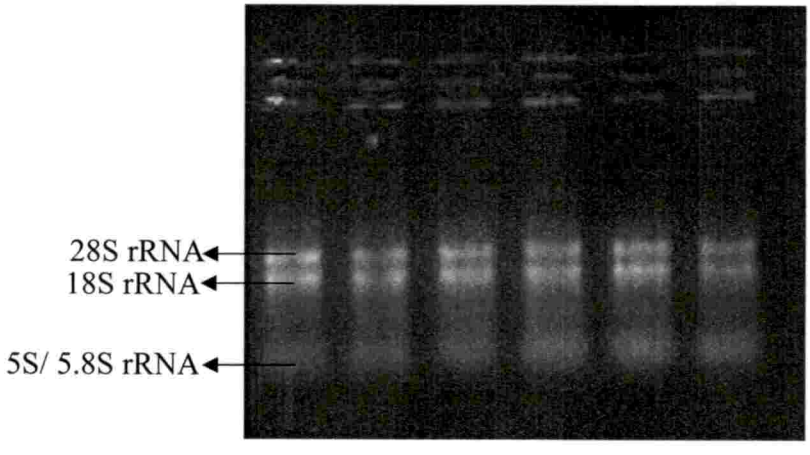
5.2.1 Isolation of total RNA

In the present study total RNA was isolated from the leaves of the ginger plant. The modified protocol suggested by Sreeja (2017) was followed. The protocol followed with certain modifications as mentioned in 3.5.1.1.1 helped in better recovery of high quality total RNA from the leaves which was confirmed upon agarose gel electrophoresis. All the RNA samples possessed three distinct bands that corresponded to 28S rRNA, 18S rRNA and 5S rRNA +tRNA (Plate 12).

The leaf samples were homogenized thoroughly with liquid nitrogen so as to enhance the disruption of cells along with increased recovery of nucleic acids. Trizol which served as a monophasic solution constituting of phenol and guanidiumisothiocyanate is known for its instant solubilisation of biological materials and protein denaturation. The phenol aids in the phase separation and promotes the separation of the upper and the lower organic phases with adequate denaturation of protein whereas guanidium salts facilitates the removal of the effect of nucleases. The homogenate was laid down horizontally at room temperature after trizol addition. The tubes were laid down horizontally so as to enhance the area of contact for adequate nucleoprotein complex dissociation. The addition of chloroform enhance the removal of protein impurities such as chlorophyll. The



RNA isolated from ginger leaves using the protocol (Sreeja, 2017)



RNA isolated by the modified protocol

Plate 12. Comparison of the quality of RNA isolated from different protocols

addition of chloroform along with phenol was found to have reduced the of poly(A) from mRNA into the organic phase thus reducing the formation of RNA-protein complexes at the interphase that are insoluble (Perry *et al.*, 1972). Another extraction with chloroform was followed so as to remove the some left over impurities such as insoluble lipids. The addition of chloroform over the solubilized product and centrifugation enhance the phase separation with the proteins in the organic phase, DNA at the interphase and RNA retained in the aqueous phase. Addition of sodium acetate and ice-cold isopropanol was followed so as to precipitate the RNA. Sodium acetate is known to neutralize the negative charge on the sugar phosphate backbone in the RNA with its positively charged sodium ions which makes the molecule less hydrophilic and thus reducing the solubility in water. The precipitation of larger nucleic acids is facilitated by the addition of lower concentrations of isopropanol. After the precipitation of the sample with isopropanol and sodium acetate in freezer it was washed with ice cold 75 per cent DEPC ethanol. A white pellet obtained was then dried (not over dried) and dissolved in nuclease-free water.

The spectrophotometric analysis of the RNA samples showed that the total RNA isolated was free from carbohydrate, protein and polyphenol with A260/A280 and A260/A230 of 1.8 to 2.3 and above 1.0 respectively.

5.2.2 First strand cDNA synthesis

The cDNA was synthesized from the isolated total RNA using First Strand cDNA Synthesis Kit (Thermoscientific). The kit employs ReverseAid Reverse Transcriptase with lower RNase H activity with an optimum activity between 42 and 50^0C. This is suitable for the synthesis of cDNA up to 13 kb. The RiboLock RNase inhibitor protects RNA from degradation at 55^0C. The oligo $(dT)_{18}$ primer was annealed to the tail poly(A) tail of mRNA. The synthesized first strand cDNA was directly employed as a template in PCR and qRT-PCR.

5.2.2.1 Confirmation of the synthesis of first strand cDNA using primers

The relative gene expression of *chalcone synthase* was analysed in the present study using *Actin* as the reference gene. The *Actin* gene specific primers were designed from the sequence (EST clone ZO__Ed0002F02: DY357890) and the first strand cDNA was amplified using *Actin* and *Chalcone synthase* gene specific primers. The desired band size of 141 and 138 bp was observed for *CHS* and *Actin* gene specific primers when run on 1.8 per cent agarose gel.

The housekeeping gene acts as the internal control which has sequences different from the target. The reference gene must be consistent and must remain unaffected by the experimental factors and should exhibit a minimum variability in the expression between the tissue and physiological states of the organism (Chervoneva *et al.*, 2010). The housekeeping genes are the basic metabolism genes that accomplished these conditions and are found to be involved in the essential processes for the cell survival and thus showing a consistent expression (Thellin *et al.*, 2009).

The commonly employed reference genes in qRT-PCR studies are *Actin* gene, *ubiquitin-conjugating enzyme* (*UBI-1*), *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*), *β-tubulin* (*β-TUB*), *ubiquitin-conjugating* protein (*UBI-3*), *eukaryotic initiation factor 5A2* (*EIF5A2*) and *α-tubulin* (*α-TUB*). geNorm, BesKeeper and NormFinder are some of the statistical algorithms used commonly for the identification of expression stability and promote the selection of accurate housekeeping genes. The expression stability of the "classical" reference gene *Actin*, was analysed using genorm^{PLUS}, NormFinder and BestKeeper algorithms (Manoli *et al.*, 2012). *Actin* gene was found to play an important role in plant cell cytoplasmic streaming, cell shape determination, cell division, organelle movement and extension growth (Staiger and Schliwa, 1987). In the present study, *Actin* gene is considered the optimal reference gene in *Zingiber officinale* Rosc. for qRT-

PCR. The target gene to be studied using qRT-PCR is the *Chalcone synthase* (*CHS*) gene. The *CHS* gene specific primers were designed from the sequence (EST clone ZO__Ed0002F02: DY357890) and used for the amplification of cDNA from different treatments. The desired band size was 141 bp for *CHS* gene and *Actin* gene respectively.

5.2.2.2 Real Time PCR

A well-developed platform for the quantification of gene expression levels is the Real Time PCR that detects the amount of amplicon using fluorescence in every cycle of the PCR.

The number of cycles of PCR that is necessary to achieve a given level of fluorescence is regarded the C_T value and read out in Real-Time PCR. C_T value was fixed in the exponential phase of the PCR. The use of fluorescent dyes such as SYBR Green and their binding to the double-stranded DNA favoured the Real-Time imaging and observed by about 1000-fold increased intensity of fluorescence (Hugget *et al.*, 2005). Another approach is the use of fluorescent-labelled oligonucleotides in which the signals are generated when the probes that are specific to amplicons hybridise to the complementary region. The reaction can be made efficiently specific using chemistry such as "TaqMan" probes (hydrolysis probes) or the "molecular probes" (structured probes). These are very epensive as compared to that of SYBR Green. SYBR® Premix Ex Taq™ (Tli RNaseH Plus) provided by TaKaRa was used in the present study. In the initial PCR reaction cycles, the fluorescence signal emitted by the PCR product bound with SYBR® Premix Ex Taq™ was weak. Also until 16 and 22 PCR cycles any difference was not defined for *Actin* and *CHS* genes respectively. The PCR cycles reached saturation at 35 cycles during which the intensity of the fluorescent signal began to plateau. The threshold cycle can be defined as the cycle at which the fluorescence emitted by the target is above the threshold and baseline and within the exponential

region of the amplification curve. The lower the target DNA lower is the increase in the fluorescence and higher will be the C_T and vice versa.

5.2.2.3 Relative gene expression analysis

The gene expression can be analysed by either absolute or relative quantification. In relative method, the expression of a gene can be determined in which one gene is set as a calibrator *i.e.* the gene that is not exposed to the experimental factor and against to which the change is given (Tyburski *et al.*, 2008). The difference between ΔC_T of the target gene and the control gene is calculated for each sample, then subtracted between ΔC_T of sample with unknown concentration and ΔC_T of the calibrator. The relative quantification was done using $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

In the study endogenous control *Actin* was used, the C_T values gave nearly the same values in control *i.e.*, in the treatment T1 (Nutrient management as per PoP (KAU)) in open condition and polyhouse and all the treatments in open condition and polyhouse. This indicated that the cDNA samples from all the treatments were normalized. The expression patterns of the genes were analysed by relative quantification.

5.2.2.3.1 Relative expression analysis of *CHS* gene

Chalcone synthase (CHS) superfamily is of plant type III polyketide synthases (PKSs). CHS is known to catalyze the iterative decarboxylative condensations of malonyl with a CoA-linked initiator molecule leading to the production of important plant secondary metabolites that are structurally diverse, and pharmaceutically important (Abe and Morita, 2010).

In the present study the influence of nutrient management and spraying of elicitors on *Chalcone synthase* gene expression in ginger was analysed under polyhouse and open conditions.

The *CHS* gene showed 1.166 and 1.346 fold increase in the treatment T2 (PoP Soil test based nutrient management) of polyhouse and open conditions respectively and was found to be the highest. The neutral pH of the soil, high amounts of organic carbon, available form of phosphorous, potassium and sufficient amounts of calcium, magnesium and Sulphur along with the foliar spray of borax and the timely application of recommended amounts of nitrogenous, phosphatic and potassic fertilizers led to the increased amount of total gingerol in both polyhouse and open conditions.

The increase in relative *CHS* gene expression in the treatment T4 (PoP nutrient management (KAU) + Salicylic acid foliar spray (100 µM)) of polyhouse and open condition was found to be 1.049 and 1.21 folds respectively. Salicylic acid is also found to have involved in some signal transduction system that can induce the enzymes that have roles in the biosynthesis of secondary metabolites (Taguchi *et al.*, 2001 and Chen *et al.*, 2006).

However T2 (PoP Soil test based nutrient management) yielded increased *CHS* expression than T4 (PoP nutrient management (KAU) + Salicylic acid foliar spray (100 µM)), there was no significant difference in their expressions in both the polyhouse and open conditions. The increased expression of *Chalcone synthase* gene in the treatment T2 (PoP Soil test based nutrient management) can be attributed to the adequate supply of nutrients which created a conducive environment for the expression of *Chalcone synthase* gene in the leaves which enhanced the synthesis of the metabolite in the leaves during the active growth phase of up to four MAP. Zunun-Perez *et al.* (2017) could find no significant increase in the expression of these genes in salicylic acid treatment.

In the present study the relative expression of *CHS* gene in the plants of treatment T5 (PoP nutrient management (KAU) + methyl jasmonate foliar spray (100 µM)) showed only 1.001 and 1.010 fold level of increased expression in the polyhouse and open conditions respectively. This shows slight upregulation of the expression of the *CHS* gene but the upregulation was found to be non-significant.

Methyl jasmonate induced and coordinated the suppression of key enzyme, Chalcone synthase in flavonoid biosynthesis in *Scutellaria viscidula* (Wei *et al.*, 2010). Also the interaction of methyl jasmonate with the specific receptors in the nucleus and membranes can activate a signaling pathway that can induce the transcription factors by the activating or repressing the methyl jasmonate regulated genes (Kazan and Manners, 2008).

5.3 Harvesting of ginger rhizomes and gingerol estimation

5.3.1 Fresh yield of rhizomes

The treatments T1 (Nutrient management as per PoP (KAU)), T2 (PoP Soil test based nutrient management) and T3 (Adhoc PoP organic (KAU)) recorded yield on par in the polyhouse condition. This may be because of the enhanced availability of nutrients as per crop demand and the mineralization of organic compounds in available form created an environment that is conducive enough for the plants to grow. Also the use efficiency in terms of inorganic nitrogenous fertilizer would have further enhanced the plant growth leading to the increased yield in the treatment Srinivasan *et al.* (2019) observed that the yield of rhizomes obtained from organic nutrient management and chemical nutrient management to be identical but the integrated nutrient management recorded an increased yield as compared to the other two treatments.

. However as compared to the average yield reported for the variety Karthika, the recorded yield in the present study was low. This could be because of the severe flooded situation experienced during July and August, 2018.

5.3.2 Yield of oleoresin

In the present study significant variations were noticed among the treatments with respect to oleoresin yield. The treatment T2 (PoP Soil test based nutrient management) recorded the highest oleoresin yield in both polyhouse and open conditions which may be due to the adequate supply of nutrients required for the translocation of the secondary metabolites synthesized in the leaves.

5.3.3 Estimation of gingerol by High Performance Liquid Chromatography (HPLC)

The treatment T2 (PoP Soil test based nutrient management) of polyhouse and open conditions recorded the highest 6-gingerol content, total gingerol content in polyhouse and open conditions, 10-gingerol in polyhouse condition. The neutral pH of the soil, high amounts of organic carbon, available form of phosphorous, potassium and sufficient amounts of calcium, magnesium and sulphur along with the foliar spray of borax and the timely application of recommended amounts of nitrogenous, phosphatic and potassic fertilizers led to the increased amount of total gingerol in both polyhouse and open conditions. The influence of boron in translocation of photosynthates might have contributed to the higher recovery of gingerol in the treatment T2 (PoP Soil test based nutrient management). The higher recovery observed in the open condition might be due to the severe stress situation experienced in the open condition as compared to the polyhouse. The influence of light on the upregulation of *Chalcone synthase* gene expression also cannot be ruled out (Fuglevand *et al.*, 1996).

The treatment T4 (PoP nutrient management (KAU) + Salicylic acid foliar spray (100 µM)) recorded the highest 10-gingerol content in open condition. Idrees *et al.* (2011) suggested that salicylic acid enhanced the accumulation of phenolic, terpenoid and alkaloid compounds. Salicylic acid is also found to have involved in some signal transduction system that can induce the enzymes that have roles in the biosynthesis of secondary metabolites (Taguchi *et al.*, 2001 and Chen *et al.*, 2006). Salicylic acid showed only a minor increase of 6 per cent in the capsiate contents in the fruits of pepper in the day before harvest foliar sprays at 0.1 mM, 1 mM and 1 mM weekly foliar spray (Zunun-Perez *et al.*, 2017).

The rhizomes obtained from the treatment T5 (PoP nutrient management (KAU) methyl jasmonate foliar spray (100 µM)) recorded the highest 8-gingerol content in open condition. Methyl jasmonate an important signaling compound in the process of elicitation leading to the increased production of secondary metabolites (Walker et al., 2002). A key role is played by methyl jasmonate in signal transduction processes that effectively regulate and enhance the production of secondary metabolites (Zhao *et al.*, 2010).

5.3.5 The expression of *Chalcone synthase* gene versus total gingerol content

The treatment T2 (PoP Soil test based nutrient management) of polyhouse and open conditions recorded the highest total gingerol content. The highest relative *CHS* gene expression was found to be 1.166 and 1.346 fold in the treatment T2 (PoP Soil test based nutrient management) of polyhouse and open conditions respectively and was found to be the highest. This can be substantiated by the findings of Ghosh and Mandi (2015) which showed that *Chalcone synthase* gene expression was higher in high gingerol containing landraces as compared to the intermediate and low gingerol containing landraces of ginger. This suggests that gingerol content is associated with the expression of *CHS* gene.

The major outcome of the present investigations is that *Chalcone synthase* gene expression is influenced by various management practices and spraying of elicitors. The *Chalcone synthase* gene expression is related to gingerol content. Soil test based nutrient management recorded the highest *Chalcone synthase* gene expression and high gingerol recovery. The treatment T2 (PoP soil test based nutrient management) recorded the highest expression of *Chalcone synthase* gene under polyhouse condition with a fold increase of 1.346 and under open condition with a fold increase of 1.166. The treatment T2 (PoP Soil test based nutrient management) recorded higher recovery of oleoresin under polyhouse condition with an increase of 32.11 per cent over the control and under open condition with an increase of 71.16 per cent over the control. The treatment T2 (PoP Soil test based nutrient management) recorded higher recovery of total gingerol under polyhouse condition with an increase of 15.28 per cent over the control and under open condition with an increase of 31.98 per cent over the control. Spraying of abiotic elicitors could not improve the gingerol content.

Soil test based management practices can be recommended for recovery of high gingerol. Soil test based management is cost effective in terms of application of fertilisers at the same it is effective for high gingerol recovery. The results from present investigations has to be validated in large scale field experiments. Expression studies of other genes involved in gingerol biosynthetic pathway has to be elucidated for further metabolic pathway engineering.

Summary

6. SUMMARY

The research work entitled "Expression of *Chalcone synthase* gene in ginger (*Zingiber officinale* Rosc.) as influenced by various management practices" was carried out at Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture, Kerala Agricultural University, Thrissur, from 2017-2019, with the objective to analyse the influence of nutrient management and spraying of elicitors on *Chalcone synthase* gene expression in ginger under polyhouse and open conditions.

The salient findings of the study are summarised below:-

- The plants raised under polyhouse condition recorded the highest length of the pseudostem at 60, 90 and 120 DAP.

- An increased tiller production was observed in the plants raised under open condition at 60, 90 and 120 DAP.

- The treatment T3 (Adhoc PoP organic- KAU) recorded the highest number of leaves per tiller irrespective of the growing conditions.

- The highest leaf area was recorded by the plants under polyhouse condition at 90 and 120 DAP.

- All the treatments recorded a higher expression of *Chalcone synthase* gene than the control treatment T1 (Nutrient management as per PoP- KAU).

- The treatment T2 (PoP Soil test based nutrient management) recorded the highest relative expression of *Chalcone synthase* gene both under polyhouse and open conditions with a fold increase of 1.346 and 1.116 over the control respectively.

- The plants under polyhouse recorded higher fresh yield of rhizomes per plant than the plants raised under open condition.

- The treatment T1 (Nutrient management as per PoP- KAU) recorded the highest fresh yield of rhizomes per plant irrespective of the conditions.
- The rhizome characters were better in the treatments T1 (Nutrient management as per PoP- KAU), T2 (PoP Soil test based nutrient management) and T3 (Adhoc PoP organic- KAU).

- There was no significant difference in the dry ginger recovery for the rhizomes harvested from various treatments both in polyhouse and open conditions.

- The treatment T2 (PoP Soil test based nutrient management) recorded the highest yield of oleoresin both under polyhouse and open conditions with 5.39 and 9.91 per cent respectively.

- The treatment T2 (PoP Soil test based nutrient management) recorded 32.11 per cent and 71.16 per cent increase of oleoresin yield under polyhouse and open conditions over the control respectively.

- The highest total gingerol content was recorded in the treatment T2 (PoP Soil test based nutrient management) both in polyhouse and open conditions with 18.94 and 19.77 per cent respectively.

- An increase of 15.28 per cent and 31.98 per cent of total gingerol was recorded in the treatment T2 (PoP Soil test based nutrient management) both in polyhouse and open conditions over the control respectively.

References

REFERENCES

Abdel - Wahed, M.S.A., Amin, A.A., and El- Rashad, S. M. 2006. Physiological effect of some bioregulators on vegetative growth, yield and chemical constituents of yellow maize plants. *World J. Agric. Sci.* 2(2): 149-155.

Abe, I. and Morita, H. 2010. Structure and function of the chalcone synthase superfamily of plant type III polyketide synthases. *Natural Product Reports 27* (6): 809-838.

Ali, B. H., Blunden, G., Tanira, M. O., and Nemmar, A. 2008. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): A review of recent research. *Food Chem. Toxicol.* 46(2)**:** 409-420.

AOAC. 1980. Official Methods of Analysis of the Association of Official Analyticalchemists. Thirteenth edition. Association of Official Analytical Chemists, Washington D. C., 525p.

Awasthi, P., Mahajan, V., Jamwal, V. L., Kapoor, N., Rasool, S., Bedi, Y. S., and Gandhi, S. G. 2016. Cloning and expression analysis of *Chalcone synthase* gene from *Coleus forskohlii. J. Genet.* 95(3): 647-657.

Bailey-Shaw, Y. A., Williams, L. A., Junor, G. A. O., Green, C. E., Hibbert, S. L., Salmon, C. N., and Smith, A. M. 2008. Changes in the contents of oleoresin and pungent bioactive principles of Jamaican ginger (*Zingiber officinale* Roscoe.) during maturation. *J. Agric. Food Chem. 56*(14): 5564-5571.

Balladin, D. A., Headley, O., Chang-Yen, I., and McGaw, D. R. 1998. High pressure liquid chromatographic analysis of the main pungent principles of solar dried West Indian ginger (*Zingiber officinale* Roscoe). *Renewable Energy* 13(4): 531-536.

Baranowski, J. D. 1986. Changes in solids, oleoresin, and 6-gingerol content of ginger during growth in Hawaii. *Hort. Science* 21: 145-6.

Beritognolo, I., Magel, E., Abdel-Latif, A., Charpentier, J. P., Jay-Allemand, C., and Breton, C. 2002. Expression of genes encoding chalcone synthase, flavanone 3-

hydroxylase and dihydroflavonol 4-reductase correlates with flavanol accumulation during heartwood formation in *Juglans nigra*. *Tree physiol. 22*(5): 291-300.

Bhattarai, K., Pokharel, B., Maharjan, S., and Adhikari, S. 2018. Chemical constituents and biological activities of ginger rhizomes from three different regions of Nepal. *J. Nutri. Diet Probiotics* 1(1): 180005.

Boughton, A. J., Hoover, K., and Felton, G. W. 2005. Methyl jasmonate application induces increased densities of glandular trichomes on tomato, *Lycopersicon esculentum*. *Journal of chemical ecology* 31(9): 2211-2216.

Chen, C. C., Kuo, M. C., and Ho, C. T. 1986. High performance liquid chromatographic determination of pungent gingerol compounds of ginger (*Zingiber officinale* Roscoe). *J. Food Sci.* 1(5): 1364-1365.

Chen, J. Y., Wen, P. F., Kong, W. F., Pan, Q. H., Zhan, J. C., Li, J. M., Wan, S. B., and Huang, W. D. 2006. Effect of salicylic acid on phenylpropanoids and phenylalanine ammonia-lyase in harvested grape berries. *Postharvest Biology and Technology* 40(1): 64-72.

Chervoneva, I., Li, Y., Schulz, S., Croker, S., Wilson, C., Waldman, S. A., and Hyslop, T. 2010. Selection of optimal reference genes for normalization in quantitative RT-PCR. *BMC Bioinforma*. 11: 253-256.

Chrubasik, J. E., Roufogalis, B. D., and Chrubasik, D. 2007. Evidence of effectiveness of herbal anti-inflammatory drugs in the treatment of painful osteoarthritis and chronic low back pain. *Phyto. Res.* 21: 675-683.

Connell, D. and Sutherland, M. 1969. A re-examination of gingerol, shogaol and zingerone, the pungent principles of ginger (*Zingiber officinale* Roscoe). *Aust. J. Chem.* 22: 1033–1043.

Connell, D.W. 1970. Natural pungent compounds. III. The paradols and associated compounds. *Aust. J. Chem. 23*(2): 369-376.

Conrad, A.C. and Mathabatha, M. F. 2016. Characterization and expression analyses of *Chalcone synthase* (*CHS*) and *Anthocyanidin synthase* (*ANS*) genes in *Cliviaminiata*. *Transcriptomics* 4(136): 2.

de Lima, R. M. T., dos Reis, A. C., de Menezes, A. A. P. M., Santos, J. V. D. O., Filho, J. W. G.D. O., Ferreira, J. R. D. O., de Alencar, M. V. O. B., da Mata, A. M. O. F., Khan, I. N., Islam, A., and Uddin, S. J. 2018. Protective and therapeutic potential of ginger (*Zingiber officinale*) extract and 6-gingerol in cancer: A comprehensive review. *Phytother. Res.* 32(10): 1885-1907.

de Lima, R. M. T., dos Reis, A. C., de Oliveira Santos, J. V., de Oliveira Ferreira, J. R., Braga, A. L., de Oliveira Filho, J. W. G., de Menezes, A. A. P. M., da Mata, A. M. O. F., de Alencar, M. V. O. B., do Nascimento Rodrigues, D. C.. and Ferreira, P. M. P. 2019. Toxic, cytogenetic and antitumor evaluations of 6-gingerol in non-clinical *in vitro* studies. *Biomedicine and Pharmacotherapy* 115: 108873.

Denniff, P., Macleod, I., and Whiting, D. A. 1980. Studies in the biosynthesis of 6-gingerol, pungent principle of ginger (*Zingiber officinale*). *J. Chem. Soc. Perkin Trans.* 1: 2637-2644.

Du, L., Guo, J., Zhang, W., Wu, H., and Yang, J. 2014. Comparative antioxidant properties of some gingerols and shogaols, and the relationship of their contents with the antioxidant potencies of fresh and dried ginger (*Zingiber officinale* Roscoe). *J. Agr. Sci. Tech.* 16: 1063-1072.

Dugasani, S., Pichika, M. R., Nadarajah, V. D., Balijepalli, M. K., Tandra, S., and Korlakunta, J. N. 2010. Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. *J. Ethnopharmacol.* 127: 515–520.

Ekundayo, O., Laakso, I., and Hiltunen, R. 1988. Composition of ginger (*Zingiber officinale* Roscoe) volatile oils from Nigeria. *Flavour and fragrance J.* 3(2): 85-90.

Elwan, M. W. M. and El-Hamahmy, M. A. M. 2009. Improved productivity and quality associated with salicylic acid application in greenhouse pepper. *Sci. Hortic.* 122(4): 521-526.

Evans, W. C. 2002. Ginger Trease and Evans Pharmacognosy (15th Ed.). W. B. Saunders, Edinburgh, pp. 277–280.

Feng, Q., Gui-Gong, G., Yang, Z., Hui-Chun, X., Lan, J., Jun, S., and Zhi, C. 2015. Molecular cloning and expression profiling of a chalcone synthase gene from *Lamiophlomisrotata. J. Genet.* 94(2): 193-205.

Fuglevand, G., Jackson, J. A. and Jenkins, G. I. 1996. UV-B, UV-A, and blue light signal transduction pathways interact synergistically to regulate chalcone synthase gene expression in Arabidopsis. *The Plant Cell* 8(12): 2347-2357.

George, M. V. 1997. Status of plantation crops in India. *Indian J. Plantation Crops 25: 1-14.*

Ghasemzadeh, A. and Ghasemzadeh, N. 2011. Effects of shading on synthesis and accumulation of polyphenolic compounds in ginger (*Zingiber officinale* Roscoe) varieties. *J. Med. Plants Res. 5*(11): 2435-2441.

Ghasemzadeh, A., Jaafar, H. Z. E., and Karimi, E. 2012b. Involvement of salicylic acid on anti-oxidant and anti-cancer properties, anthocyanin production and *Chalcone synthase* activity in ginger (*Zingiber officinale* Roscoe) varieties. *Int. J. Mol. Sci.* 13: 14828-14844.

Ghasemzadeh, A., Jaafar, H. Z. E., Karimi, E., and Ibrahim, M. H. 2012a. Combined effect of CO_2 enrichment and foliar application of salicylic acid on the production and anti-oxidant activities of anthocyanin, flavonoids and isoflavonoids from ginger. *BMC Complement. Altern. Med.* 12: 229.

Ghosh, S. and Mandi, S. S. 2015. SNP in *Chalcone synthase* gene is associated with variation of 6-gingerol content in contrasting land races of *Zingiber officinale* Roscoe. *Gene* 566: 184-188.

Gould, N., Reglinski, T., Northcott, G. L., Spiers, M., and Taylor, J. T. 2009. Physiological and biochemical responses in *Pinusradiata* seedlings associated with methyl jasmonate-induced resistance to *Diplodiapinea*. *Physiol. Mol. Plant Path.* *74*(2): 121-128.

Govindarajan, V. 1982. Ginger-chemistry technology and quality evaluation: Part-I CRC. *Crit. Rev. Food Sci. Nutr.* 17: 1-96.

Guchhait, S., Chakravarthy, A., Hore, J. K., and Mukherjee, D. 2005. Effect of micro-nutrients on some biochemical properties of rhizosphere soil and yield of ginger. *J. Plant. Crops* 33(2): 103-106.

Haroun, S. A., Aldesuqy, H. S., W. M. Shukry., and A. M. Gaber. 1998. Regulation of growth and metabolism in Lupinustermis plant by sodium salicylate. *Egypt J. physiol. Sci.* 22: 75.

He, X. G., Bernart, M. W., Lian, L. Z., and Lin, L. Z. 1998. High-performance liquid chromatography–electrospray mass spectrometric analysis of pungent constituents of ginger. *J. Chromatogr. A* 796(2): 327-334.

Heijari, J., Nerg, A. M., Kainulainen, P., Viiri, H., Vuorinen, M., and Holopainen, J. K. 2005. Application of methyl jasmonate reduces growth but increases chemical defence and resistance against *Hylobiusabietis* in Scots pine seedlings. *Entomol. Exp. Appl.* 115: 117–124.

Hu, B., Yao, H., Peng, X., Wang, R., Li, F., Wang, Z., Zhao, M., and Jin, L. 2019. Overexpression of Chalcone synthase improves flavonoid accumulation and drought tolerance in tobacco. *Preprints* 2019060103.

Huggett, J., Dheda, K., Bustin, S., and Zumla, A. 2005. Real-time RT-PCR normalization; strategies and considerations. *Genes Immun.* 6 : 279-284.

Idrees, M., Naeem, M., Aftab, T., and Khan, M. M. A. 2011. Salicylic acid mitigates salinity stress by improving antioxidant defence system and enhances vincristine

and vinblastine alkaloids production in periwinkle [*Catharanthus roseus* (L.) G. Don]. *Acta Physiologiae Plantarum 33*(3): 987-999.

Jiang, H., Solyom, A. M., Timmermann, B. N., and Gang, D. R. 2005. Characterization of gingerol-related compounds in ginger rhizome (*Zingiber officinale* Rosc.) by high-performance liquid chromatography/electrospray ionization mass spectrometry. *Rapid Communications in Mass Spectrometry* 19(20): 2957-2964.

Jiang, Y., Liao, Q., Zou, Y., Liu, Y., and Lan, J. 2017. Transcriptome analysis reveals the genetic basis underlying the biosynthesis of volatile oil, gingerols, and diarylheptanoids in ginger (*Zingiber officinale* Rosc.). *Bot. Stud.* 58(1): 41.

Jyotsna, N., Ghosh, C., and Meitei, W. I. 2012. Study of growth, yield and quality of organically grown ginger varieties under rainfed condition of Manipur. *J. Crop Weed. 8*: 17-21.

Kawaguchi, M., Fujioka, S., Sakurai, A., Yamaki, Y. T., and Syōno, K. 1993. Presence of a pathway for the biosynthesis of auxin via indole-3-acetamide in trifoliata orange. *Plant and Cell Physiology* 34(1): 121-128.

Kazan, K. and Manners, J. M. 2008. Jasmonate signaling: toward an integrated view. *Plant Physiology 146*(4): 1459-1468.

Kazemi, M. 2014. Foliar application of salicylic acid and methyl jasmonate on yield, yield components and chemical properties of Tomato. *Jordan J. Agric. Sci.* 173(3834): 1-8.

Kerala Agricultural University 2016. *Package of Practices Recommendations: Crops* 15th edition. Kerala Agricultural University, Thrissur, 392 p.

Kerala Agricultural University 2017. *Package of Practices Recommendations (Organic): Crops* 2017 2nd edition. Kerala Agricultural University, Thrissur, 328 p.

Kim, J., Chang, C., and Tucker, M. L. 2015. To grow old: regulatory role of ethylene and jasmonic acid in senescence. *Front. Plant Sci. 6*: 20.

Kizhakkayil, J. and Sasikumar, B. 2012. Characterization of ginger (*Zingiber officinale* Rosc.) germplasm based on volatile and non-volatile components. *Afr. J. Biotechnol.* 4: 777-786.

Kord, M. and Hathout, T. 1992. Changes on some growth criteria, metabolic activities and endogenous hormones in tomato plants consequent to spraying with different concentrations of salicyladhyde. *Egypt. J. Physiol. Sci.* 16: 117.

Kundu, J. K., Na, H. K., and Surh, Y. J. 2009. Ginger-derived phenolic substances with cancer preventive and therapeutic potential. *Food Factors for Health Promotion* (Vol. 61, pp. 182-192). Karger Publishers.

Langner, E., Greifenberg, S., and Gruenwald, J. 1998. Ginger: history and use. *Adv. Ther.* 15: 25-44.

Latha, P., Giridharan, M. P. and Naik, B. J. 1995. Performance of turmeric (*Curcuma longa* L.) cultivars in open and partially shaded conditions under coconut. *J. Spices and Aromatic Crops 4* (2): 139-144.

Lee, E. and Surh, Y. J. 1998. Induction of apoptosis in HL-60 cells by pungent vanilloids, [6]-gingerol and [6]-paradol. *Cancer letters* 134(2): 163-168.

Li, C., Wang, P., Menzies, N. W., Lombi, E., and Kopittke, P. M. 2018. Effects of methyl jasmonate on plant growth and leaf properties. *J. Plant Nutr. Soil Sci. 181*(3): 409-418.

Li, Y., Hong, Y., Han, Y., Wang, Y., and Xia, L. 2016. Chemical characterization and antioxidant activities comparison in fresh, dried, stir-frying and carbonized ginger. *J. Chromatogr. B* 1011: .223-232.

Liu, L., Yao, L., Wang, S., Chen, Z., Han, T., Ma, P., Jiang, L., Yuan, C., Li, J., Ke, D., and Li, C. 2019. 6-Gingerol improves ectopic lipid accumulation, mitochondrial dysfunction, and insulin resistance in skeletal muscle of ageing rats: dual stimulation of the AMPK/PGC-1α signaling pathway via plasma adiponectin and muscular AdipoR1. *Mol. Nutr. Food Res.* 63(6): 1800649.

Livak, K. J. and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods 25*(4): 402-408.

Mabberley, D. J. 1997. *The plant-book: a portable dictionary of the vascular plants*. Cambridge university press, New York, 858p.

Majumdar, B., Venkatesh, M. S., Kumar, K., and Patiram. 2005. Effect of potassium and farmyard manure on yield, nutrient uptake and quality of ginger (*Zingiber officinale*) in a TypicHapludalf of Meghalaya. *Indian J. Agric. Sci.* 75(12): 809-811.

Manaf, A. 2013. Molecular cloning and expression profiling of a chalcone synthase gene from *Ginkgo biloba*, *Scutellaria viscidula* Bunge and Tree peony (*Paeonias uffruticosa*). *Int. J. Biosci. 3*(10): 187-202.

Manoj, M. 2017. *Effect of metabolite elicitors on curcumin content in turmeric (Curcuma longa L.) cv. Mydukur* Ph. D. Thesis, Dr. YSR Horticultural University, West Godavari, 101 p.

Manoli, A., Sturaro, A., Trevisan, S., Quaggiotti, S., and Nonis, A. 2012. *Evaluation* of candidate reference genes for qPCR in maize. *J. Plant Physiology* 169 (8): 807–815.

McHale, D., Laurie, W. A., and Sheridan, J. B. 1989. Transformations of the pungent principles in extracts of ginger. *Flavour Fragr. J.* 4(1): 9-15.

Mian, S. S., Upadhayay, S., and Naqvi, S. N. 2019. Physicochemical analysis of ginger (*Zingiber officinale* Rosc.) rhizome along with its TLC, HPLC and HPTLC Profile. *Pharma. Methods* 10(1).

Mishra, A. P., Saklani, S., and Chandra, S. 2013. Estimation of gingerol content in different brand samples of ginger powder and their anti-oxidant activity: A comparative study. *Recent Res. Sci. Technol.* 5(1).

Moreira, X., Zas, R., and Sampedro, L. 2012. Methyl jasmonate as chemical elicitor of induced responses and anti-herbivory resistance in young conifer trees. In *Plant defence: Biological control* 345-362.

Natarajan, C. P., Bai, R. P., Krishnamurthy, M. N., Raghawan, B., Shankaracharya, N. B., Kuppuswamy, S., Govinbarajan, V. S., and Lewis, Y. S. 1972. Chemical composition of ginger varieties and dehydration studies on ginger. *Indian J. Food Sci. Technol.* 9: 120-124.

Ohnishi, M., Ohshita, M., Tamaki, H., Marutani, Y., Nakayama, Y., Akagi, M., Miyata, M., Maehara, S., Hata, T., and Inoue, A. 2019. Shogaol but not gingerol has a neuroprotective effect on hemorrhagic brain injury: Contribution of the α, β-unsaturated carbonyl to heme oxygenase-1 expression. *Eur.J.Pharmacol. 842:* 33-39.

Padmapriya, S., and N. Chezhiyan. 2009. Effect of shade, organic, inorganic and biofertilizers on morphology, yield and quality of turmeric. *Indian J. Hortic.* 66 (3): 333-339.

Padmapriya, S., Chezhiyan, N., and Sathiyamurthy, V. A. 2007. Effect of shade and integrated nutrient management on biochemical constituents of turmeric (*Curcuma longa* L.). *J. Hortic. Sci. 2*(2), pp.123-129.

Pahalwan, D. K., Dubey, O. P., and Wasnikar, A. R. 2004. Performance assessment of ginger under different fertility levels. In: *National Seminar on Opportunities and Potentials of Spices for Crop Diversification. Jan.* 19-21.

Park, G. H., Park, J. H., Song, H. M., Eo, H. J., Kim, M. K., Lee, J. W., Lee, M.H., Cho, K. H., Lee, J. R., Cho, H. J., and Jeong, J. B. 2014. Anti-cancer activity of Ginger (*Zingiber officinale*) leaf through the expression of activating transcription factor 3 in human colorectal cancer cells. *BMC complementary and alternative medicine.* 14(1): 408.

Parthasarathy, V. A., Chempakam, B., and Zachariah, T. J. 2008. *Chem. Spices*. 45p.

Pawar, N., Pai, S., Nimbalkar, M., and Dixit, G. 2011. RP-HPLC analysis of phenolic antioxidant compound 6-gingerol from different ginger cultivars. *Food Chem.* 126(3): 1330-1336.

Peerapak, T. and Tongchitpakdee, S. 2010. Effect of drying method on 6-gingerol content total phenolic content and antioxidant capacity of ginger (*Zingiber officinale*). In: *Proceedings of the 48th Kasetsart University Annual Conference;* 3-5 Mar. 2010, Kasetsar. *Subject: Agro-Industry*. Kasetsart University.

Perry, R. P., La Torre, J., Kelley, D. E., and Greenberg, J. R. 1972. On the lability of poly(A) sequences during extraction of messenger RNA from polyribosomes. *Biochem. Biophys .Acta.* 262(2): 220-226.

Prasad, S., Gupta, S. C., and Aggarwal, B. B. 2012. Micronutrients and cancer: Add spice to your life. In *Nutrition, Diet and Cancer* (pp. 23-48). Springer, Dordrecht.

Radhakrishnan, E. K. and Soniya, E. V. 2009. Molecular analysis of type III polyketide synthase (PKS) gene family from *Zingiber officinale* Rosc. *Afr. J. Plant Sci.* 3(3): 044-048.

Radhakrishnan, E. K., Rintu, T. V., and Soniya, E. V. 2010. Unusual intron in the second exon of a type III polyketide synthase gene of *Alpiniacalcarata*Rosc. *Genet. Mol. Biol.* 33(1): 141-145.

Radhakrishnan, E. K., Sivakumar, K. C., and Soniya, E. V. 2009. Molecular characterization of novel form of type III polyketide synthase from *Zingiber officinale* Rosc. and its analysis using bioinformatics method. *J. Proteomics Bioinform.* 2: 310-315.

Rafi, M., Lim, L. W., Takeuchi, T., and Darusman, L. K. 2013. Simultaneous determination of gingerols and shogaol using capillary liquid chromatography and

its application in discrimination of three ginger varieties from Indonesia. *Talanta* 103: 28-32.

Rai, S., Mukherjee, K., Mal, M., Wahile, A., Saha, B. P., and Mukherjee, P. K. 2006. Determination of 6-gingerol in ginger (*Zingiber officinale*) using high-performance thin-layer chromatography. *J. Sep. Sci. 29*(15): 2292-2295.

Ramirez-Ahumada, M. D., Timmermann, B. N., and Gang, D. R. 2006. Biosynthesis of curcuminoids and gingerols in turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*): identification of curcuminoid synthase and hydroxycinnamoyl-CoA thioesterases. *Phytochem.* 67(18): 2017-2029.

Rastogi, N., Gara, R. K., Trivedi, R., Singh, A., Dixit, P., Maurya, R., Duggal, S., Bhatt, M. L. B., Singh, S., and Mishra, D. P. 2014. (6)-Gingerol induced myeloid leukemia cell death is initiated by reactive oxygen species and activation of miR-27b expression. *Free Radic.Biol. Med.* 68:288-301.

Rhode, J., Fogoros, S., Zick, S., Wahl, H., Griffith, K. A., Huang, J., and Liu, J.,R. 2007. Ginger inhibits cell growth and modulates angiogenic factors in ovarian cancer cells. *BMC Complementary and Alternative Medicine.* 7(1): 44.

Rowshan, V. and Bahmanzadegan, A. 2013. Effects of salicylic acid on essential oil components in Yarrow (*Achillea millefolium* Boiss). *Int. J. Basic Sci. Appl. Res.* 2: 347-351.

Rowshan, V., Khoi, M. K., and Javidnia, K. 2010. Effects of salicylic acid on quality and quantity of essential oil components in *Salvia macrosiphon*. *J. Biol. Environ. Sci.* 4(11): 77-82.

Sampedro, L., Moreira, X., and Zas, R. 2011. Resistance and response of *Pinus pinaster* seedlings to *Hylobius abietis* after induction with methyl jasmonate. *Plant Ecol. 212*(3): 397-401.

Schröder, J. 1977. A family of plant-specific polyketide synthases: facts and predictions. *Trends Plant Sci.* 2(10): 373-378.

Seyie, A., Naruka, I. S., Singh, P. P., and Kushwah, S. S. 2013. Nutrient Management and Its Effect on Growth, Yield and Quality of Ginger Cultivars. *Indian J.Hortic.* 70(1): 65-70.

Shylaja, M. R., 2017. Metabolome analysis in ginger and product development using gingerol, Project Completion report submitted to BIRAC, Department of Biotechnology, New Delhi.

Shylaja, M. R., Nybe, E. V., Nazeem, P. A., Mathew, S. K., Krishnan, S., and Paul, R. 2014. Aswathy, a new ginger variety from Kerala Agricultural University for green ginger. *Indian J. Arecanut Spices Med. Plants.* 16(2): 18-20.

Shylaja, M. R., Paul, R., Nybe, E. V., Abraham, K., Nazeem, P. A., Valsala, P. A., and Krishnan, S. 2010. Two new ginger varieties from Kerala Agricultural University. *Indian J. Arecanut, Spices and Med. Plants* 12(2): 3-4.

Singh, B. and Usha, K. 2003. Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regulation* 39(2): 137-141.

Singh, K., Kumar, A., Kajal, M., and Singh, B. 2019. Characterization and expression analysis of chalcone synthase and chalcone isomerase genes in *Phyllanthusemblica* (L.). *J. Plant Biochem.Biotechnol.* 28(1): 105-113.

Singh, S. P. and Dwivedi, D. K. 2007. Impact of zinc, boron and iron elements on yield and economics of ginger. *Int. J. Agric. Sci.* 3 (1): 136-138.

Sreeja, S. 2017. Metabolite profiling and gene expression analysis for gingerol production in selected somaclones of ginger (*Zingiber officinale* Rosc.). Ph.D. Thesis, Kerala Agricultural University, Thrissur, 276 p.

Srinivasan, V., Thankamani, C. K., Dinesh, R., Kandiannan, K., Hamza, S., Leela, N. K., and Zachariah, T. J. 2019. Variations in soil properties, rhizome yield and quality as influenced by different nutrient management schedules in rainfed ginger. *Agric. Res.* 8(2): 218-230.

Staiger, C. J. and Schliwa, M. 1987. Actin localization and function in higher plants. *Protoplasma, 141* (1): 1-12.

Suhaimi, M. Y., Adzemi, M. S., and Zaliha, W. S. W. 2018. Effect of organic substrates on ginger growth, yield and [6]-gingerol content cultivated using soilless culture system. *Agrofor. Int. J. 3* (1): 122- 131.

Sutarno, H., Hadad, E. A., and Brink, M. 1999. *Zingiber officinale* Roscoe. *Plant Resources of South-East Asia.* 13, pp.238-244.

Swiatek, A., Azmi, A., Stals, H., Inze, D., and Onckelen, V. H. 2004. Jasmonic acid prevents the accumulation of cyclin B1; 1 and CDKB in synchronized tobacco BY-2 cells. *FEBS Lett.* 572: 118–122.

Świątek, A., Lenjou, M., Van Bockstaele, D., Inzé, D., and Van Onckelen, H. 2002. Differential effect of jasmonic acid and abscisic acid on cell cycle progression in tobacco BY-2 cells. *Plant Physiol. 128* (1): 201-211.

Taguchi, G., Yazawa, T., Hayashida, N., and Okazaki, M. 2001. Molecular cloning and heterologous expression of novel glucosyltransferases from tobacco cultured cells that have broad substrate specificity and are induced by salicylic acid and auxin. *European J. Biochem.* 268(14): 4086-4094.

Tajik, S. and Niknam, V. 2015. Effects of salicylic acid on carotenoids and antioxidant activity of saffron (*Crocus sativus* L.). *Appl. Food Biotechnol.* 2(4): 33-37.

Thellin, O., ElMoualij, B., Heinen, E., and Zorzi. 2009. A decade of improvements in quantification of gene expression and internal standard selection. *Biotechnol. Adv.* 27: 323-333.

Tyburski, J., Studzinska, A., Daca, P., and Tretyn, A. 2008. PCR in real time: methods of data analysis. *Biotechnologia* 1(80): 86-96.

Walker, T. S., Bais, H. P., and Vivanco, J. M. 2002. Jasmonic acid-induced hypericin production in cell suspension cultures of *Hypericum perforatum* L. (St. John's wort). *Phytochem. 60*(3): 289-293.

Wang, J., Ke, W., Bao, R., Hu, X., and Chen, F. 2017. Beneficial effects of ginger Zingiber officinale Roscoe on obesity and metabolic syndrome: a review. *Ann. N. Y. Acad. Sci.* 1398(1): 83-98.

Wei, L., Shao-Hu, T., Ke-Ming, L., and Min, S. 2010. Molecular cloning and expression profiling of *Chalcone synthase* gene from hairy root cultures of *Scutellaria viscidula* Bunge. *Gen. Mol. Biol.* 33 (2): 285-291.

Wilson, H. and Ovid, A. 1993. Growth and yield responses of ginger (*Zingiber officinale* Roscoe) as affected by shade and fertilizer applications. *J. Plant Nutr.* 16(8): 1539-1545.

Wohlmuth, H. 2008. Phytochemistry and pharmacology of plants from the ginger family, Zingiberaceae. Ph. D Thesis, Southern Cross University, Lismore, 237p.

Zhang, C., Yao, X., Ren, H., Wang, K., and Chang, J. 2019. Isolation and Characterization of Three Chalcone Synthase Genes in Pecan (*Caryaillinoinensis*). *Biomolecules.* 9(6): 236.

Zhang, W., Liu, X., Jiang, Y., Wang, N., Li, F., and Xin, H. 2019. 6-Gingerol Attenuates Ischemia-Reperfusion-Induced Cell Apoptosis in Human AC16 Cardiomyocytes through HMGB2-JNK1/2-NF-κB Pathway. *Evid. Based Complementary and Alternat. Med.* 2019.

Zhang, Y. X., Li, J. S., Chen, L. H., Peng, W. W., and Cai, B. C. 2012. Simultaneous determination of five gingerols in raw and processed ginger by HPLC. *Chin. Pharm. J.* 47(6): 471-474.

Zhao, T., Krokene, P., Björklund, N., Långström, B., Solheim, H., Christiansen, E., and Borg-Karlson, A. K., 2010. The influence of Ceratocystis polonica inoculation and

methyl jasmonate application on terpene chemistry of Norway spruce, Piceaabies. *Phytochemistry* 71(11-12):1332-13.

Zunun-Pérez, A. Y., Guevara-Figueroa, T., Jimenez-Garcia, S. N., Feregrino-Pérez, A. A., Gautier, F., and Guevara-González, R. G. 2017. Effect of foliar application of salicylic acid, hydrogen peroxide and a xyloglucan oligosaccharide on capsiate content and gene expression associated with capsinoids synthesis in *Capsicum annuum* L. *J. Biosci.* 42(2): 245-250.

Appendices

APPENDIX I

Soil test report for the treatment T2- PoP Soil test based nutrient management

Parameters	Value	Remarks
pH	6.80	Neutral
Electrical conductivity	1.25 dSm^{-1}	High
Organic Carbon	2.47 %	High
Available Phosphorous	455.63 kg ha^{-1}	High
Available Potassium	2074.80 kg ha^{-1}	High
Available Calcium	763.21 mg kg^{-1}	Sufficient
Available Magnesium	450.89 mg kg^{-1}	Sufficient
Available Sulphur	132.24 mg kg^{-1}	Sufficient
Copper	1.47 mg kg^{-1}	Sufficient
Iron	72.09 mg kg^{-1}	Sufficient
Zinc	8.20 mg kg^{-1}	Sufficient
Manganese	79.54 mg kg^{-1}	Sufficient
Boron	0.39 mg kg^{-1}	Deficient

Recommendations as per soil test report for the treatment T2 - PoP Soil test based nutrient management

Fertiliser	Basal	60 days after planting	120 days after planting
Urea	-	0.244 g/growbag	0.244 g/growbag
Rock Phosphate	0.403 g/growbag	-	-
Muriate of Potash	0.060 g/growbag	-	0.060 g/growbag

Foliar spray of 0.1 per cent borax solution was at weekly intervals from one month after planting to four months after planting.

APPENDIX II

Composition of buffers and dyes used for agarose gel electrophoresis

1. 50X TAE buffer 50X (for 1 L)

242 g tris base

57.1 ml glacial acetic acid

100 ml 0.5 M EDTA (pH 8.0)

1000 ml distilled water

The solution was autoclaved and stored at room temperature.

2. Loading dye/tracking dye (6X)

0.25% bromophenol blue

0.25% xylene cyanol

30% glycerol in water

The dye was stored at 4°C

3. Ethidium bromide (intercalating dye)

The dye was prepared as a stock solution of 10mg/ml in water and was stored at room temperature in a dark bottle.

APPENDIX III

Reagents provided in the kit for first strand cDNA preparation

Oligo$(dT)_{18}$ primer (100 µM)

Nuclease free water

5X reaction buffer

RiboLock RNase Inhibitor (20 U/µl)

10 mM dNTP Mix

RevertAid M-MuLV RT (200 U/µl)

Expression of *Chalcone synthase* gene in ginger (*Zingiber officinale* Rosc.) as influenced by various management practices

By

Archita Unnikrishnan

(2017-11-004)

Abstract of Thesis

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ABSTRACT

Ginger (*Zingiber officinale* Rosc.), a rhizomatous spice crop is known for its nutraceutical potential due to the presence of non-volatile pungent principles, gingerols. The pungent principles in ginger are derived *via* "stilbenoid, diarylheptanoid and gingerol biosynthesis" pathway and the key enzyme involved in gingerol biosynthesis is Chalcone synthase.

The present research work was undertaken at Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture, Kerala Agricultural University, Thrissur from 2017 to 2019 to analyse the influence of nutrient management and spraying of elicitors on *Chalcone synthase* gene expression in ginger.

The study was conducted under two situations, in high tech polyhouse available at CPBMB and in open conditions. Various treatments *viz.*, T1 (Nutrient management as per PoP, KAU), T2 (PoP soil test based nutrient management), T3 (Adhoc PoP organic, KAU), T4 (PoP nutrient management, KAU + salicylic acid foliar spray - 100 µM) and T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray, 100 µM) were imposed. The KAU released ginger variety Karthika, known for its high gingerol content was used for the experiment.

The morphological observations such as length of pseudostem, number of tillers per plant, number of leaves per tiller and leaf area were recorded at 60, 90 and 120 Days After Planting (DAP). All the treatments except T4 (PoP nutrient management KAU + salicylic acid foliar spray, 100 µM) and T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray, 100 µM) recorded higher pseudostem length irrespective of the growing conditions. The plants under polyhouse recorded higher pseudostem length than the plants raised under open condition. The tiller production was higher in plants grown in open condition. The number of leaves per tiller was less in treatment T5 (PoP nutrient management, KAU + methyl jasmonate foliar spray - 100 µM) both under polyhouse and open conditions. In polyhouse condition, the leaf area was higher than in open condition.

The relative expression of *Chalcone synthase* gene was studied from the leaves of various treatments at 120 DAP. All the treatments in polyhouse and open conditions recorded higher gene expression over the control treatment T1 (Nutrient management as per PoP, KAU). The treatment T2 (PoP soil test based nutrient management) recorded the highest expression of *Chalcone synthase* gene both under polyhouse and open conditions with a fold increase of 1.346 and 1.166 respectively.

The plants under polyhouse recorded higher fresh yield of the rhizomes than plants under open condition. The treatment T1 (Nutrient management as per PoP, KAU) was the best irrespective of the growing conditions with regard to fresh yield. The rhizome characters such as number, length and girth of primary, secondary, tertiary fingers, thickness of inner core, colour and plumpiness of rhizomes were recorded. Rhizome characters were good in the treatments T1 (Nutrient management as per PoP, KAU), T2 (PoP soil test based nutrient management) and T3 (Adhoc PoP organic-KAU). There was no significant difference in the dry ginger recovery for the rhizomes harvested from both polyhouse and open conditions. The treatment T2 (PoP soil test based nutrient management) recorded higher recovery of both oleoresin and gingerol. The increased recovery of oleoresin in the treatment T2 (PoP soil test based nutrient management) was 71.16 per cent over the control in open condition and 32.11 per cent in polyhouse over the control. Similarly, the total gingerol content recorded an increase of 15.28 per cent over the control in polyhouse and 31.98 per cent over the control in open condition.

The major outcome of the present investigations is the high recovery of total gingerol in soil test based nutrient management in ginger. The abiotic elicitors like salicylic acid and methyl jasmonate sprayed could not improve the recovery of gingerols. The soil test based nutrient management recorded higher recovery of both oleoresin and gingerol and was found cost-effective when compared to the other treatments.